Ilam University of Medical Sciences

Plant Biotechnology Persa

Online ISSN: 2676-7414

Homepage: https://pbp.medilam.ac.ir

Effect of Different Drying Methods on Hypericin Quantity in Adventitious Roots of St. John's Wort (*Hypericum perforatum* L.)

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Article Info

Article type:
Original Article

Article History:
Received: 05 Jul 2025
Revised: 19 Sep 2025
Accepted: 21 Sep 2025
Published Online: 23 Sep 2025

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A B S T R A C T

Objective: *Hypericum perforatum* L. synthesizes hypericin, a bioactive compound with antidepressant and antiviral properties. Optimizing post-harvest processing is crucial to preserve metabolite content, as drying conditions can markedly affect compound stability. This study evaluated the impact of shade-drying, oven-drying, and freeze-drying on hypericin accumulation in adventitious roots of H. perforatum.

Methods: Fresh adventitious roots (~90% moisture, wet basis) underwent shade-drying, oven-drying, or freeze-drying until final moisture reached 37%, 8%, and 5.3%, respectively. Hypericin content was quantified via high-performance liquid chromatography (HPLC). Four independent root lines were analyzed to assess biological variability. Hierarchical cluster analysis with heatmap visualization examined the relationships between drying treatments and root lines.

Results: Drying method significantly influenced hypericin retention. Freeze-drying preserved the highest levels, with peak concentrations of 0.166 mg/g DW, while oven-drying retained intermediate levels and shade-drying caused the greatest losses. Across all drying conditions, line 4 consistently exhibited higher hypericin content, indicating potential genetic or physiological advantages in metabolite biosynthesis or storage. Cluster analysis revealed a clear separation of freeze-dried samples, with line 4 displaying distinctly superior performance.

Conclusion: Freeze-drying is the most effective method for maintaining hypericin in *H. perforatum* adventitious roots, whereas oven- and shade-drying substantially reduce content. Additionally, root line selection critically affects metabolite accumulation, with line 4 demonstrating the highest potential. These findings highlight that both post-harvest processing strategies and genotype optimization are essential for maximizing hypericin yield in biotechnological and industrial applications.

Key words: *Hypericum perforatum*, Hypericin, Adventitious Roots, Drying Methods, High-Performance Liquid Chromatography, Genotype

➢ **How to cite this paper**
Shafaei M, Ebrahimi M, Mokhtari A. Effect of Different Drying Methods on Hypericin Quantity in Adventitious Roots of St. John's Wort. Plant Biotechnology Persa. 2026; 8(2): Proof.

[Downloaded from pbp.medilam.ac.ir on 2026-11-05]

[DOI: 10.66224/pbp.8.3.4]

Introduction

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Publisher: Ilam University of Medical Sciences

Hypericum perforatum L. (St. John's Wort) is a widely used medicinal plant with a long history of application in both traditional and modern medicine. It is best known for producing hypericin, a naphthodianthrone derivative that exhibits antidepressant, antiviral, and anticancer properties [1, 2]. Owing to its broad pharmacological activities, hypericin is regarded as one of the most valuable secondary metabolites of *H. perforatum*.

By focusing on the adventitious roots of *H. perforatum* var. Topaz, this study addresses a key knowledge gap and evaluates how alternative drying strategies influence hypericin content. This approach highlights both the importance of optimizing processing methods and the potential of specific genotypes for improving metabolite yield.

The plant material used in this study was *H. perforatum* var. Topaz, a cultivar previously identified as a high-hypericin–producing variety with stable biosynthetic capacity under in vitro culture conditions [3]. Adventitious roots of this variety provide a renewable and controlled source of hypericin, yet the impact of different drying methods on metabolite stability in roots has received little attention compared with aerial tissues.

Freeze-drying reduces oxidative and thermal stress, and in this study, an 8-hour duration was selected based on efficient desiccation of fine root particles, in line with prior reports on *Hypericum* tissues [4].

Oven-drying provides faster processing, and the chosen duration was optimized to achieve stable moisture levels below 10%, ensuring data reliability while minimizing thermal degradation [5].

Drying is a critical step in processing medicinal plant materials, as it reduces moisture content, prevents microbial growth, and enhances long-term stability [6]. However, the choice of drying technique has a substantial effect on the retention of bioactive compounds. Shade-drying, oven-drying, and freeze-drying are among the most common methods, each with advantages and limitations. Shade-drying is simple and low-cost, but its long duration results from reliance on ambient airflow and temperature, which slows moisture reduction and increases the risk of enzymatic or microbial activity [7].

Despite its importance, hypericin is inherently unstable, being sensitive to degradation by light, elevated temperatures, and oxygen [8]. Such instability presents a major challenge during post-harvest processing and storage, underscoring the importance of identifying methods that minimize degradation and preserve compound stability.

The objective of this study was to evaluate the effects of three drying methods shade-drying, oven-drying, and freeze-drying on the preservation of hypericin quantity in the adventitious roots of *Hypericum perforatum* L., and to compare variations in hypericin levels among different root lines.

Materials and Methods

Plant Material

Adventitious roots were induced from *Hypericum perforatum* L. var. Topaz (strain previously characterized as a high hypericin–producing cultivar [3]. Petioles were removed, and leaf explants were cultured in a liquid medium containing 2 mg/L indole-3-butyric acid (IBA) within half-strength Murashige and Skoog (MS) salts and B5 vitamins. Adventitious root formation was observed after one month of culture, and root biomass was subsequently maintained on a biomass-enhancing medium before harvest.

Drying techniques and equipment

Three drying methods were employed:

Shade-drying: Samples were dried under controlled ambient airflow (0.3–0.5 m/s) at 26 ± 2 °C, with relative humidity of 55–65%.

Oven-drying: Samples were dried at 45 °C in a ventilated oven (airflow 1.5 m/s; MEMMERT ULE 500).

Freeze-drying: Samples were dried in a laboratory freeze-dryer (MEMMERT; V 400, pressure 200 mbar) at –52 °C for 8 h. The relatively short drying time was sufficient due to the fine particle size of the milled roots and has been previously reported for *H. perforatum* tissues [9].

Moisture quantity

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Moisture quantity of fresh and dried roots was determined on both wet basis (%) and dry basis (g water/g dry matter), following standard gravimetric methods [10].

Preparation of root extract

Fifty milligrams of milled dried roots were extracted with 500 µL pure methanol by ultrasonic extraction for one hour, followed by centrifugation at 7000 g for five minutes. The residue was re-extracted, and the combined supernatants were adjusted to 1 mL total extract volume.

Determination of total hypericin quantity

Hypericin was quantified using a validated HPLC method described in the European Pharmacopoeia (Ph. Eur.,10th edition) and adapted from Cui et al. (2010). Chromatographic separation was achieved on a Reprosphere 100 C18 column (5 µm, 250 × 4.6 mm) using a mobile phase of methanol, ethyl acetate, and sodium dihydrogen phosphate buffer (pH 2.0). Detection was performed by fluorescence (λ_ex 315 nm, λ_em 593 nm). Method validation included determination of limit of detection (LOD = 0.002 mg/g DW), limit of quantification (LOQ = 0.006 mg/g DW), and recovery rate (98.3 ± 2.1%). Method validation included determination of the limit of detection (LOD = 0.002 mg/g DW), limit of quantification (LOQ = 0.006 mg/g DW), and recovery rate (98.3 ± 2.1%). These parameters confirmed the method's sensitivity and accuracy for quantifying hypericin in dried root samples.

Statistical analysis

All analyses were conducted using SAS 9.2. One-way ANOVA was performed to compare hypericin yields across treatments. Duncan's multiple range test was selected for post-hoc analysis due to its higher sensitivity for detecting differences among a small number of treatment groups, compared with more conservative tests such as Tukey or Bonferroni. Duncan's multiple range test was selected for post-hoc analysis because it offers greater sensitivity in distinguishing differences among a relatively small number of treatment groups (three drying methods × four root lines). While Tukey and Bonferroni tests are more conservative and reduce Type I error, they often mask meaningful differences in small experimental designs. Duncan's test was therefore appropriate for our aim of detecting subtle but biologically relevant differences in hypericin content. Euclidean distance reflects the quantitative dissimilarity in hypericin content between samples, such that smaller distances indicate closer similarity in metabolite retention. Ward's method minimizes the total variance within clusters, ensuring that groups represent distinct drying outcomes. In this context, clusters correspond to sets of root lines and drying treatments that share similar hypericin quantities, with larger distances between clusters representing stronger separation in drying efficiency or genetic performance.

Cluster analysis

Hierarchical cluster analysis (HCA) was performed using SPSS v27.0 with Euclidean distance and Ward's minimum variance method as the clustering algorithm. Euclidean distance reflects the quantitative dissimilarity in hypericin content between samples, such that smaller distances indicate closer similarity in metabolite retention. Ward's method minimizes the total variance within clusters, ensuring that groups represent distinct drying outcomes. In this context, clusters correspond to sets of root lines and drying treatments that share similar hypericin quantities, with larger distances between clusters representing stronger separation in drying efficiency or genetic performance.

Results

Dry product characteristics

Fresh adventitious roots of *H. perforatum* contained ~90% (w/w) moisture at harvest. After drying, residual moisture differed by method (Figure 1). Shade-drying for five days left 37% moisture, indicating incomplete desiccation. Oven-drying at 45 °C reduced moisture to 8% within 24 h, while freeze-drying achieved 5.3% within 8 h. As moisture quantities below 10% are generally recommended to prevent microbial growth and enzymatic activity[10]. Only oven- and freeze-drying met this criterion. Shade-dried samples, therefore, presented higher risks of instability during storage.

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Hypericin quantity under different drying methods

Drying method strongly influenced hypericin quantity (Table 2). Freeze-dried samples retained the highest concentrations, ranging from 0.142 to 0.167 mg/g DW across lines. Oven-dried samples showed intermediate values (0.059–0.095 mg/g DW), whereas shade-drying caused the greatest losses, with hypericin reduced to ≤0.038 mg/g DW, and in some cases, undetectable. When averaged across lines, freeze-drying preserved 0.155 mg/g DW, oven-drying 0.076 mg/g DW, and shade-drying only 0.021 mg/g DW. These differences represent more than a seven-fold variation between the best and worst-performing treatments.

Variation among root lines

Significant differences were also observed among the four adventitious root lines (Figure 3). Line 4 consistently accumulated the most hypericin, reaching 0.167 mg/g DW under freeze-drying, 0.095 mg/g DW under oven-drying, and 0.038 mg/g DW under shade-drying. Line 3 exhibited intermediate levels (0.155, 0.083, and 0.024 mg/g DW, respectively), whereas Line 2 showed the lowest overall accumulation (0.142, 0.059, and undetectable under shade-drying). Line 1 fell between these extremes.

These data indicate that both genotype (root line) and post-harvest treatment contribute substantially to final metabolite yield. Importantly, even under suboptimal methods such as oven- or shade-drying, Line 4 outperformed the other lines, suggesting inherent biosynthetic or stability advantages.

Cluster analysis of drying outcomes

Hierarchical cluster analysis (HCA) grouped the data into six distinct clusters (Figure 2, Table 1). Clusters 3 and 6, dominated by freeze-dried samples, were characterized by the highest hypericin concentrations (0.110 and 0.167 mg/g DW, respectively). Clusters 4 and 5 included oven-dried samples with intermediate values (0.062–0.081 mg/g DW). Clusters 1 and 2 corresponded to shade-dried samples, with very low or absent hypericin (0.000–0.033 mg/g DW).

This clustering pattern highlights the biological effect of drying conditions: freeze-drying consistently segregated into high-hypericin clusters, oven-drying into intermediate clusters, and shade-drying into low-value clusters. The use of Euclidean distance and Ward's method ensured that samples were grouped according to similarity in quantitative hypericin retention rather than random variation.

Integrated interpretation of drying × line interactions

Table 2 (Line × Method matrix) provides a detailed breakdown of mean hypericin quantities for each root line under each drying method. These values allow direct quantitative comparisons:

Freeze-drying consistently maximized retention across all lines, with Line 4 reaching the highest concentration (0.167 mg/g DW).

Oven-drying preserved moderate amounts, with Line 4 again highest (0.095 mg/g DW) and Line 2 lowest (0.059 mg/g DW).

Shade-drying resulted in drastic losses, with only trace levels detected in Line 4 (0.038 mg/g DW) and no detectable hypericin in Line 2.

These results confirm that both processing method and genetic background interact to determine final metabolite yield. From a practical perspective, selecting superior lines such as Line 4 and combining them with optimized drying methods is necessary to maximize hypericin-rich raw material production.

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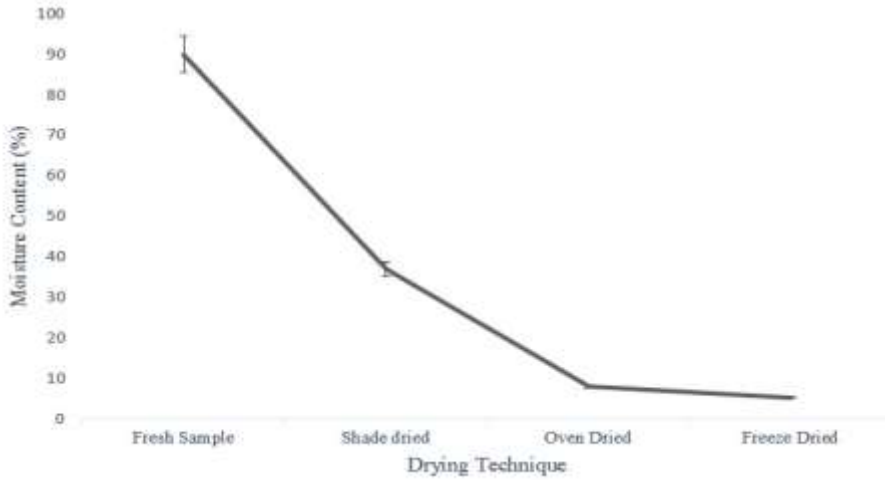


Figure 1: Moisture quantity (%) in the adventitious root of *H. perforatum* samples that were dried using different methods

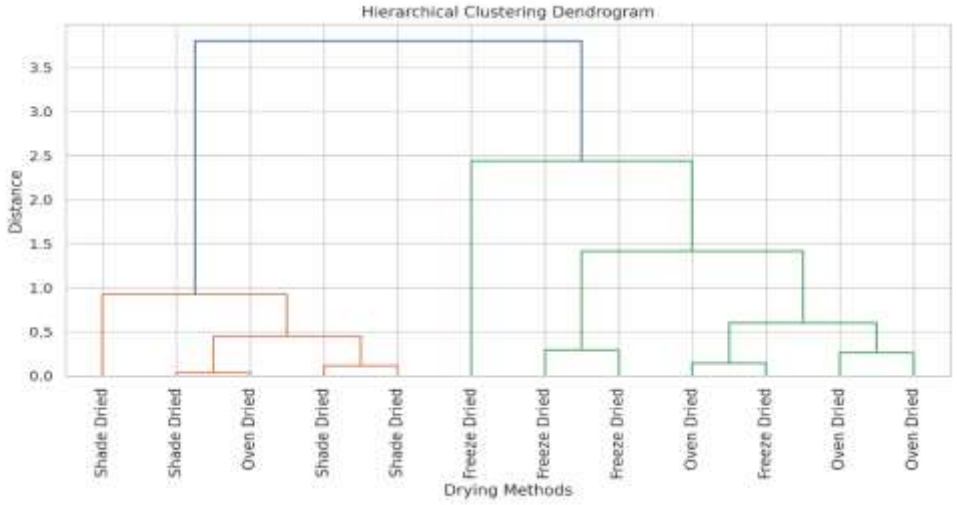


Figure 2: The hierarchical clustering analysis revealed how different drying methods group based on their hypericin quantity. The dendrogram illustrates the relationships among the methods, indicating that certain methods are more similar in their effects on hypericin levels.

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Table 1: The characteristics of each cluster based on the hypericin quantity

Cluster	Hypericin (mg/g DW) mean
1	0.032
2	0
3	0.1
4	0.08
5	0.061
6	0.166

Table 2: The table of drying methods associated with each cluster

Cluster	Drying methods
1	Shade Dried - Oven Dried
2	Shade Dried
3	Freeze Dried
4	Oven Dried - Freeze Dried
5	Oven Dried
6	Freeze Dried

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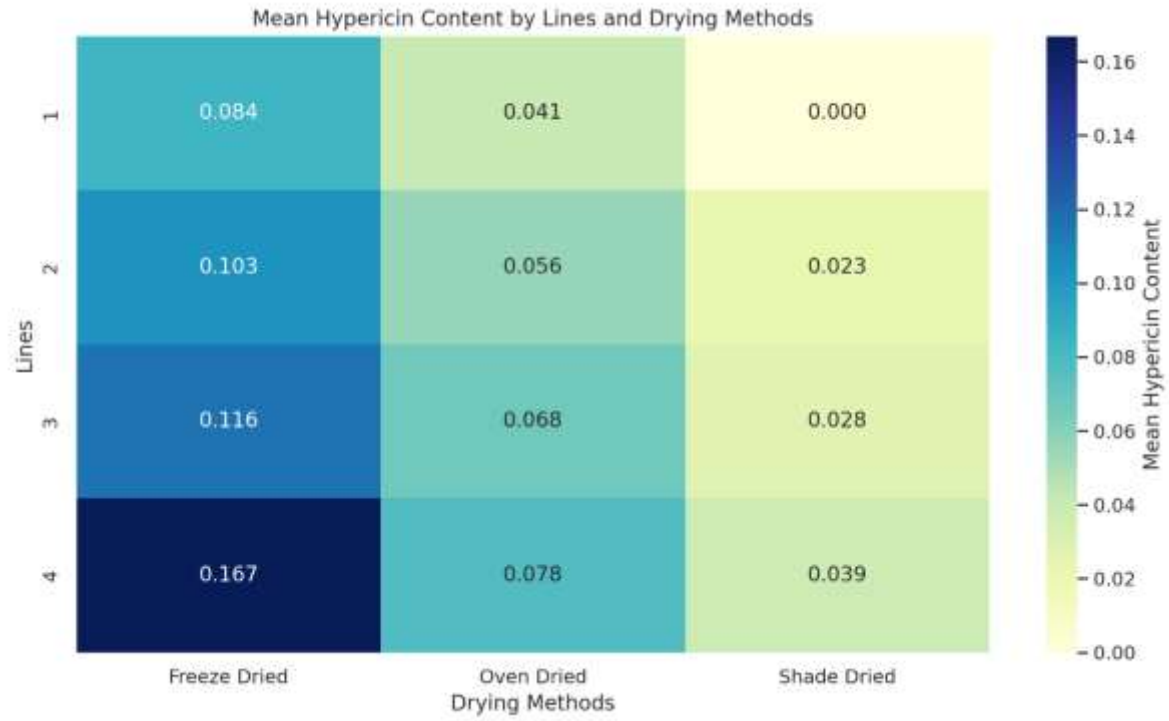


Figure 3: The heatmap of mean hypericin quantity by lines and drying methods

Discussion

Freeze-drying produced the lowest residual moisture levels and retained higher hypericin concentrations than oven- and shade-drying, confirming its effectiveness without repeating earlier statements.

This study confirms that drying methods exert a strong influence on the stability of hypericin in *Hypericum perforatum* adventitious roots. Freeze-drying produced the lowest residual moisture levels and retained higher hypericin concentrations than oven- and shade-drying. These findings align with earlier reports showing that lyophilization better preserves hypericin and other thermolabile compounds by minimizing oxidation and enzymatic degradation [2, 4] Similar results were obtained by Rainha et al. (2013), who observed significantly higher hypericin retention in freeze-dried aerial parts compared with air- and oven-dried samples[11].

Oven-drying, although less effective than freeze-drying, maintained moderate hypericin levels in our study. Comparable outcomes were reported by Blanco et al. (2000), who noted compound losses in oven-dried rosemary extracts when drying temperatures exceeded 40 °C [6]. In contrast, Gadzovska et al. (2013) found that certain phenolic compounds in *Hypericum* leaves were relatively stable during hot-air drying, suggesting that compound-specific stability may vary and that oven-drying can be optimized for selected metabolites [12]. Shade-drying led to substantial hypericin losses in the present study, which agrees with Zhang et al. (2022), who documented oxidative degradation of hypericin under prolonged exposure to light and ambient oxygen [13]. However, other studies have noted that shade-drying preserved certain phenolics in Myrtus communis more effectively than hot-air drying [14], indicating that shade drying may still be valuable for compounds less sensitive to oxidative stress.

From an industrial perspective, the choice of drying method must balance preservation efficiency with operational costs. Freeze-drying ensures excellent compound stability but is associated with high energy consumption and long processing times, limiting scalability. Industrial-scale freeze-dryers require significant investment, and energy costs are multiple

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times higher than hot-air systems. Oven-drying, although retaining less hypericin, offers faster throughput, lower costs, and simpler scalability for industrial applications.

Line 4 consistently exhibited higher hypericin levels across drying methods, whereas Line 2 showed the lowest. While this observation may suggest favorable physiological or biochemical traits in certain lines, this remains a hypothesis in the absence of supporting molecular or physiological data.

Taken together, our findings emphasize the dual importance of processing and genotype in optimizing hypericin yield. At a practical level, combining moderately efficient and cost-effective drying (e.g., optimized oven-drying) with the selection of high-yielding root lines could represent a balanced strategy for industry. For applications requiring maximum compound stability, freeze-drying remains the benchmark. Ultimately, integrating biotechnological selection with tailored drying techniques will advance the sustainable production of hypericin-rich raw materials for pharmaceutical use.

Conclusion

The findings of this study highlight two complementary strategies for improving hypericin yield: optimizing drying protocols and selecting high-performing root lines. From a practical perspective, freeze-drying offers excellent preservation but is limited by high energy costs and scalability challenges, whereas oven-drying provides a more feasible industrial option. Integrating biotechnological line selection with cost-effective drying technologies will advance the sustainable production of hypericin-rich raw materials.

The study is limited by the absence of molecular or physiological data to explain line-to-line differences and by the lack of a cost–benefit analysis of drying methods. Addressing these limitations will be essential to guide both fundamental research and industrial applications.

Acknowledgment

The authors are grateful to the Agricultural Biotechnology Research Institute of Iran (ABRII) for their support.

Compliance with ethical standards

The authors declare no conflict of interest.

Funding Statement

No financial support was provided for this study.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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