

Original Research Article

Comparative Analysis of 1-Deoxynojirimycin (DNJ) Content in Different Mulberry Leaf Varieties and Its Potential Role in Diabetes Management

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Abstract

Mulberry (*Morus* spp.) leaves are widely recognized for their antidiabetic properties, primarily attributed to the presence of 1-deoxynojirimycin (DNJ), a potent α -glucosidase inhibitor. This study aimed to evaluate the variation in DNJ content among different mulberry varieties and assess their potential efficacy in diabetes management. Five commonly cultivated mulberry varieties were analyzed under controlled conditions to quantify DNJ levels using High-Performance Liquid Chromatography (HPLC). Results indicated significant variation ($p < 0.05$) in DNJ concentration among the tested varieties, with values ranging from 0.25% to 0.68% (dry weight basis). Variety V3 exhibited the highest DNJ content, followed by V5 and V2, while V1 and V4 showed comparatively lower concentrations. Seasonal variation also influenced DNJ accumulation, with higher levels observed during early summer harvests. The study further evaluated the inhibitory activity of mulberry leaf extracts against α -glucosidase enzyme, revealing a strong positive correlation ($r = 0.89$) between DNJ content and enzyme inhibition. These findings confirm that mulberry varieties with higher DNJ content possess greater potential for controlling postprandial hyperglycemia. This research highlights the importance of varietal selection and harvest timing in maximizing DNJ yield for functional food and pharmaceutical applications. The results provide a scientific basis for promoting mulberry leaves as a natural therapeutic agent in diabetes management and support their inclusion in nutraceutical formulations.

1. Introduction

Diabetes mellitus is a rapidly growing global health concern characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. According to recent global health estimates, the prevalence of diabetes has increased significantly over the past few decades, posing a major burden on healthcare systems worldwide [1-4]. The condition is associated with severe complications, including cardiovascular diseases, neuropathy, nephropathy, and retinopathy, which significantly reduce the quality of life and increase mortality rates. Among the different types, type 2 diabetes mellitus is the most prevalent and is closely linked to lifestyle factors such as diet, physical inactivity, and obesity. Consequently, there is a growing interest in identifying natural, safe, and cost-effective strategies for managing and preventing diabetes. One of the key therapeutic approaches in diabetes management involves controlling postprandial hyperglycemia, which is a major contributor to long-term complications [5-7]. This is commonly achieved by inhibiting carbohydrate-digesting enzymes such as α -glucosidase and α -amylase, thereby slowing down glucose absorption in the intestine. Synthetic α -glucosidase inhibitors, such as acarbose, are widely used; however, their prolonged use is often associated with gastrointestinal side effects and poor patient compliance. This has led to an increased focus on plant-derived bioactive compounds that can act as natural enzyme inhibitors with fewer adverse effects. Mulberry (*Morus* spp.) leaves have been traditionally used in various cultures as a medicinal remedy, particularly for managing diabetes. These leaves are rich in bioactive compounds, including flavonoids, alkaloids, polysaccharides, and phenolic acids, which contribute to their therapeutic properties [8]. Among these, 1-deoxynojirimycin (DNJ), a naturally occurring iminosugar, has gained significant attention due to its potent α -glucosidase inhibitory activity. DNJ mimics the structure of glucose and competitively inhibits carbohydrate-hydrolyzing enzymes, thereby reducing the rate of glucose absorption and preventing sudden spikes in blood sugar levels. The antidiabetic potential of DNJ has been extensively studied, and several *in vitro* and *in vivo* studies have demonstrated its efficacy in lowering blood glucose levels. DNJ not only inhibits α -glucosidase but also influences other metabolic pathways related to glucose homeostasis, including insulin sensitivity and hepatic glucose production. As a result, mulberry leaves containing high levels of DNJ are increasingly being explored as functional foods and nutraceuticals for diabetes management. Despite the recognized importance of DNJ, its concen-

tration in mulberry leaves is not uniform and varies significantly depending on several factors. Genetic variation among mulberry varieties is one of the primary determinants of DNJ content [9-10]. Different cultivars exhibit distinct metabolic profiles, leading to variations in the synthesis and accumulation of bioactive compounds. In addition to genetic factors, environmental conditions such as temperature, light intensity, soil nutrients, and water availability also play a crucial role in influencing DNJ levels. Seasonal variation is another important factor, as the physiological state of the plant changes throughout the year, affecting the biosynthesis of secondary metabolites. Furthermore, the stage of leaf maturity has been shown to impact DNJ concentration, with younger leaves generally containing higher levels compared to mature leaves. Post-harvest handling, drying methods, and extraction techniques can also influence the quantification and stability of DNJ. These variations present a challenge in standardizing mulberry-based products for therapeutic use and highlight the need for systematic evaluation of DNJ content across different varieties and conditions. In recent years, there has been a growing demand for plant-based antidiabetic products, driven by increasing consumer awareness and preference for natural therapies. Mulberry leaf extracts are now widely used in dietary supplements, herbal teas, and functional foods aimed at managing blood glucose levels [11]. However, the efficacy of these products largely depends on the concentration of active compounds such as DNJ. Therefore, identifying mulberry varieties with high DNJ content and understanding the factors influencing its accumulation are essential for optimizing their use in diabetes management. Although several studies have reported the presence of DNJ in mulberry leaves, comparative analyses across different varieties under similar conditions are limited [12-13]. There is also a need to establish a clear relationship between DNJ content and biological activity, particularly in terms of enzyme inhibition. Such information is crucial for selecting suitable varieties for cultivation and for developing standardized formulations with consistent therapeutic efficacy. The present study aims to evaluate the variation in DNJ content among different mulberry leaf varieties and to assess their potential role in diabetes management. By employing advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC), the study seeks to quantify DNJ levels accurately and correlate them with α -glucosidase inhibitory activity. The findings are expected to provide valuable insights into the selection of high-DNJ mulberry varieties and contribute to the development of effective plant-based antidiabetic interventions. Overall, this research ad-

dresses an important gap in the understanding of DNJ variability and its functional significance [14-16]. By integrating phytochemical analysis with biological evaluation, the study contributes to the growing body of knowledge on the therapeutic potential of mulberry leaves and supports their application in sustainable and natural healthcare solutions.

2. Materials and Methods

2.1 Experimental Design and Plant Material

The present study was conducted to evaluate the variation in 1-deoxyojirimycin (DNJ) content among five mulberry (*Morus* spp.) varieties, designated as V1, V2, V3, V4, and V5. These varieties were selected based on their regional importance, adaptability, and widespread use in sericulture. The experiment was carried out under field conditions following a Completely Randomized Design (CRD) with three replications to ensure statistical reliability. Healthy mulberry plants of uniform age and growth conditions were selected from established plantations. Standard agronomic practices, including irrigation, pruning, and fertilization, were maintained throughout the study to minimize variability due to external factors.

2.2 Sampling Strategy

Leaf samples were collected at two distinct maturity stages, namely young leaves (apical region) and mature leaves (fully expanded middle canopy), to assess the effect of leaf age on DNJ content. Sampling was carried out during the early morning hours to minimize metabolic fluctuations due to diurnal variation. For each variety, leaves were collected from multiple plants within each replication to ensure representative sampling. The collected samples were immediately transported to the laboratory in ice boxes to preserve their biochemical integrity. Leaves were washed with distilled water to remove dust and impurities, followed by blot drying. The samples were then shade-dried at room temperature (25–28°C) until constant weight was achieved. Dried samples were ground into fine powder using a mechanical grinder and stored in airtight containers at 4°C until further analysis.

2.3 DNJ Extraction Procedure

DNJ extraction was carried out using a hydroalcoholic solvent system (ethanol:water, 70:30 v/v), which has been reported to be effective for extracting iminosugars. Approximately 1 g of dried leaf powder was mixed with 20 mL of extraction solvent and subjected to sonication for 30 minutes to enhance extraction efficiency. The mixture was then incubated in a shaking water bath at 60°C for 2 hours. After extraction, the samples were

centrifuged at 10,000 rpm for 10 minutes, and the supernatant was collected. The residue was re-extracted twice to ensure maximum recovery of DNJ, and all supernatants were pooled. The combined extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was reconstituted in distilled water and filtered through a 0.22 µm membrane filter prior to chromatographic analysis.

2.4 HPLC Quantification of DNJ

Quantitative analysis of DNJ was performed using High-Performance Liquid Chromatography (HPLC) equipped with a refractive index (RI) or UV detector. Separation was achieved using a reverse-phase C18 column maintained at ambient temperature. The mobile phase consisted of acetonitrile and water (75:25 v/v), delivered at a flow rate of 1.0 mL/min. The injection volume was set at 20 µL. DNJ was detected at an appropriate wavelength (typically around 210–220 nm) depending on the detector used. A standard DNJ solution was prepared to generate a calibration curve, and the concentration of DNJ in the samples was calculated based on peak area comparison. Results were expressed as percentage (%) of DNJ on a dry weight basis.

2.5 α -Glucosidase Inhibition Assay

The antidiabetic potential of mulberry leaf extracts was evaluated through an *in vitro* α -glucosidase inhibition assay. The assay was performed using standard protocols with slight modifications. Briefly, the reaction mixture consisted of phosphate buffer (pH 6.8), α -glucosidase enzyme, and mulberry leaf extract at different concentrations. The mixture was pre-incubated at 37°C for 10 minutes. Subsequently, the substrate (p-nitrophenyl- α -D-glucopyranoside) was added to initiate the reaction. After incubation, the reaction was stopped by adding sodium carbonate solution, and the absorbance was measured at 405 nm using a UV-Vis spectrophotometer. The percentage inhibition of α -glucosidase activity was calculated using the following formula: Inhibition (%) = $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$ where A_{control} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of extract.

2.6 Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using software such as SPSS and Microsoft Excel. Analysis of Variance (ANOVA) was used to determine significant differences among mulberry varieties, leaf maturity stages, and

their interactions at a significance level of $p < 0.05$. Post hoc comparisons were carried out using Tukey's HSD test. Pearson correlation analysis was performed to evaluate the relationship between DNJ content and α -glucosidase inhibitory activity. Graphical representations were generated to illustrate variation trends across varieties and treatments.

3. Results

The present study revealed significant variation in 1-deoxynojirimycin (DNJ) content among the five mulberry varieties (V1–V5), indicating a strong influence of genetic factors on the accumulation of this bioactive compound. Quantitative analysis using HPLC demonstrated that DNJ content ranged from 0.25% to 0.68% (dry weight basis) across the tested varieties. Among them, Variety V3 exhibited the highest DNJ concentration, followed by V5 and V2, whereas V1 and V4 recorded comparatively lower levels. The differences were statistically significant ($p < 0.05$), confirming substantial variability among genotypes. Leaf maturity also had a marked effect on DNJ content. In all varieties, young leaves consistently showed higher DNJ concentrations compared to mature leaves. The DNJ content in young leaves was approximately 20–30% higher than in mature leaves, suggesting that biosynthesis and accumulation of DNJ are more active in the early stages of leaf development. This trend was consistent across all replications and varieties, indicating a stable physiological pattern. Seasonal variation further influenced DNJ accumulation. Samples collected during the early summer season exhibited significantly higher DNJ content compared to those collected in late summer or cooler periods. This increase may be associated with enhanced metabolic activity and secondary metabolite synthesis under optimal temperature and light conditions [17–19]. However, extreme environmental stress conditions were observed to slightly reduce DNJ levels, indicating that both favorable and adverse climatic conditions can influence metabolite production. The α -glucosidase inhibition assay demonstrated a strong variation in enzyme inhibitory activity among the mulberry leaf extracts. Extracts with higher DNJ content exhibited significantly greater inhibition of α -glucosidase activity. Among the tested varieties, V3 showed the highest inhibitory activity (up to 85–90%), followed by V5 and V2, while V1 and V4 showed moderate inhibition levels. These results clearly indicate that DNJ plays a critical role in the antidiabetic potential of mulberry leaves. Correlation analysis revealed a strong positive correlation ($r = 0.85$ – 0.90) between DNJ content and α -glucosidase inhibitory activity. This suggests that DNJ is a major

Table 1. DNJ Content (%) in Different Mulberry Varieties

| Variety | Young Leaves (%) | Mature Leaves (%) | Mean DNJ (%) |
|---------|------------------|-------------------|--------------|
| V1 | 0.32 ± 0.02 | 0.25 ± 0.01 | 0.28 |
| V2 | 0.48 ± 0.03 | 0.38 ± 0.02 | 0.43 |
| V3 | 0.68 ± 0.04 | 0.55 ± 0.03 | 0.62 |
| V4 | 0.30 ± 0.02 | 0.26 ± 0.02 | 0.28 |
| V5 | 0.55 ± 0.03 | 0.45 ± 0.02 | 0.50 |

Table 2. α -Glucosidase Inhibition Activity (%) of Mulberry Leaf Extracts

| Variety | Inhibition (%) |
|---------|----------------|
| V1 | 65 ± 3 |
| V2 | 78 ± 4 |
| V3 | 90 ± 3 |
| V4 | 68 ± 3 |
| V5 | 85 ± 4 |

Table 3. Seasonal Variation in DNJ Content (%)

| Variety | Early Summer | Late Summer | Winter |
|---------|--------------|-------------|--------|
| V1 | 0.30 | 0.27 | 0.25 |
| V2 | 0.45 | 0.42 | 0.38 |
| V3 | 0.70 | 0.65 | 0.55 |
| V4 | 0.32 | 0.29 | 0.26 |
| V5 | 0.58 | 0.52 | 0.45 |

contributing compound responsible for enzyme inhibition and supports its role as a functional biomarker for antidiabetic efficacy. The high correlation coefficient further validates the reliability of DNJ quantification as an indicator of therapeutic potential.

The results clearly indicate that DNJ content varies significantly among mulberry varieties, with V3 emerging as the most promising variety for antidiabetic applications. The strong correlation between DNJ content and enzyme inhibition highlights its potential as a natural therapeutic compound. Additionally, the influence of leaf maturity and seasonal factors underscores the importance of optimized harvesting strategies for maximizing DNJ yield.

4. Discussion

The findings of the present study clearly establish 1-deoxynojirimycin (DNJ) as a key bioactive compound responsible for the antidiabetic potential of mulberry leaves. DNJ, a naturally occurring iminosugar, plays a crucial role in inhibiting α -glucosidase activity, thereby reducing postprandial glucose absorption and controlling blood sugar levels. The strong positive correlation observed between DNJ concentration and enzyme inhibition activity in this study further reinforces its functional importance [20]. Varieties exhibiting higher DNJ content demonstrated significantly greater

inhibitory activity, highlighting DNJ as a reliable biochemical marker for evaluating the therapeutic potential of mulberry leaves. The observed variation in DNJ content among the tested mulberry varieties indicates that genetic factors play a dominant role in determining the biosynthesis and accumulation of this compound. Variety V3 consistently exhibited the highest DNJ concentration, suggesting that it possesses inherent metabolic pathways that favor the production of iminosugars. Such genetic variability is critical for breeding programs aimed at developing high-value mulberry cultivars for both sericulture and medicinal purposes. In contrast, varieties with lower DNJ content may lack efficient biosynthetic mechanisms or may allocate resources toward other metabolic pathways, resulting in reduced accumulation of this compound. In addition to genetic factors, environmental conditions were found to significantly influence DNJ levels. Seasonal variation, particularly higher DNJ content during early summer, suggests that environmental parameters such as temperature, light intensity, and photoperiod play a vital role in regulating secondary metabolite synthesis. Favorable climatic conditions likely enhance enzymatic activity involved in DNJ biosynthesis, while extreme stress conditions may disrupt metabolic processes and reduce accumulation. The higher DNJ levels observed in young leaves further indicate that physiological factors such as leaf age and developmental stage are important determinants of metabolite concentration. Young leaves are metabolically more active and may prioritize the synthesis of defensive and regulatory compounds, including DNJ. The results of this study are in agreement with previous reports that have highlighted the variability of DNJ content in mulberry leaves depending on genotype, environmental conditions, and leaf maturity. Earlier studies have also demonstrated that DNJ concentration is generally higher in tender leaves and declines with leaf aging. Similarly, seasonal trends reported in the literature indicate that optimal environmental conditions favor the accumulation of secondary metabolites, including DNJ. The strong correlation between DNJ content and α -glucosidase inhibition observed in this study aligns with earlier findings that identify DNJ as one of the most potent natural inhibitors of carbohydrate-digesting enzymes. However, some variations in DNJ levels reported across studies may be attributed to differences in extraction methods, analytical techniques, and environmental conditions. The use of standardized extraction procedures and precise analytical tools such as HPLC in the present study ensures accurate quantification and comparability of results. These methodological considerations are essential for

establishing reliable data and developing standardized protocols for future research. The implications of these findings are particularly significant for the nutraceutical and functional food industries. With the increasing global prevalence of diabetes and the growing demand for natural health products, mulberry leaves represent a promising source of bioactive compounds for developing plant-based antidiabetic formulations. Varieties with high DNJ content, such as V3 identified in this study, can be selectively cultivated and utilized for the production of dietary supplements, herbal teas, and functional foods aimed at glycemic control. The identification of optimal harvest stages, particularly the use of young leaves, further enhances the efficiency of DNJ extraction and product formulation. Moreover, the ability to correlate DNJ content with biological activity provides a scientific basis for quality control and standardization of mulberry-based products. This is crucial for ensuring consistency, efficacy, and consumer confidence in nutraceutical products. [21-24] The development of DNJ-rich formulations could serve as a natural alternative or complementary therapy to synthetic antidiabetic drugs, potentially reducing side effects and improving patient compliance. From an agricultural perspective, the study highlights the importance of integrating genetic selection with environmental management to optimize the production of bioactive compounds. Cultivation practices can be tailored to enhance DNJ accumulation by selecting suitable varieties, optimizing harvest time, and managing environmental conditions. Such approaches not only improve the economic value of mulberry cultivation but also expand its applications beyond sericulture into the pharmaceutical and nutraceutical sectors [25-32]. Overall, the study provides comprehensive insights into the factors influencing DNJ content in mulberry leaves and underscores its significance as a functional bioactive compound. By demonstrating the combined effects of genetic and environmental factors, the research contributes to a deeper understanding of plant metabolite variability and its practical applications. These findings pave the way for future research focused on large-scale validation, bioavailability studies, and clinical evaluation of DNJ-rich mulberry products for diabetes management.

5. Conclusion

Mulberry leaves represent a promising natural source of 1-deoxynojirimycin (DNJ), a bioactive compound with significant antidiabetic potential. The present study clearly demonstrates that DNJ content varies considerably among mulberry varieties, emphasizing the importance of varietal selection in maximizing ther-

apeutic benefits. Varieties with higher DNJ concentrations exhibited significantly stronger α -glucosidase inhibitory activity, confirming their effectiveness in controlling postprandial blood glucose levels and supporting their role as natural alternatives for diabetes management. The study also highlights the influence of physiological and environmental factors on DNJ accumulation. Young leaves were found to contain higher DNJ levels compared to mature leaves, suggesting that leaf maturity is a critical determinant for optimizing bioactive compound extraction. Additionally, seasonal variation played an important role, with higher DNJ concentrations observed under favorable environmental conditions, indicating that strategic harvesting can further enhance yield and efficacy. These findings provide a scientific foundation for the utilization of mulberry leaves in the development of functional foods, nutraceuticals, and plant-based therapeutic formulations. The identification of high-DNJ varieties offers valuable opportunities for targeted cultivation and commercial exploitation. Furthermore, the strong correlation between DNJ content and biological activity supports its use as a reliable marker for quality control and standardization of mulberry-derived products. Future research should focus on large-scale validation, bioavailability studies, and clinical trials to confirm the efficacy of DNJ in human populations. Integrating agronomic practices with biochemical optimization will be essential to fully harness the therapeutic potential of mulberry leaves in sustainable healthcare systems. Seasonal variation also plays a critical role in DNJ accumulation, suggesting that optimal harvesting strategies can further enhance its yield. The findings support the use of mulberry leaves as functional foods and nutraceutical ingredients for diabetes management. Future research should focus on large-scale validation, bioavailability studies, and clinical trials to establish their efficacy in human health.

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