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Effect of mannitol, sucrose, and hydrolyzed casein on hypericin content, gland number, and red pigment synthesis in the *in vitro* culture of *Hypericum perforatum* cv. Helos

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Abstract

Objective: This study aimed to establish a protocol for enhancing the quantity of hypericin, glands, and red pigments in *Hypericum perforatum* cv. Helos.

Methods: The effects of hydrolyzed casein (0 and 500 mg/L), mannitol (0, 5, and 10 g/L), and sucrose (20 and 30 g/L) on leaf explants derived from the *in vitro* plantlets were evaluated. The factors were arranged in a factorial experiment using a completely randomized design with three replications and five samples per experimental unit.

Results: The concentrations of hydrolyzed casein, mannitol, and sucrose markedly influenced the proportions of callus formation and the shoots displaying red pigments. The highest proportion of red pigment-bearing calli was observed in the media containing either the control or 500 mg/L hydrolyzed casein with 20 g/L sucrose, without mannitol. Glands were observed on all shoots that underwent development. The maximum gland number and the highest percentage of shoots with glands were recorded for the explants cultured on media containing 30 g/L sucrose in combination with 5 or 10 g/L mannitol, and similarly for the medium with 20 g/L sucrose and 5 g/L mannitol. These results indicated that the MS composition with sucrose concentration and the addition of hydrolyzed casein significantly enhance hypericin production in the calli and shoots.

Conclusion: Overall, optimization of MS via adjustments to sucrose and the incorporation of hydrolyzed casein increased the production of hypericin, glands, and red pigments in *H. perforatum*.

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Introduction

Hypericum perforatum, which is commonly known as St. John's Wort, is a perennial flowering species recognized for its medicinal applications, particularly for its use as a herbal remedy for conditions such as depression and anxiety. The medicinal efficacy of *H. perforatum* is mainly attributed to its bioactive constituents, particularly hypericin ($C_{30}H_{16}O_8$) and pseudohypericin, which are categorized as anthraquinones (Kwiecień *et al.* 2015). These bioactive compounds are produced in specialized glandular structures found on the leaves and stems of the plant. The production of these secondary metabolites is impacted by various environmental and nutritional factors; thus, optimizing cultivation conditions is crucial for enhancing their performance (Khakpour *et al.*, 2015).

In vitro culture methods have emerged as valuable strategies for the propagation and boosting the production of secondary metabolites in medicinal plants (Rashidi *et al.* 2018; Singh and Singh 2021). These techniques provide researchers with precise management of growth conditions, enabling the manipulation of various factors, including nutrient composition, light intensity, temperature, and the application of plant growth regulators (Al-Khateeb *et al.* 2019; Mohammadrezakhani *et al.* 2022). The application of Murashige and Skoog (MS) medium, along with various supplements, can notably affect the growth and development of plant tissues (Dorani and Sadeghi 2024; Akyüz 2025). Among the various additives, carbohydrates play a critical role in plant metabolism and can directly influence the production of secondary metabolites. Mannitol and sucrose are two carbohydrates that have demonstrated potential in increasing the production of secondary metabolites in a variety of plant species (Amiri and Kazemitabar 2011; Ullah and Khan 2022).

Mannitol, recognized as a sugar alcohol, has gained prominence in plant tissue culture for its dual function as both an osmotic agent and a promoter of metabolite production (Linjikao *et al.* 2024). Mannitol helps to regulate osmotic pressure in plant cells and promotes callus formation, which is crucial for the effective propagation of plant species (El-Azraq *et al.* 2018). The callus tissue acts as an undifferentiated mass of cells, which can develop into diverse plant structures, such as roots and shoots (Ghassemi *et al.* 2015). In facilitating this process, mannitol not only assists in the early phases of tissue culture but also boosts the overall stress resistance of plant tissues. This stress tolerance is especially beneficial under *in vitro* conditions, where environmental factors such as nutrient availability and light intensity may vary considerably (Matter *et al.* 2017). Moreover, mannitol can influence the physiological responses in plant cells, promoting the increased production of secondary

metabolites, including phenolic compounds and flavonoids, which are essential for the plant's defense systems and can have beneficial effects on human health (Jan *et al.* 2020).

On the other hand, sucrose plays a crucial role as the primary carbon source in plant tissue cultures. It provides energy for metabolic activities, such as cellular respiration and biosynthetic pathways (Malik *et al.* 2017). The amount of sucrose in the growth medium can significantly impact critical physiological functions, including cell division, growth rate, and the production of secondary metabolites (Khaliluev *et al.* 2014). Higher concentrations of sucrose can lead to boosted osmotic potential in the culture medium, which can stimulate cell division and enhance overall growth (Gao *et al.* 2021). Moreover, sucrose has been shown to contribute to the accumulation of vital metabolites by offering the necessary precursors for biosynthetic pathways (Martinez *et al.* 2021). However, maintaining the right balance of sucrose concentration is essential; insufficient levels may hinder growth, while excessive levels can result in osmotic stress, negatively impacting the health and viability of the plant tissues.

Additionally, adding hydrolyzed casein to the culture medium introduces another level of complexity to the nutritional dynamics of plant tissue cultures. Hydrolyzed casein is a protein hydrolysate that provides a rich source of amino acids and peptides, which are vital for the growth and development of plants (Bělonožníková *et al.* 2023). These amino acids function as fundamental components for protein synthesis, participate in numerous metabolic pathways, and play crucial roles in signaling processes within the plant (Al-Asadi Ahmed *et al.* 2024). The addition of hydrolyzed casein has been found to promote shoot and root development by supplying growth factors that enhance cellular activities and promote differentiation (Grzegorczyk-Karolak *et al.* 2021). This role is particularly significant in tissue culture, where the accurate balance of nutrients can significantly affect the outcomes of plant regeneration (Sadat-Hosseini and Soleimani 2024).

Although these additives show enormous promise, there is currently limited research on their collective effects on *H. perforatum*. It is necessary to learn how mannitol, sucrose, and hydrolyzed casein influence the number of glands and red pigment formation to develop the most favorable cultivation conditions. This study aimed to investigate the influence of different concentrations of mannitol, sucrose, and hydrolyzed casein on the production of red pigment, the number of glands, and hypericin content in the *in vitro* cultures of *H. perforatum*. By defining such interactions, we aimed to further boost the optimization of cultivation techniques with the ultimate goal of raising the production of valuable secondary metabolites for pharmaceutical applications. In addition, the morphological variations noted during the growth of leaf explants in response to different levels of these additives will offer more detailed knowledge regarding the developmental biology of *H.*

perforatum. The implications of this research are expected to have significant effects on both experimental botany and the pharmaceutical industry, providing a pathway for the sustainable production of high-quality *H. perforatum* extracts. The successful development of a reliable protocol for boosting gland development and pigment synthesis will not only facilitate the cultivation of this important medicinal plant but also advance our knowledge of the underlying biochemical mechanisms involved in the production of secondary metabolites.

Materials and Methods

This study was conducted as a factorial experiment in a completely randomized design with three replications and five samples per experimental unit. The factors to be evaluated consisted of hydrolyzed casein (0 and 500 mg/L), mannitol (0, 5, and 10 g/L), and sucrose (20 and 30 g/L). The *H. perforatum* seeds were sterilized to provide a clean environment for germination and further experimentation. This procedure consisted of submerging the seeds in a 75% ethanol solution for 30 seconds to eliminate surface contaminants, followed by a 10-minute immersion in a 5% (v/v) sodium hypochlorite solution, which further ensured sterilization. After this, the seeds were washed three times with sterile distilled water to eliminate any remaining chemicals that could inhibit germination. After sterilization, the sterile seeds were germinated on a hormone-free MS solidified medium. Once germination occurred, the elongated shoots were chosen as the experimental material for subsequent examination. The leaf explants were sectioned into four to five mm pieces from the posterior surface of the leaves and then cultured on the modified MS medium supplemented with 0.5 mg/L benzylaminopurine (BAP), a cytokinin that promotes shoot proliferation. The cultures were incubated in the dark at a constant temperature of 25 °C to create favorable conditions for growth. To preserve the cultures, subculturing was carried out every 30 days to replenish nutrients and promote further development. Two months after planting, all samples in each experimental plot were evaluated. Throughout the experiment, specific characteristics were carefully recorded, including the percentage of callus production, the proportion of red-pigmented shoots, the number of glandular structures developed, and the percentage of gland development in the shoots.

Extraction of and quantification of hypericin

Freshly harvested shoots and calli were thoroughly rinsed with distilled water and immediately stored in a deep freeze at -80 ºC. The frozen samples were then subjected to lyophilization using a freeze dryer. Once dried, the plant materials were ground to obtain a uniform powder. The powdered samples were extracted using chloroform, and the resulting solution was filtered and subsequently evaporated

to dryness. The remaining residue was then re-extracted with methanol, and the filtrate was allowed to evaporate in a water bath. Following this, chloroform was added to the residue, and the mixture was shaken. The supernatant was discarded, leaving behind a solid phase that contained hypericin and its derivatives, which was then dissolved in methanol and filtered. The hypericin content in the methanol extracts was quantified using a spectrophotometer (Biowave, 2100S, UK). The methanol extracts were prepared from plant materials and filtered to remove any suspended particles. A standard curve was established using various concentrations of hypericin to determine its concentration accurately. The samples and standards were measured at a wavelength of 592 nm, and the absorbance was recorded using the spectrophotometer. The concentration of hypericin in the extract samples was then calculated based on the standard curve, providing a precise measure of the hypericin content and ensuring reliable results (Koperdáková *et al.* 2007).

Statistical analysis

After the analysis of variance, Duncan's multiple range test at a 5% probability level was employed to compare the means. The data were analyzed using SPSS software version 16.

Results

In all culture media that included hydrolyzed casein, sucrose, and mannitol, induction of callus was observed to begin between the 7^{th} and 8^{th} days following the start of the culture. By 14 to 20 days, noticeable growth of the calli was evident, with diameters measuring between 10 and 20 mm (Figure 1). Across all treatment groups, the callus displayed a soft consistency and exhibited a range of colors, displaying shades of green and yellow, often adorned with red or brown speckles (Figure 2).

Adventitious shoot induction was noted in all treatments for 20 to 30 days following the initiation of culture. After the induction and development of shoots, callus growth stopped, and lateral shoots were produced after one month on the induced shoots. The outcome was the formation of clusters or masses of shoots on the calli. Shoot regeneration was completed successfully in all treatments. The shoots were green and sometimes had red spots (Figure 3). In a limited number of explants, direct shoot induction was observed.

Glands were present on all shoots, with diameters ranging from 0.1 to 0.2 mm (Figure 4). The average number of glands on each shoot ranged from 7 to 10. The impacts of various concentrations of hydrolyzed casein, sucrose, and their interaction were found to be insignificant on calli containing red pigments. However, calli containing red pigments were significantly affected by different concentrations of mannitol, mannitol × sucrose, and mannitol × sucrose × hydrolyzed casein

Figure 1. Callus diameter: A callus with a diameter of 5 mm (left) and a callus with a diameter of 20 mm (right)

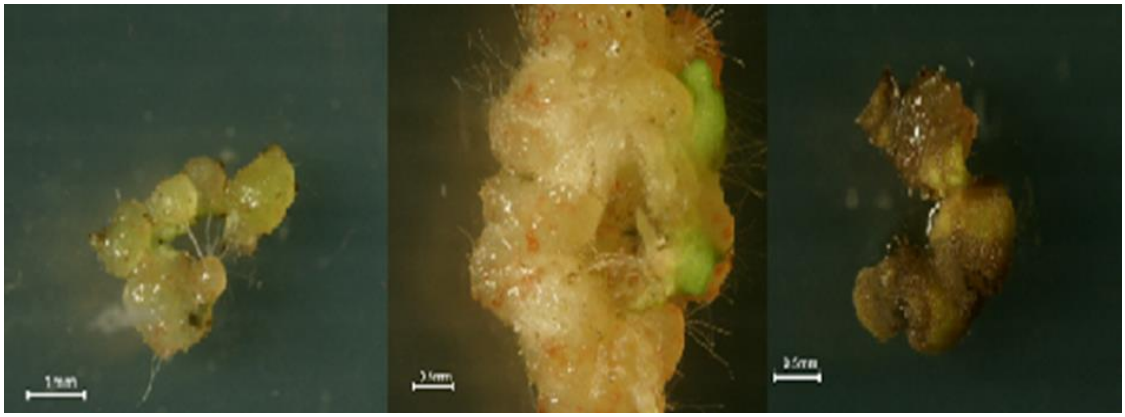

Figure 2. Callus color: Green callus (left), yellow callus with red dots (center), and brown callus (right)

Figure 3. Shoot color: Shoots exhibiting a yellow color with red spots (left) and green shoots (right)

Figure 4. Direct regeneration of shoots from plant tissues. The images show the stages of shoot formation, highlighting the successful development of new shoots from explants under optimized growth conditions.

interactions ($p < 0.01$). The highest percentage of calli containing red pigments was found in the culture medium supplemented with control or 500 mg/L hydrolyzed casein and 20 g/L sucrose, without the addition of mannitol. Thus, the highest percentage of calli containing red pigments was noted in the media supplemented with control or 500 mg/L hydrolyzed casein. Therefore, the addition of hydrolyzed casein was not required to enhance the production of calli containing red pigments. Conversely, the number of calli containing red pigments decreased as the amounts of mannitol increased, with the lowest percentage observed in the medium containing 10 g/L mannitol. Calli exhibiting red pigments were not produced in the medium containing 20 g/L sucrose and 10 g/L mannitol without the hydrolyzed casein. The reason was probably attributed to a reduction in the energy source and an increase in osmotic pressure. The media lacking hydrolyzed casein and mannitol, supplemented with either 20 or 30 g/L of sucrose, met the relative cell requirements for the production of secondary metabolites. Notably, the calli that developed red pigments in the sucrose-containing media were significantly more abundant than those in the media that included mannitol. In the medium with 500 mg/L hydrolyzed casein, the highest percentage of calli exhibiting red pigments was found in the medium containing 20 g/L sucrose, without any mannitol. The results of this study indicated that the inclusion of osmotic materials, like mannitol, resulted in a reduction in calli containing red pigments (Figure 5).

The occurrence of red pigments in shoots was significantly influenced by the different concentrations of mannitol, sucrose, hydrolyzed casein, and their interactions ($p < 0.01$). The highest percentage of shoots with red pigments was found in the culture medium enriched with 30 g/L sucrose, excluding hydrolyzed casein and mannitol, as well as in a medium containing 500 mg/L hydrolyzed casein. The highest percentage of shoots containing red pigments was recorded in the presence of 20 g/L sucrose, without mannitol. There were significant differences in the levels of red pigments in shoots across the different concentrations of mannitol. The highest percentage of shoots containing red pigments was recorded in the culture medium supplemented with 30 g/L sucrose, excluding hydrolyzed casein. However, the absence of hydrolyzed casein resulted in a decrease in the percentage of shoots containing red pigments at high mannitol concentrations, ultimately reaching zero at 10 g/L mannitol. In the media lacking hydrolyzed casein, the percentage of shoots containing red pigments was zero in the media containing 20 g/L sucrose in all concentrations of mannitol (Figure 6).

In a medium with 500 mg/L of hydrolyzed casein, the highest proportion of shoots exhibiting red pigments was found in the concentrations of sucrose at 20 and 30 g/L, in the absence of mannitol. Nonetheless, there were significant differences in the amount of red pigment observed in shoots

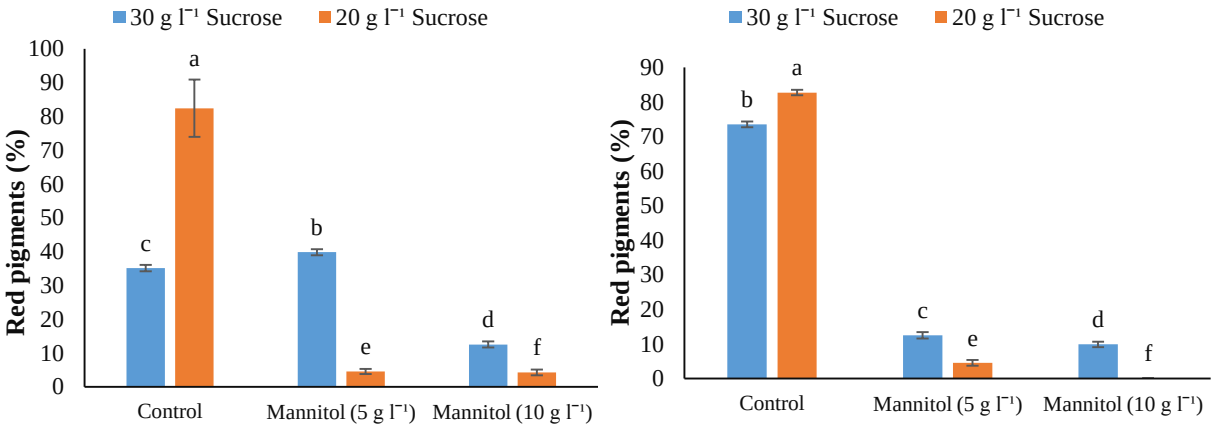

Figure 5. The percentage of calli of the St. John's Wort exhibiting red pigments in various concentrations of sucrose and mannitol as well as in the presence of hydrolyzed casein [(The medium without hydrolyzed casein (right) and the medium containing 500 mg/L hydrolyzed casein (left)]; The data are represented as mean ± standard error; Means with different letters are significantly different at $p \leq 0.05$.

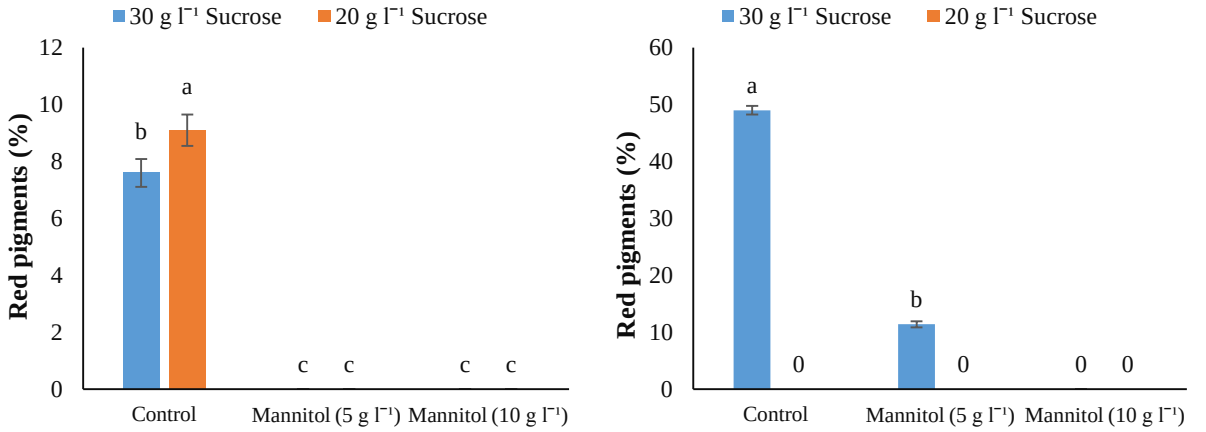

Figure 6. Percentage of shoots of the St. John's Wort plant exhibiting red pigments in various concentrations of sucrose and mannitol, as well as in the presence of hydrolyzed casein [(The medium without hydrolyzed casein (right) and the medium containing 500 mg/L hydrolyzed casein (left)]. The data are represented as mean ± standard error. Means with different letters are significantly different at $p \leq 0.05$.

between the two sucrose concentrations. The highest percentage of shoots exhibiting red pigments was recorded at a sucrose concentration of 20 g/L. This suggests that the inclusion of hydrolyzed casein as an amino acid source, particularly at lower sucrose levels, enhances the percentage of shoots that contain red pigments. However, the percentage of shoots containing red pigments reached zero with increasing mannitol in both concentrations of sucrose.

The effects of various concentrations of hydrolyzed casein and their interaction were found to be insignificant for the number of glands and the percentage of shoots containing glands. While hydrolyzed casein is known to be an effective organic complex for *in vitro* morphogenesis, its use in this research did not yield significant results regarding the number of glands or the percentage of shoots containing glands. As there is a lack of reports regarding the application of hydrolyzed casein

in the medium for gland quantity and the percentage of shoots with glands in *H. perforatum*, this study indicates that using 500 mg/L of this material does not effectively increase gland production. The number of glands and the percentage of shoots containing glands were significantly influenced by the concentrations of mannitol and sucrose, as well as their interaction ($p < 0.01$). The percentage of shoots that contained glands was significantly elevated in the culture medium with 30 g/L sucrose than in the medium with 20 g/L sucrose (Figure 7). The inclusion of mannitol in the media containing 30 g/L sucrose influenced the number of glands. Although insignificant differences were noted between 5 and 10 g/L mannitol for gland number, in the medium with 20 g/L sucrose, the highest gland number was recorded in 5 g/L mannitol (Figure 8).

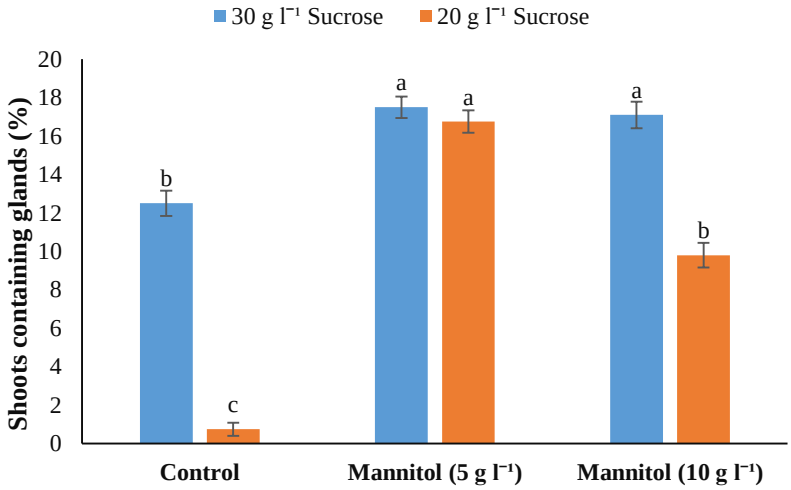

Figure 7. Number of shoots of the St. John's Wort plant containing glands in different concentrations of sucrose and mannitol. The data are represented as mean ± standard error. Means with different letters are significantly different at $p \leq 0.05$.

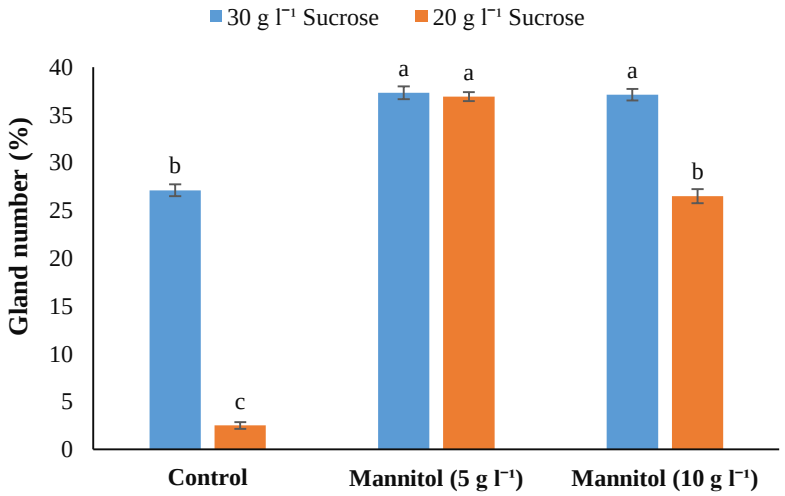

Figure 8. Number of glands of the St. John's Wort plant in different concentrations of sucrose and mannitol. The data are represented as mean ± standard error. Means with different letters are significantly different at $p \leq 0.05$.

The number of shoots containing glands after 3 months was significantly influenced by the concentrations of mannitol ($p < 0.01$). The highest number of shoots with glands was recorded in the medium with 5 and 10 g/L mannitol, suggesting that mannitol boosted osmotic pressure, which in turn promoted the formation of dark glands on the shoot surfaces (Figures 9 and 10).

The production of hypericin in calli and the produced shoots was not significantly affected by different concentrations of mannitol, sucrose, and hydrolyzed casein. However, the interaction between different concentrations of sucrose and hydrolyzed casein had a significant effect on the hypericin content in both calli and shoots ($p < 0.05$). The increase of hydrolyzed casein to 500 g/L in the presence of 20 g/L of sucrose resulted in the highest accumulation of hypericin in calli, which was significantly greater than that observed under other tested conditions. Additionally, in the plant shoots, the highest production of hypericin was observed under culture conditions with 30 g/L of sucrose and 500 g/L of hydrolyzed casein, which was statistically significantly higher compared to control conditions and other treatments (Table 1).

Figure 9. St. John's Wort shoots, which had red pigments after three months.

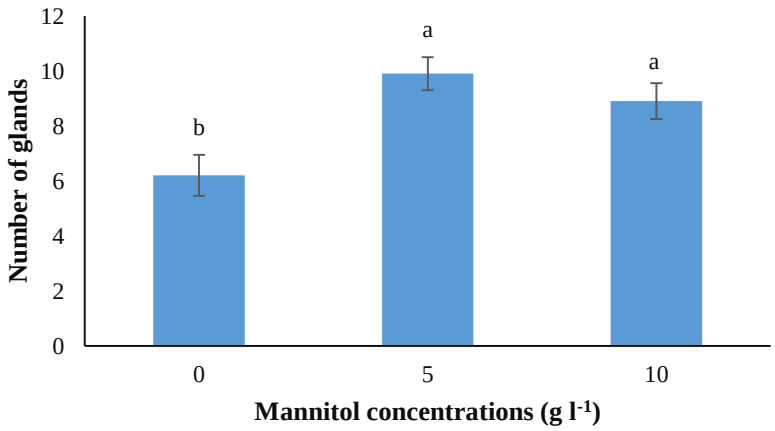

Figure 10. Number of glands of the St. John's Wort plant in different concentrations of mannitol. The data are represented as mean ± standard error. Means with different letters are significantly different at $p \leq 0.05$.

Table 1. Hypericin content in the calli and shoots grown on the MS medium containing different concentrations of sucrose and hydrolyzed casein in the St. John's Wort plant.

Sucrose concentration (g/L)	Hydrolyzed casein concentration (g/L)			
	0 (Control)	500	0 (Control)	500
	Hypericin content in the calli (%)		Hypericin content in the shoots (%)	
20	75[b]	80[a]	75[c]	90[b]
30	60[c]	50[d]	70[d]	110[a]

Means with different letters in each trait are significantly different at $p \leq 0.05$, using the Duncan's multiple range test.

Discussion

The findings of this research indicate the impact of different nutrient combinations on the induction of callus, the development of shoots, the production of glands, and the synthesis of red pigments in *H. perforatum*. This research contributes to a more thorough understanding of the physiological and biochemical mechanisms associated with plant tissue culture. It provides guidance on refining conditions to enhance the production of secondary metabolites, which are of significant importance in pharmaceutical applications (Singh and Singh 2021). Callus tissues, as undifferentiated and non-specialized structures, play a crucial function in the processes of regeneration and the biosynthesis of secondary metabolites (Ghassemi *et al.* 2015). In the course of this study, callus induction was recorded between the 7th and 8th days, reflecting the activation of cell division and differentiation processes that are impacted by the composition of the nutrient medium. The results revealed that in every culture condition containing hydrolyzed casein, sucrose, and mannitol, the callus was visibly growing, with diameters measuring between 10 and 20 mm from 14 to 20 days after culturing. The variation in color and the soft texture of the calli indicate significant metabolic activity and the potential synthesis of secondary metabolites, including anthocyanins and carotenoids, which are known for their antioxidant properties and potential health advantages (Kwiecień *et al.* 2015).

The detection of red and brown pigments in the callus may be attributed to the production of secondary metabolites, which are affected by the culture conditions and different concentrations of sucrose. The findings showed that calli containing red pigments were impacted by the varying concentrations of mannitol and their interactions with sucrose. Specifically, the highest percentage of calli exhibiting red pigments was found in the culture medium enriched with either zerio or 500 mg/L hydrolyzed casein and 20 g/L sucrose, without the addition of mannitol. This indicates that sucrose, as the main carbon source, plays a vital role in metabolite biosynthesis, providing the necessary energy and substrates for the biochemical pathways involved in pigment production (Martinez *et al.* 2021).

The rise in mannitol concentration, functioning as an osmotic agent, may lead to boosted osmotic pressure, which in turn restricts water and nutrient uptake, ultimately inhibiting the production of red pigments. The results revealed that as the concentration of mannitol increased, the number of calli exhibiting red pigments diminished, finally reaching zero in the medium containing 10 g/L mannitol. This influence is especially pronounced at high concentrations of mannitol, highlighting the negative influence of osmotic pressure on metabolic activities and the synthesis of secondary metabolites, likely due to stress-induced responses that divert resources away from pigment biosynthesis (Linjikao *et al.* 2024).

The successful induction of shoots within 20 to 30 days of culturing is a significant achievement in the regeneration protocols for *H. perforatum*. The results indicated that green shoots with red spots were observed in every treatment group, indicating differences in secondary metabolite accumulation in various conditions (El-Azraq *et al.* 2018). The highest percentage of shoots displaying red pigments was observed in the culture medium containing 30 g/L sucrose, and without mannitol. This observation highlights the importance of high sucrose levels as a carbon source in facilitating the production of secondary metabolites, aligning with established research that underscores the role of carbon sources in boosting metabolic pathways (Gao *et al.* 2021). Significantly, the absence of hydrolyzed casein combined with high mannitol concentrations resulted in a significant reduction in the production of red pigments in shoots. The results indicated that the percentage of shoots with red pigments significantly decreased in the medium with 30 g/L sucrose when hydrolyzed casein was absent, ultimately reaching zero at high levels of mannitol (Khakpour *et al.* 2015).

This finding highlights the importance of hydrolyzed casein as a source of amino acids that can boost the production of secondary metabolites, especially in conditions of low sucrose, where amino acids serve as precursors for the biosynthesis of different metabolites (Bělonožníková *et al.* 2023). The production of glands was also notably influenced by various concentrations of sucrose and mannitol. The findings indicated that the number of glands and the percentage of shoots with glands were elevated in the culture medium with 30 g/L sucrose compared to 20 g/L sucrose. This suggests that elevated sucrose levels may enhance gland formation, which serves as a storage structure for secondary metabolites (Rajesh *et al.* 2014). The formation of glands is critical for the accumulation of bioactive compounds, and their increased development in response to nutrient composition may enhance the overall yield of valuable metabolites. This synergistic interaction may lead to enhanced production of secondary metabolites, as osmotic stress is known to activate various signaling pathways that promote the synthesis of protective and beneficial substances (Ullah and Khan 2022).

Based on the results obtained from the present study, it can be concluded that variations in the

composition of the MS culture medium, particularly the adjustment of sucrose concentration and the addition of hydrolyzed casein, have significant and determining effects on the production of hypericin in callus tissues and plant shoots (Table 1). The increase in sucrose concentration, serving as the primary source of metabolizable carbohydrates, enhances the supply of energy and carbon required for cellular metabolic processes (Khakpour *et al.* 2015; Grzegorczyk-Karolak *et al.* 2021), thereby stimulating metabolic activities associated with the biosynthesis of secondary compounds, including hypericin. Furthermore, hydrolyzed casein acts as a rich source of nitrogen, amino acids, and growth factors, which can positively influence the metabolic pathways involved in hypericin synthesis. This component can enhance the physiological status of the cells and activate effective metabolic pathways, leading to an increased rate of production of hypericin (Khakpour *et al.* 2015; Bělonožníková *et al.* 2023). Statistical analyses indicate significant differences in the levels of hypericin produced across various treatments, underscoring the high sensitivity of the biosynthetic pathways of this compound to changes in environmental conditions. These findings emphasize the importance of precise regulation of the culture medium composition as an effective strategy for optimizing the productivity of plant tissue cultures and the industrial production of valuable pharmaceutical metabolites. Moreover, the significant impact of hydrolyzed casein on increasing hypericin production may be attributed to the regulation of gene expression related to biosynthetic pathways and the enhancement of the protein composition of the culture medium. This aspect particularly necessitates further molecular and genetic research to elucidate the precise mechanisms by which hydrolyzed casein influences hypericin biosynthesis. Future investigations in this area could contribute to optimizing culture conditions and ultimately lead to sustainable production and increased yield of valuable pharmaceutical metabolites (Khakpour *et al.* 2015; Coste *et al.* 2019).

Conclusion

This study demonstrated the significant effects of varying levels of mannitol, sucrose, and hydrolyzed casein on the synthesis of red pigments and glandular structures in the *in-vitro* cultures of *H. perforatum*. The results highlighted the importance of optimizing nutrient formulations to enhance the production of valuable secondary metabolites, particularly hypericin and pseudohypericin, known for their therapeutic properties, including antidepressant and antiviral activities. The optimal conditions identified were 500 mg/L hydrolyzed casein combined with 20 g/L sucrose for calli, and 500 mg/L hydrolyzed casein with 30 g/L sucrose for shoots. These findings underscore the potential for improving hypericin biosynthesis in commercial applications, contributing to the development of effective therapeutic agents derived from *H. perforatum*.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics declaration

Not applicable.

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