Scientific Papers. Series B, Horticulture. Vol. LXX, No. 1, 2026
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

IN VITRO CULTURE, ACCLIMATIZATION AND BIOCHEMICAL TRAITS OF *HYPERICUM PERFORATUM* L.

Doina CLAPA[1], Monica HÂRȚA[1], Ana Maria RADOMIR[2], Adrian PETICILĂ[3], Dorin Ioan SUMEDREA[4]

[1]Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Calea Mănăştur, 400372, Cluj-Napoca, Romania
[2]National Research and Development Institute for Biotechnology in Horticulture Ștefănești-Argeș, 37 Bucharest-Pitești Road, 117715, Ștefănești, Argeş County, Romania
[3]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[4]Academy of Agricultural and Forestry Sciences, 61 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: monica.harta@usamvcluj.ro

Abstract

The present study aims to develop an efficient in vitro culture and acclimatization protocol for Hypericum perforatum and to provide a preliminary biochemical assessment of micropropagated plants compared to acclimatized plants. In vitro cultures were initiated from nodal explants collected from greenhouse-grown plants and established on Murashige and Skoog (MS) medium. Shoot multiplication was evaluated on Driver and Kuniyuki Walnut (DKW) medium supplemented with different cytokinins, while rooting was assessed on DKW medium with and without indole-3-butyric acid. Ex vitro acclimatization was performed in perlite under controlled humidity conditions, followed by transfer to a peat substrate under greenhouse conditions. The highest shoot multiplication rate was obtained on medium supplemented with benzyladenine, while the best rooting response was recorded on auxin-free medium, which resulted in high explant viability. Acclimatization rates were high for both rooted and non-rooted shoots, with superior growth performance observed in plantlets rooted in vitro. The biochemical analysis revealed comparable levels of phenolic compounds and flavonoids in both plant categories, suggesting that in vitro-derived biomass may represent a potential source of phenolic compounds.

Key words*: micropropagation, 6-benzyladenine, total phenolic content (TPC), total flavonoid content (TFC).*

INTRODUCTION

Hypericum perforatum L. (St. John's wort) is a medicinal plant of significant pharmacological interest due to its rich content of bioactive secondary metabolites, especially phenolic compounds and flavonoids, which contribute to antioxidant, anti-inflammatory, and therapeutic effects (Al-haliem et al., 2025; Al Nooh et al., 2025; Amooaghaie & Rajaie, 2025; Clapa et al., 2025; Kilibarda et al., 2025; Mišina et al., 2025; Rusanova et al., 2025). The continued scientific interest in *H. perforatum* and related Hypericum species is reflected in recent research on in vitro propagation and phytochemical analysis, highlighting the need for robust protocols to produce uniform plant material with consistent biochemical profiles (Kwiecień et al., 2023; Ravindran et al., 2023; Shasmita et al., 2023; Tzatzani et al., 2023; Ahmed et al., 2025).

Conventional propagation and wild harvesting may lead to variable phytochemical profiles in *H. perforatum*, depending on environmental factors, genotype, and developmental stage (Barnes et al., 2001; Southwell & Bourke, 2001; Zobayed et al., 2024; Kilibarda et al., 2025).

Additionally, intensive harvesting from natural habitats can raise sustainability concerns (Chen et al., 2016). These limitations emphasize the need for alternative propagation strategies that ensure both uniformity in plant material and stability in phytochemical composition (Selvakesavan & Franklin, 2021).

In vitro culture techniques are widely adopted for rapid micropropagation of medicinal plants, offering advantages such as year-round production, genetic uniformity, and pathogen-free material (Hasnain et al., 2022; Anuruddi et al., 2023; Rahimi et al., 2023; Thiem et al., 2023; Samridha & Chandra, 2024). Recent studies

have demonstrated efficient shoot initiation and multiplication in several Hypericum species, including *H. perforatum*, with optimized responses to plant growth regulators and improved acclimatization strategies under controlled conditions (Khakpour et al., 2015; Sood et al., 2015; Abdollahpoor et al., 2017; Fascella et al., 2017; Syahid & Wahyuni, 2019; Ravindran et al., 2023).

In addition to morphological development, in vitro conditions and acclimatization may influence secondary metabolism (Espinosa-Leal et al., 2018; Grzelak et al., 2024). Phenolic compounds and flavonoids are important for plant defense and stress adaptation and are often used as indicators of biochemical stability in medicinal plants (Reshi et al., 2023). Assessing total phenolic and flavonoid contents in both in vitro cultured and acclimatized plants provides insight into the impact of micropropagation on phytochemical traits and biomass quality (Moraes et al., 2012; Kikowska et al., 2020; Custódio et al., 2022).

Therefore, the present study aims to develop an efficient in vitro culture and acclimatization protocol for *H. perforatum* and to perform a preliminary assessment of total phenolic and total flavonoid contents in *in vitro* cultured and acclimatized plants.

MATERIALS AND METHODS

In vitro culture conditions

All *in vitro* culture stages were performed under the same controlled environmental conditions. Culture initiation was carried out in test tubes containing 5 mL of culture medium, while shoot multiplication and rooting stages were conducted in 720 mL culture jars containing 100 mL of medium. All culture media were supplemented with 30 g/L sucrose and solidified with 5 g/L plant agar. The pH of the media was adjusted to 5.8 prior to autoclaving at 120°C for 20 min. Cultures were maintained at 23 ± 1°C under a 16-h light/8-h dark photoperiod, with a light intensity of 32.4 µmol m^{-2} s^{-1}. Unless otherwise stated, all chemicals and culture media components were purchased from Duchefa Biochemie B.V. (Haarlem, The Netherlands).

Initiation of *in vitro* cultures

In vitro cultures of *H. perforatum* were initiated using nodal explants excised from juvenile shoots collected from greenhouse-grown plants. The explants were surface-sterilized using a 20% (v/v) commercial bleach solution (5% active chlorine) for 20 minutes and rinsed three times with sterile distilled water under aseptic conditions. Single-node explants were inoculated individually onto Murashige and Skoog (MS) (Murashige & Skoog, 1962) basal medium without plant growth regulators (PGRs). The cultures were maintained under the environmental conditions described above. After four weeks of culture, the percentage of culture initiation was recorded based on the number of viable, regenerated explants.

In vitro shoot multiplication

For the shoot multiplication stage, Driver and Kuniyuki Walnut (DKW) (Driver and Kuniyuki, 1984) basal medium was supplemented with different plant growth regulators (PGRs): 2 mg/L kinetin (Kin), 2 mg/L meta-topolin (mT), 1 mg/L zeatin (Z), 5 mg/L 2-isopentenyladenine (2iP), 0.1 mg/L forchlorfenuron (CPPU), and 0.1 mg/L benzyladenine (BA). Five nodal explants were inoculated per culture vessel. After 30 days of culture, the multiplication rate (number of shoots per initial explant) and shoot length were recorded.

In vitro rooting and *ex vitro* acclimatization

For the *in vitro* rooting stage, explants with apical buds of *H. perforatum* were cultured on DKW basal medium supplemented with indole-3-butyric acid (IBA) at concentrations of 0.5, 1.0, and 1.5 mg/L, as well as on an auxin-free control medium. Fifteen explants were inoculated per culture vessel. After 30 days of culture, rooting percentage, number of roots per explant, root length, and shoot viability were recorded.

Ex vitro acclimatization was performed using both *in vitro*-rooted plantlets and non-rooted shoots. The plant material was transferred to trays containing moistened perlite and maintained under high humidity conditions, which were gradually reduced over two weeks. During acclimatization, plantlets were regularly misted to prevent dehydration. After six weeks, acclimatized plants were transferred to peat substrate in alveolar trays and maintained under greenhouse conditions. Survival rates were assessed after 30 days.

Biochemical analysis (TPC and TFC)

Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophoto-

metrically in *in vitro* cultured and acclimatized plants of *H. perforatum*. Fresh plant material (0.5 g) was extracted with acidified methanol (1% HCl) and the obtained extracts were used for biochemical analyses.

TPC was determined using the Folin-Ciocalteu method (Singleton et al., 1999; Puia et al., 2025) with gallic acid as the standard, and the results were expressed as µg gallic acid equivalents per g fresh weight (µg GA/g FW). TFC was determined using the aluminium chloride colorimetric method, with quercetin as the standard, and the results were expressed as µg quercetin equivalents per g fresh weight (µg Q/g FW) (Zhishen et al., 1999; Molnar et al., 2024). The concentration of TPC and TFC was calculated using standard calibration equations derived from gallic acid and quercetin, respectively. For TPC, the calibration equation was $y = 0.0011x + 0.1319$, with $R^2 = 0.9981$. For TFC, the calibration equation was $y = 0.001x + 0.0313$, with $R^2 = 0.9952$, where y represents the absorbance and x represents the concentration of standard equivalents used for calibration.

Absorbance was measured using a UV-Vis spectrophotometer.

Statistical analysis

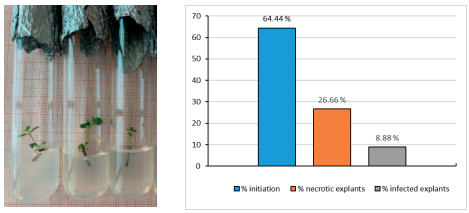
Experiments were arranged in three replicates, and data were expressed as mean ± standard error. Statistical analysis was performed using OriginPro software.

RESULTS AND DISCUSSIONS

In vitro culture initiation

H. perforatum responded satisfactorily to the applied *in vitro* initiation protocol. After four weeks of culture, an initiation rate of64.44% was recorded, while 8.89% of explants showed microbial contamination (Figure 1).

A relatively high percentage of non-developing but non-contaminated explants (26.67%) was also observed. Similar responses have been reported in *H. perforatum*, where explant sensitivity to surface sterilization and oxidative stress may negatively affect culture establishment (Tzatzani et al., 2023). Nevertheless, the obtained initiation rate enabled the successful establishment of viable *in vitro* cultures for subsequent multiplication stages.

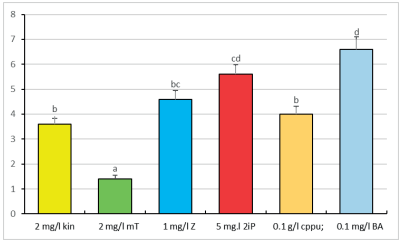


(a) (b)

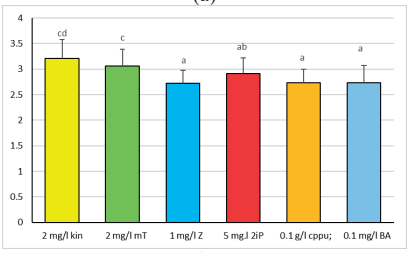
Figure 1. Initiation of in vitro culture of *H. perforatum*: (a) - initiation of in vitro culture from mini-cuttings with one node; (b) - Percentage of initiation

In vitro shoot multiplication

Shoot multiplication was significantly influenced by the type of cytokinin supplemented in the culture medium. The lowest number of shoots per explant was recorded in the presence of meta-topolin (1.6 ± 0.17), while the highest number (6.6 ± 0.50) was obtained on DKW medium supplemented with benzyladenine (Figure 2a, Figure 3). These results confirm the high responsiveness of *H. perforatum* to benzyladenine, which has been widely reported as an efficient cytokinin for shoot proliferation in this species (Savio et al., 2012).



(a)



(b)

Figure 2. Influence of growth regulators on the number of shoots/explant (a) and shoot length (b), in *H. perforatum*, cultivated *in vitro* on DKW medium supplemented with the following growth regulators: kinetin (2 mg/L kin); metatopolin (2 mg/L mT); zeatin (1 mg/L Z); 2-isopentenyladenine (5 mg/L 2iP); forchlorfenuron (0.1 mg/L CPPU); benzyladenine (0.1 mg/L BA)

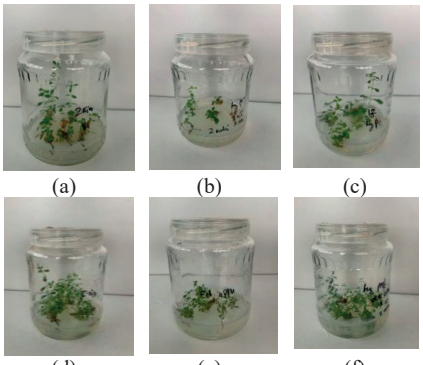


Figure 3. *In vitro* cultures of *H. perforatum* on DKW medium supplemented with different growth regulators: (a) - 2 mg/L kinetin (kin); (b) - 2 mg/L metatopolin (mT); (c) - 1 mg/L zeatin (Z); (d) - 5 mg/L 2-isopentenyladenine (2iP).; (e) - 0.1 mg/L forchlorfenuron (CPPU); (f) - 0.1 mg/L benzyladenine (BA)

Shoot length was less affected by cytokinin type, showing relatively uniform values across treatments. The longest shoots were obtained in the presence of kinetin, while slightly shorter but morphologically uniform shoots were observed on media supplemented with zeatin, CPPU, and benzyladenine (Figures 2b and 3).

The balance between shoot number and shoot quality indicates that benzyladenine is the most suitable cytokinin for efficient multiplication of *H. perforatum* under the tested conditions (Santarém & Astarita, 2003; Tzatzani et al., 2023).

***In vitro* rooting and ex vitro acclimatization**

Rooting response was influenced by the presence and concentration of IBA (Figures 4 and 5). Interestingly, the highest rooting percentage (83.33%) and the longest roots were obtained on auxin-free medium, where explant viability reached 100%. Similar auxin-independent rooting has been reported in *Hypericum microshoots*, indicating that rooting can occur on hormone-free media depending on genotype and culture conditions (McCoy & Camper, 2002). In contrast, media supplemented with IBA resulted in lower rooting percentages, which is consistent with reports indicating that the effect of exogenous auxins on in vitro rooting may be genotype- and medium-strength-dependent, and that IBA may reduce rooting performance under certain conditions (Coste et al., 2012; Fascella et al., 2017).

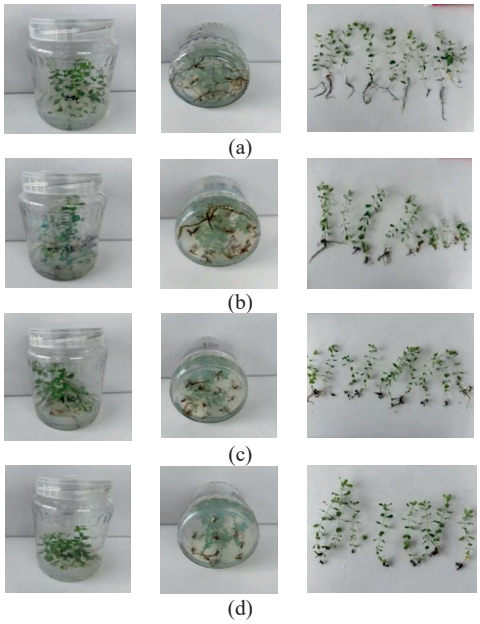


Figure 4. *In vitro* rooting of explants with apical bud in *H. perforatum* on DKW culture medium without auxins (a) and supplemented with 0.5 mg/L IBA (b), 1 mg/L IBA (c), 1.5 mg/L IBA (d)

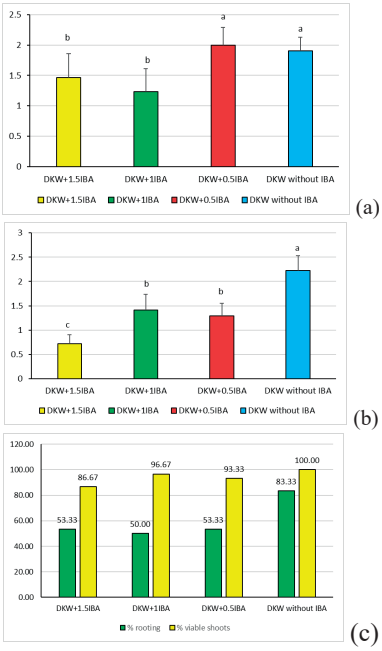
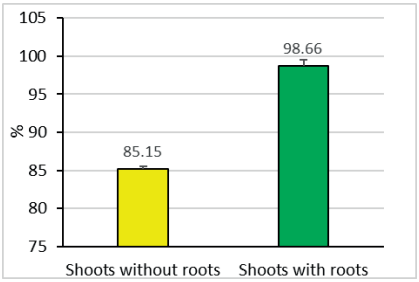
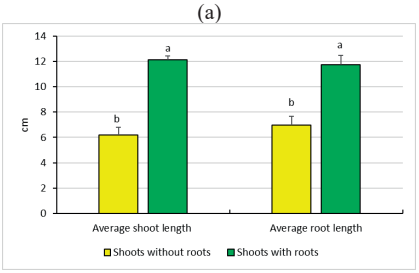


Figure 5. *In vitro* rooting on DKW culture medium without IBA and supplemented with 0.5 mg/L IBA, 1 mg/L IBA, and 1.5 mg/L IBA in *H. perforatum*: (a) average number of roots/initial explant; (b) average root length; (c) rooting percentage and percentage of viable shoots

These results suggest that endogenous auxin levels and physiological status of explant can play a major role in *in vitro* rooting, while exogenous auxin supplementation may not always be required (Koperdakova et al., 2009).
Ex vitro acclimatization in perlite (Figure 7a,b) was successful for both in vitro-rooted plantlets and non-rooted shoots. However, significantly higher survival rates were recorded in plantlets rooted in vitro, which showed an acclimatization percentage of 98.66%, compared with 85.15% in non-rooted shoots (Figure 6a).



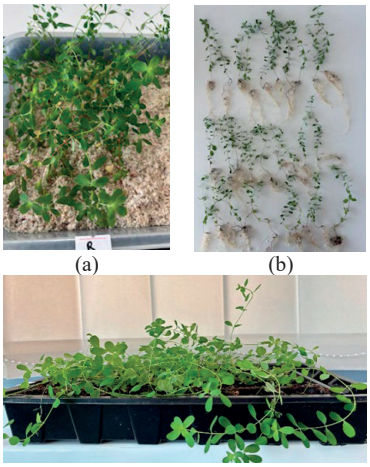
(a)



(b)

Figure 6. Perlite acclimation of rooted and unrooted shoots of *H. perforatum*: (a) percentage of acclimatization; (b) average shoot and root length

In addition, plantlets rooted in vitro exhibited superior growth performance, expressed by greater shoot and root length, compared with non-rooted shoots (Figure 6b).
After transfer to peat substrate and greenhouse conditions (Figure 7c), acclimatized plants derived from in vitro-rooted plantlets showed a high establishment rate, with 93% successful establishment inalveolar trays.
These results demonstrate the efficiency of the proposed micropropagation and acclimatization protocol and highlight the advantage of in vitro rooting prior to *ex vitro* transfer.


(a) (b)

(c)

Figure 7. Rooting and acclimatization in *H. perforatum*: shoots acclimatized in perlite (a); acclimatized plants transferred to peat (b, c)

Biochemical traits (TPC and TFC)
As shown in Table 1, TPC and TFC exhibited comparable values in samples obtained from in vitro cultures and potted plants of *H. perforatum*.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) in *H. perforatum* samples obtained from in vitro cultures and potted plants. Values are expressed as mean ± standard error (n = 3)

Parameter	Potted plants	In vitro plants
Total phenolic content (µg GA g^{-1} FW)	6539.67±30.46	6395.17±29.55
Total flavonoid content (µg Q g^{-1} FW)	5882.33±36.19	5846.10±21.39

TPC reached 6539.67 ± 30.46 µg GA g^{-1} fresh weight in potted plants and 6395.17 ± 29.55 µg GA g^{-1} fresh weight in *in vitro*-derived plants. Similarly, TFC showed close values between the two plant categories, with 5882.33 ± 36.19 µg Q g^{-1} fresh weight in potted plants and 5846.10 ± 21.39 µg Q g^{-1} fresh weight in in vitro cultures.
In contrast to the present results, other studies have reported significant differences between in vitro derived and field-grown plants. For instance, Yaman et al. (2022) reported significantly lower phenolic and flavonoid contents in in vitro samples compared to field-grown plants, with all samples differing statistically at $p \leq 0.01$.

In their study, *in vitro* plantlets and callus exhibited the lowest TPC and TFC values, while field-grown plants showed substantially higher levels of secondary metabolites.

The larger differences observed in that study may be attributed to the use of mature field-grown plants, which are known to accumulate higher levels of phenolic compounds due to prolonged development and exposure to environmental stimuli. In the present study, both in vitro and potted plants originated from micropropagated material and were at relatively early developmental stages, which may explain the smaller differences observed between the two plant categories. These findings suggest that plant developmental stage and growth conditions play a major role in secondary metabolite accumulation in *H. perforatum*.

The present study demonstrated that *H. perforatum* can be efficiently micropropagated through *in vitro* culture using nodal explants, followed by successful rooting and ex vitro acclimatization. Among the tested cytokinins, benzyladenine proved to be the most effective for shoot multiplication, while auxin-free medium ensured the highest rooting percentage and explant viability.

Ex vitro acclimatization was achieved with high survival rates for both rooted and non-rooted shoots, although superior growth performance was observed in plantlets rooted *in vitro*. The high establishment rate after transfer to greenhouse conditions confirms the reliability of the proposed micropropagation protocol for producing healthy planting material.

Biochemical analysis revealed comparable levels of total phenolic and flavonoid contents in in vitro cultured and acclimatized plants, indicating that secondary metabolism was not negatively affected by in vitro conditions or by the acclimatization process. These results suggest that biomass obtained through in vitro culture may represent a potential source of phenolic compounds.

CONCLUSIONS

The developed protocol provides a practical and efficient approach for the propagation of *H. perforatum*, while also highlighting the potential of *in vitro*-derived biomass for further applications. Additional studies focusing on detailed phytochemical profiling and biomass yield optimization are required to further explore this potential.

ACKNOWLEDGEMENTS

This research was funded by the Romanian Ministry of Agriculture and Rural Development, contract ADER 5.2.3. / 2023.

REFERENCES

Abdollahpoor, M., Kalantari, S., Azizi, M., & Saadat, Y. A. (2017). *In vitro* shoot proliferation of *Hypericum perforatum* L. through indirect and direct plant regeneration. *Journal of Medicinal Plants and By-products*, 6(1), 81-89.

Ahmed, H., A. A., Uranbey, S., Salaj, T., & Mistrikova, V. (2025). Cell suspension cultures and High Frequency Shoot Regeneration of some Hypericum species. *Journal of Agricultural Sciences*, 31(2), 319-331.

Al-haliem, S., M., Mohammed, M., J., Hesarinejad, M., A., & Abedelmaksoud, T. G. (2025). Antimicrobial, Anti-Biofilm Activity and antioxidants of Phenolic Compounds Isolated From *Hypericum perforatum* on Periodontal Pathogenic Oral Bacteria. *Food Science & Nutrition*, 13(6), e70336.

Al Nooh, K., M., Salim, A., M., Faizy, W., S., Dargiri, S., A., & Meftahizade, H. (2025). Synergistic efFects Of plant growth regulators and Fe_3O_4 nanoparticles on in vitro organogenesis and bioactive compound production in *Hypericum perforatum*. *BMC Plant Biology*, 25(1), 1335.

Amooaghaie, R., & Rajaie, N. (2025). Foliar application of yeast extract mitigates water deficit stress and elicits hypericin and phenolic production in *Hypericum perforatum* L. *Scientific Reports*, 15(1), 20896.

Anuruddi, H., I. G. K., Nakandalage, N., & Fonseka, D. K. (2023). New insights for the production of medicinal plant materials: *Ex vitro* and in vitro propagation. In *Biosynthesis of Bioactive Compounds in Medicinal and Aromatic Plants: Manipulation by Conventional and Biotechnological Approaches* (pp. 181-212). Cham: Springer Nature Switzerland.

Barnes, J., Anderson, L., A., & Phillipson, J. D. (2001). St John's wort (*Hypericum perforatum* L.): A review of its chemistry, pharmacology, and clinical properties. *Journal of Pharmacy and Pharmacology*, 53(5), 583–600.

Chen, S. L., Yu, H., Luo, H. M., Wu, Q., Li, C. F., & Steinmetz, A. (2016). Conservation and sustainable use of medicinal plants: Problems, progress, and prospects. *Chinese medicine*, 11(1), 37.

Clapa, D., Hârța, M., Radomir, A. M., Peticilă, A. G., Leopold, L., Ranga, F., & Sumedrea, D. I. (2025). Effects of Culture Period and Plant Growth Regulators on *In Vitro* Biomass Production and Phenolic Compounds in Seven Species of Hypericum. *Plants*, 14(15), 2437.

Coste, A., Halmagyi, A., Butiuc-Keul, A., Deliu, C., Coldea, G., & Hurdu, B. (2012). In vitro propagation and cryopreservation of Romanian endemic and rare Hypericum species. *Plant Cell, Tissue and Organ Culture*, 110(2), 213–226.

Custódio, L., Slusarczyk, S., Matkowski, A., Castañeda-Loaiza, V., Fernandes, E., Pereira, C., & Rodrigues, M. J. (2022). A first approach for the micropropagation of the medicinal halophyte *Polygonum maritimum* L. and phenolic profile of acclimatized plants. *Frontiers in plant science*, 13, 960306.

Driver, J. A., & Kuniyuki, A. H. (1984). *In vitro* propagation of Paradox walnut rootstock. *HortScience*, 19(4), 507-509.

Espinosa-Leal, C. A., Puente-Garza, C. A., & García-Lara, S. (2018). In vitro plant tissue culture: Means for production of biologically active compounds. *Planta*, 248(1), 1–18.

Fascella, G., Airò, M., Mammano, M., M., Giardina, G., Carrubba, A., & Lazzara, S. (2017). Rooting and acclimatization of micropropagated *Hypericum perforatum* L. native to Sicily. *Acta Horticulturae*, 1155, 543–548

Grzelak, M., Pacholczak, A., & Nowakowska, K. (2024). Challenges and insights in the acclimatization step of micropropagated woody plants. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 159(3), 72.

Hasnain, A., Naqvi, S. A. H., Ayesha, S. I., Khalid, F., Ellahi, M., Iqbal, S., ... & Abdelhamid, M. M. (2022). Plants in vitro propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario and future approaches. *Frontiers in plant science*, 13, 1009395.

Khakpour, S., Motallebi-Azar, A., Hosseini, B., Alizadeh-Salte, S., & Hasani, A. (2015). Optimization of micropropagation by different concentrations of vitamins and sucrose in St. John's Wort (*Hypericum perforatum*). *Plant Breeding and Seed Science*, 71(1), 67.

Kikowska, M., Thiem, B., Szopa, A., & Ekiert, H. (2020). Accumulation of valuable secondary metabolites: phenolic acids and flavonoids in different in vitro systems of shoot cultures of the endangered plant species - *Eryngium alpinum* L. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 141(2), 381-391.

Kilibarda, S., Jović, M. D., Milinčić, D., D., Vuković, S., Trifković, J. Đ., Pešić, M., B., & Kostić, A. Ž. (2025). Phytochemical Profile and Biological Activities of Rtanj's *Hypericum perforatum* Infusion Tea and Methanolic Extracts: Insights from LC-MS/MS and HPTLC–Bioautography. *Plants*, 14(9), 1377.

Koperdakova, J., Katkoveinová, Z., U. Z. A. N. A., Košuth, J., Giovannini, A., & Cellarova, E. (2009). Morphogenetic response to plant growth regulators in transformed and untransformed *Hypericum perforatum* L. clones. *Acta Biol Cracov Ser Bot*, 51, 61-70.

Kwiecień, I., Miceli, N., Kędzia, E., Cavò, E., Taviano, M., F., Beerhues, L., & Ekiert, H. (2023). Different types of *Hypericum perforatum* cvs. (Elixir, Helos, Topas) *in vitro* cultures: a rich source of bioactive metabolites and biological activities of biomass extracts. *Molecules*, 28(5), 2376.

McCoy, J., A., & Camper, N. D. (2002). Development of a micropropagation protocol for St. John's wort (*Hypericum perforatum* L.). *HortScience*, 37(6), 978-980.

Mišina, I., Perkons, I., Siger, A., Soliven, A., & Górnaś, P. (2025). Residues of St. John's wort (*Hypericum perforatum*) tea infusions/water extracts as a valuable source of tocotrienols: An extraction study. *Applied Sciences*, 15(4), 2047.

Molnar, S., Clapa, D., Dagni, A., Leopold, L., Hârţa, M., & Bunea, I. C. (2024). Morpho-Biochemical Response of Blueberry Cultivar Hortblue Petite Under Drought Stress Induced by PEG 6000. *Scientific Papers. Series B. Horticulture*, 68(1), 85-93.

Moraes, R. M., Cerdeira, A., L., & Lourenço, M. V. (2021). Using micropropagation to develop medicinal plants into crops. *Molecules*, 26(6), 1752.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia plantarum*, 15(3).

Puia, A., Pandrea, S., L., Cruceru, J., Puia, I., C., Chedea, V., S., Ciont, C., ... & Pop, R. M. (2025). Phenolic Compounds with Antimicrobial Properties in Mushrooms Frequently Encountered in Temperate Deciduous Forests. *Life*, 15(11), 1653.

Rahimi, S., Bayati, M., Kordrostami, M., & Ghasemi-Soloklui, A. A. (2023). Tissue culture techniques to conserve endangered medicinal plants with antimicrobial and antiviral activities. In *Medicinal plants: Biodiversity, biotechnology and conservation* (pp. 675-710). Singapore: Springer Nature Singapore.

Ravindran, B., M., Rizzo, P., Franke, K., Fuchs, J., & D'Auria, J. (2023). Simple and robust multiple shoot regeneration and root induction cycle from different explants of *Hypericum perforatum* L. genotypes. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 152(1), 1-15.

Reshi, Z., A., Ahmad, W., Lukatkin, A., S., & Javed, S. B. (2023). From nature to lab: A review of secondary metabolite biosynthetic pathways, environmental influences, and *in vitro* approaches. *Metabolites*, 13(8), 895.

Rusanova, M., Rusanov, K., Alekova, M., Georgieva, L., Georgieva, P., Zagorcheva, T., & Atanassov, I. (2025). A simple high-throughput procedure for microscale extraction of bioactive compounds from the flowers of Saint John's Wort (*Hypericum perforatum* L.). *Applied Sciences*, 15(13), 7334.

Samridha, V., & Chandra, S. (2024). *In vitro* propagation to conserve medicinally important plants: Insight, procedures, and opportunities. In *Medicinal and Aromatic Plants: Current Research Status, Value-Addition to Their Waste, and Agro-Industrial Potential (Vol I)* (pp. 13-25). Cham: Springer Nature Switzerland.

Santarém, E., R., & Astarita, L. V. (2003). Multiple shoot formation in *Hypericum perforatum* L. and hypericin production. *Brazilian Journal of plant physiology*, 15, 43-47.

Savio, L., E. B., Astarita, L., V., & Santarém, E. R. (2012). Secondary metabolism in micropropagated

Hypericum perforatum L. grown in non-aerated liquid medium. *Plant Cell, Tissue and organ culture (PCTOC)*, 108(3), 465-472.

Selvakesavan, R., K., & Franklin, G. (2021). Robust *in vitro* culture tools suitable for sustainable bioprospecting of the genus *Hypericum*. *Industrial Crops and Products*, 170, 113715.

Shasmita, Behera, S., Mishra, P., Samal, M., Mohapatra, D., Monalisa, K. & Naik, S. K. (2023). Recent advances in tissue culture and secondary metabolite production in *Hypericum perforatum* L. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 154(1), 13-28.

Singleton, V., L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in enzymology* (Vol. 299, pp. 152-178). Academic Press.

Sood, H., Shitiz, K., & Sharma, N. (2015). Rapid method for *in vitro* multiplication of hypericin-rich shoots of *Hypericum perforatum*. *Journal of Plant Science*, 3(5), 279-284.

Southwell, I., A., & Bourke, C. A. (2001). Seasonal variation in hypericin content of *Hypericum perforatum* L. (St. John's wort). *Phytochemistry*, 56(5), 437–441

Syahid, S., F., & Wahyuni, S. (2019). Effect of silver nitrate on shoot multiplication, rooting induction, and plantlet characteristics of St. John's wort (*Hypericum perforatum* L.) *in vitro* culture. *Afr. J. Agric. Res*, 14(27), 1149-1153.

Thiem, B., Hermosaningtyas, A., A., Budzianowska, A., & Kikowska, M. (2023). Polish contributions in developing medicinal plant *in vitro* propagation system. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 155(1), 1-28.

Tzatzani, T., T., Michail, I., Samartza, I., Krigas, N., & Tsoktouridis, G. (2023). Micropropagation and molecular characterization of *Hypericum perforatum* L. subsp. Veronense (Schrank) H. Lindb., a valuable medicinal plant with ornamental value. Romanian *Journal of Horticulture*, 9.

Yaman, C., Önlü, Ş., Ahmed, H. A. A., & Erenler, R. (2022). Comparison of phytochemicals and antioxidant capacity of *Hypericum perforatum:* wild plant parts and *in vitro* samples. JAPS: *Journal of Animal & Plant Sciences*, 32(2).

Zhishen, J., Mengcheng, T. & W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, vol. 64, no. 4, pp. 555-559, 1999.

Zobayed, S., M. A., Afreen, F., & Kozai, T. (2005). Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. *Plant Physiology and Biochemistry*, 43(10-11), 977-984.