doi:10.14720/aas.2025.121.3.18392 Original research article / izvirni znanstveni članek

Sodium chloride, colchicine, and 6-benzylaminopurine can change antioxidant property and phenols content in *Hypericum perforatum* L.: An *in vitro* study

Ara MANTEGHI TAFRESHI [1], Reza MOHAMMADHASSAN [1, 2]

Received March 12, 2024, accepted August 07, 2025
Delo je prispelo 12 marec 2024, sprejeto 7. avgust 2025

Sodium chloride, colchicine, and 6-benzylaminopurine can change antioxidant property and phenols content in *Hypericum perforatum* L.: An *in vitro* study

Abstract: *Hypericum perforatum*, a medicinal plant from the Hypericaceae family, is known for its wide range of bioactive properties, including antidepressant, antiviral, antibacterial, and anti-cancer activity. These effects are attributed mainly to its antioxidants and among them phenolic secondary metabolites. This *in vitro* study aimed to investigate the impact of various concentrations of colchicine (0.05, 0.1, 0.2 mg l^{-1}), sodium chloride (NaCl; 0.5, 1. 2 mg l^{-1}), and 6-benzylaminopurine (BAP; 0.25, 0.5, 1 mg l^{-1}) on antioxidant activity and total phenol content in *H. perforatum* over three weeks. A factorial experiment was conducted in a completely randomized design with three replications. The results revealed that all treatments significantly enhanced ($p < 0.01$) antioxidant capacity, phenol content, and morphological characteristics. Notably, the highest levels of antioxidant activity and total phenol content were observed in the third week following treatment with 0.25 mg l^{-1} BAP, 2 mg l^{-1} NaCl, and 2 mg l^{-1} colchicine, separately. These findings suggest that the higher concentrations of NaCl and colchicine, along with a lower concentration of BAP, can effectively enhance the biosynthesis of phenolic compounds and antioxidant activity in *H. perforatum*.

Key words: *Hypericum perforatum*, antioxidant property, phenol content, colchicine, sodium chloride, 6-benzylaminopurine, plant tissue culture.

Natrijev klorid, kolhicin in 6-benzilaminopurin lahko spremenijo antioksidacijske lastnosti in vsebnost fenolov v šentjanževki (*Hypericum perforatum* L.): *in vitro* raziskava

Izvleček: Šentjanževka (*Hypericum perforatum* L.), zdravilna rastlina iz družine krčničevk (Hypericaceae), je zelo poznana zaradi svojih bioaktivnih lastnosti kot so antidepresivna, protivirusna, protibakterijska in protirakovna aktivnost. Ti učinki so v glavnem posledica vsebnosti antioksidantov in med njimi fenolnih sekundarnih metabolitov. Namen te *in vitro* raziskave je bil preučiti vpliv različnih koncentracij kolhicina (0,05; 0,1; 0,2 mg l^{-1}), natrijevega klorida (NaCl; 0,5; 1, 2 mg l-1) in 6-benzilaminopurina (BAP; 0,25; 0,5; 1 mg l^{-1}) na antioksidacijsko aktivnost in vsebnost celokupnih fenolov v šentjanževki v obdobju treh tednov. Popolni naključni faktorski poskus je bil zasnovan v treh ponovitvah. Rezultati so pokazali, da so vsa obravnavanja značilno povečala ($p < 0.01$) antioksidacijsko sposobnost, vsebnost phenolov in morfološke lastnosti. Največji antioksidacijska aktivnost in vsebnost celokupnih fenolov sta bili ugotovljeni tretji teden po obravnavanjih z 0,25 mg l^{-1} BAP, 2 mg l^{-1} NaCl in ločeno po obravnavanju z 2 mg l^{-1} kolhicina. Te ugotovitve nakazujejo, da lahko večje koncentracije NaCl in kolhicina hkrati z manjšimi koncentracijami BAP učinkovito pospešijo biosintezo fenolnih spojin in povečajo antioksidativno aktivnost šentjanževke.

Ključne besede: *Hypericum perforatum*, antioksidacijke lastnosti, vsebnost fenolov, kolhicin, natrijev klorid, 6-benzilaminopurin, rastlinske tkivne kulture

1 Plant Sciences Department, Amino Techno Gene Private Virtual Laboratory (NGO), Tehran, Iran.
2 corresponding author: rezarmhreza22@gmail.com

1 INTRODUCTION

Hypericum perforatum L., belonging to Hypericaceae, includes approximately 400 species, with 10 morphologically and chemically essential species worldwide. *H. perforatum* is a perennial, herbaceous, erect and rarely glabrous plant with a height of 90-100 cm. The shoot contains many branches covered by red or black, amber glands. The leaves are on smaller and narrower branches, ovate to almost linear, with 5.35×2-14 mm dimensions (Saleh, 2023; Shamilov et al., 2019).

This plant has a long history of medicinal use, initially applied for treating burns and skin injuries. In recent decades, it has gained substantial attention as an effective treatment for depression. Clinical and pharmacological investigations have indicated that *H. perforatum* exhibits antidepressant efficacy superior to several conventional antidepressants, with a notably lower incidence of side effects (Leandro et al., 2017). In addition to the neuropharmacological potential, *H. perforatum* has shown broad-spectrum antiviral activities, particularly against hepatitis C (HCV), human immunodeficiency virus (HIV), and neurotropic viruses (Tariq et al., 2019), contributing to neuroinflammation and neurodegenerative diseases (Ramedani et al., 2015; Marawne et al., 2022). The antibacterial properties of *H. perforatum* extract have also been observed against *Mycobacterium tuberculosis* Zopf 1883 and various wound-associated bacterial strains (Imreova et al., 2017). Moreover, the plant has been traditionally utilized for a wide range of therapeutic purposes, including tonic and digestive support, choleretic and urinary antiseptic actions, anti-influenza and anxiolytic effects, astringency, enhancement of respiratory and uterine function, and alleviation of arterial inflammation (Budantsev et al., 2021; Velingkar et al., 2017).

Secondary metabolites cause therapeutic effects. The hydroalcoholic extract from the upper parts of the plant contains 6 major groups of natural products, including naphthodianthrone, phloroglucinol (particularly hypericin and pseudohypericin), flavonoid, biflavone, phenylpropane, and proanthocyanidin. In addition, there are lower amounts of tannin, xanthone, and amino acids, which have been found in all parts of *Hypericum* spp (Suryawanshi et al., 2024; Saleh, 2023).

Phenol is a simple organic compound formed by bonding a hydroxyl group to a benzene ring. The compound with the formula C_6H_5OH is known as hydroxybenzene or, more commonly, phenol. It is also historically referred to as carbolic acid. Pure phenol is a white solid, usually water-soluble, with a melting point of 42 °C and low acidity (Albuquerque et al., 2021). Almost all phenolic compounds show antimicrobial activity, which is not very specific. The antimicrobial activity of phenols maight be caused by damaging the structure and changing the permeability of the cell wall and membrane in microorganisms and lysosomes (Oulahal and Degraeve, 2022). Although this antimicrobial activity is specific to some antibiotics, the general antimicrobial effects of many phenols are irreversible when diluted with water. In addition, bacteria cannot acquire immunity against the initial inhibitory concentration of a phenolic compound. Consequently, phenols are economically valuable antimicrobial agents (Lima et al., 2023). Phenol has a specific anthelmintic activity that increases with the presence of alkyl groups. The most effective anthelmintic phenolic compounds need to be low water soluble, so they are not absorbed from the intestine (Manjusa and Pradeep, 2022).

Antioxidants are chemical compounds that neutralize free radicals, an active, harmful ingredient for human health, to preserve or delay oxidative damage to biomolecules. Antioxidants are used as food additives to increase the shelf life of oils and fatty foods and maintain their quality during storage and consumption (Gulcin, 2020). Many studies indicate that some synthetic antioxidants may harm the body. Antioxidants reduce the risk of cardiovascular diseases and stroke by neutralizing free radicals. On the other hand, antioxidants can suppress the cancer process and protect cell membranes (Parcheta et al., 2021). Natural antioxidants have recently been used in food, medicine, and pharmaceutical industries. Consequently, the provision of herbal essential oils and plant-derived secondary metabolites as natural antioxidant alternatives plays a crucial role in mitigating the adverse effects of oxidative stress. Many scientists extract these compounds from medicinal plants, with fewer side effects and high efficiency (Zeb, 2020).

Salinity is a vital factor in reducing crop yield; the effects of salinity are not limited to a specific stage of plant growth but are effective throughout the entire growth period of the plant and ultimately lead to a decrease in yield (Hameed et al., 2021). Salt stress can damage plants in two ways; first, the high concentration of salts, especially the high concentration of sodium, destroys the soil structure. As soil porosity decreases, both soil ventilation and hydraulic conductivity are debilitated in such soils. Second, high salt concentrations are closely related to water stress. High concentrations of solutes reduce the water potential of the soil and make it more difficult for plants to absorb water and nutrients. In other words, some physiological drought occurs. Because both drought and salinity cause osmotic stress. Hence, the reactions of the plant are similar to the osmotic stress (Giordano et al., 2021; Etesami et al., 2021; Khan et al., 2020).

Colchicine (Col) has been used as the most effective chemical compound in studies to induce polyploidy in plants. Chol is an alkaloid extracted from the seeds and pods of *Colchicum autumnale* L.. The synthesized form of the compound is known as clomid. Col, as a natural inhibitor of mitosis, leads to the creation of a cell with a doubled number of chromosomes by creating an inhibitory state in the formation of spindle fibres and, as a result, preventing the polar migration of chromosomes and, eventually, cell division (Natt and Sattely, 2021; Gracheva et al., 2020). Technically, this compound prevents the formation and polymerization of microtubules by linking with the protein subunit of microtubules called tubulin. Finally, the chromosomes simultaneously enter the cell at the metaphase stage. Consequently, the ability has turned into a polyploid inducer. Thus, Col is the most critical chemical agent for chromosomal doubling that is widely used (Cui et al., 2023; Yuling et al., 2022).

In the present study, we aimed to analyze the effects of Col, sodium chloride (NaCl), and 6-benzylaminopurine (BAP), as the common synthetic form of cytokinin widely used in plant tissue culture, on antioxidant property (AP) and total phenol content (TPC) of *H. perforatum*, *in vitro* conditions.

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS AND CHEMICAL REAGENTS

Seeds of *H. perforatum* were provided from Pakan Bazr, Isfahan, Iran. All Media, chemical compounds and synthetic phytohormones, particularly NaCl, Col, and BAP, were from Sigma-Aldrich, St. Louis, Missouri, United States. All experiment stages were conducted in Amino Techno Gene Private Laboratory (NGO), Tehran, Iran.

2.2 PREPARING AND CULTURING EXPLANTS

The seeds were sanitized and then cultured into the basal MS agar media to germinate and grow for 2 months at 25 °C and 16/8 light and darkness. After that, the explants were regenerated in different hormone-free MS (Murashige and Skoog, 1962) agar media containing different dosages of BAP (0.25, 0.5, 1 mg l^{-1}), Col (0.05, 0.1, 0.2 mg l^{-1}), and NaCl (0.5, 1, 2 mg l^{-1}).

2.3 MORPHOLOGICAL ANALYSIS

For 3 weeks, the explants were weekly taken out from the treated media, and then different plant parts, including aerial parts, were quickly weighed to measure the fresh mass (FM; g) of the aerial parts (leaves and shoots) and other morphological characteristics, including length of shoots (cm) and the number of leaves and shoots.

2.4 EXTRACTION OF THE ESSENTIAL OIL

The essential oil of the treated *H. perforatum* was extracted with methanol solvent for measuring thymol production, elicited by different treatments. 20 ml of methanol was added to a laboratory mortar containing a sample from the aerial parts of each treatment, and then well ground with a pestle. After that, the well-ground samples were moved into a flask and covered with aluminium foil and then incubated and shaken in a shaker incubator (TSHE 53, Nour Sanat Tajhiz Co., Karaj, Alborz, Iran) at 140 rpm for 48 hours, without any set temperature. In the next step, the solutions were centrifuged (Sahand Azma Tajhiz, Tehran, Iran) at 20 °C for 15 minutes at 3000 g. The above phase was transferred into a beaker and then allowed to evaporate in the open air and darkness for 24 hours. Consequently, the solvent was removed from the concentrated extract and maintained in cool and dark conditions.

2.5 TPC ANALYSIS

First, 10 ml of methanolic extract and 490 ml methanol 80 % were poured into a 15 ml Falcon. Next, 500 ml of Folin-Ciocalteu was added to the content. After 2 min, 1 ml of sodium carbonate 7 % was added to the mixture. The final volume was made up to 6 ml using distilled water. The falcon, containing the mixture, was incubated in a bain-marie bath at 30 °C in the dark for 90 min; then, the absorption spectrum was measured using a spectrophotometer (S2100, Unico, USA) at 725 nm. Gallic acid was used as a standard solution.

2.6 2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) ANALYSIS

Different concentrations of methanolic extraction were used to analyze antioxidant potential (AP). After that, 1 ml of DPPH 0.4 mM was added to each concentration. The final volume was made up to 5 ml using pure methanol. The reaction mixture was vigorously mixed and then incubated at room temperature in the dark for 30 min. The positive control sample contained 1 ml DPPH 0.4 mM and 4 ml pure methanol. The spectrophotometer was calibrated with methanol. The absorption spectrum was measured using a spectrophotometer at 517 nm.

2.7 STATISTICAL ANALYSIS

This experiment was a factorial, completely randomized design with three replications. Duncan's multiple-range test ($p < 0.01$) was used to analyze variance and means comparison. Also, the relationship between traits was analyzed by the correlation coefficient. SAS software (Version 9.2) and Microsoft Excel (2016) were employed for statistical analysis and graph drawing, respectively.

3 RESULTS AND DISCUSSION

3.1 BAP

According to the results, BAP, duration, and BAP×duration significantly influenced all traits, including leaf number, shoot number and length, FM, AP, and TPC ($p < 0.01$; Table 1). The means of the BAP effect on the traits were compared by Duncan's multiple-range test at $p < 0.05$. The maximum and minimum numbers of leaves were observed in the highest BAP concentration, the most prolonged duration (13), and the lowest BAP × time interaction (3). Besides, the highest number of shoots was in 1 mg l^{-1} BAP in the 3rd week (7), and the lowest number of shoots was measured in 0.25 mg l^{-1} in the 1st and 2nd weeks, and 0.5 mg l^{-1} in the 2nd week (1 cm). Also, the highest shoot length was observed in 1 mg l^{-1} BAP in the 3rd week (29 cm), and the lowest length of shoot was in 0.25 mg l^{-1} in the 1st week (6 cm). In case of FM, the highest and the lowest amounts were measured in 1 mg l^{-1} BAP in the 3rd week (g) and 0.25 mg l^{-1} in the 1st week (g), respectively. However, AP and TPC were higher in the lowest BAP concentration and increased over time (233 and 8.01, respectively; Figure 1). Moreover, there are significant correlations ($p < 0.01$) among leaf number, shoot number and length, FM, AP, and TPC affected by BAP; however, the correlation between shoot length and TPC was significant at $p < 0.05$ (Table 2).

Nazir et al. (2022) studied the BAP effect on in vitro micropropagation of mg $^{-1}$ *Valeriana jatamansi* Jones ex Roxb. They found that shoot length and leaf number can be enhanced when BAP concentration increases in the MS culturing medium. In another study, growth in BAP level caused higher shoot length and number rates in *Plectranthus amboinicus* Lour. (Arumugam et al., 2020). Also, Kharel et al. (2022) found that higher BAP concentrations can enhance shoot numbers in highbush blueberry explants (*Vaccinium corymbosum* L.). In addition, a study demonstrated that the highest FM level of *Lamprocapnos spectabilis* (L.) Fukuhara was significantly induced by higher BAP concentrations (Kulus, 2020). Kozak et al. (2021) reported the same results in *Mandevilla sanderi* (Hemsl.) Woodson micropropagation. In an in vitro study on *Lycium barbarum* L. (goji berry), MS medium containing various hormones was used to investigate plant regeneration and somatic embryogenesis. Among cytokinins, BAP had a lower effect on shoot production compared to TDZ and other compounds, while the combination of TDZ and 2,4-D induced the highest number of shoots and embryos. This study showed that the selection of the appropriate type and concentration of cytokinin has a significant effect on the efficiency of regeneration and production of secondary metabolites (Verma et al., 2022). In another in vitro study on *L. barbarum*, the highest number of branches (23.33) and percentage of regeneration (100 %) were obtained from nodal explants in MS medium containing only 0.5 mg l^{-1} benzyladenine (BA). Also, the combination of BA/NAA significantly increased chlorogenic acid and caffeic acid in callus. The results showed that the type of plant growth regulator has a direct effect on regeneration and production of phenolic metabolites (Karakas, 2020). Chatoui et al (2020) found that *Lepidium sativum* L. seeds are rich in fatty acids (especially linolenic acid and oleic acid), phytosterols (β-sitosterol) and γ-tocopherol. The methanol extract showed the highest antioxidant activity and phenolic content compared to the ethanol extract. The results indicate a high potential for these seeds to be used in food supplements or additives as antioxidants. In an in vitro study on *Pyrus spinosa* Forssk., two genotypes rich in phenolic compounds and antioxidant capacity were identified from the Agia Anastasia region and used for micropropagation. Pear Medium 1, containing 5 μM BAP, provided the highest shoot regeneration (22.7 shoots per explant). This concentration of BAP was considered suitable for the efficient propagation of the rich and native germplasm of this wild species (Alexandri et al., 2023). In the *in vitro* culture of Isatis indigotica Fort. leaves, the combination of 2 mg l^{-1} BAP, 0.1 mg l^{-1} NAA, 1.5 mg l^{-1} activated carbon and 0.2 % phytagel produced the highest callus regeneration and the lowest browning. Mature leaf callus had higher total phenolic and POD activity than seedling leaves, despite the addition of browning inhibitors. Browning during the dedifferentiation process is related to phenol accumulation and PPO enzyme activity (Su et al., 2023). Also, the highest *Salvia tebesana* Bunge callus induction (100 %) was obtained in the apical meristem on MS medium containing 2,4-D and BAP. The highest accumulation of phenolic compounds, flavonoids and antioxidant activity was also observed in treatments containing 2, 4-D + BAP. The strong correlation between total phenolic content and antioxidant activities (DPPH and FRAP) indicates the efficiency of this method for mass production of medicinal metabolites (Hemmati et al., 2020).

In *H. perforatum*, BAP at higher concentrations increased vegetative growth (number and length of shoots, fresh mass), but the highest antioxidant activity and total phenolics were obtained at lower concentrations (0.25 mg l^{-1}). This pattern is consistent with results reported in plants such as *V. jatamansi*, *P. amboinicus*, and *P. spinosa*, where high concentrations of BAP increased shoot growth, but contrasts with studies such as *S. tebesana* and *I. indigotica*, where the combination of BAP with auxins produced the highest phenolics and regeneration. Therefore, the effect of BAP varies depending on the species, the type of explant, and the purpose of culture (growth or metabolite production).

BAP is a widely used synthetic cytokinin as a phytohormone with several physiological effects. Cytokinin plays critical roles in plant cell division and enlargement, consequently in plant growth and development. The phytohormone can deactivate apical dominance, on the contrary, enhance lateral buds, and then subbranches can be germinated more. Consequently, cytokinin can positively induce shoot germination, development and growth (Hallmark & Rashotte, 2019; Wybouw and De Rybel, 2019; Delche et al., 2014). Also, the phytohormone is effective in nutrient transportation into the leaves. It has been proven that cytokinin is involved in processing metabolism and preventing senescence in leaves (Mock, 2019). Cytokinin induces leaf formation by producing plant stem cells in shoot apical meristems to generate leaf primordia. Also, the phytohormone can determine the final morphology of the leaf and phyllotaxis pattern. Cytokinin decreases sugar accumulation but enhances chlorophyll levels and the period of photosynthesis in the leaf, particularly during senescence (Wu et al., 2021).

There is much evidence suggesting the role of cytokinin in responding to abiotic stresses, including drought, heat, salinity, and low temperature, as well as resisting parasites and pathogens, as the agents causing biotic stress (Li et al., 2021; Cortleven et al., 2019). Cytokinin can induce the biosynthesis and accumulation of metabolites, particularly phenolic and antioxidant compounds, to tolerate stress (Liu et al., 2020). Besides, the phytohormone increases the expression of the gene encoding an antioxidant enzyme to promote antioxidant systems. Generally, TPC, AP, and flavonoid content can be enhanced when cytokinin levels rise (Aremu et al., 2020).

3.2 NACL

The results indicated that salinity, duration, and the interaction of both treatments could significantly ($p < 0.01$) influence the number of leaves, the number of shoots and length, FM, AP, and TPC (Table 3).

Moreover, the means comparison showed that 0.5 mg l^{-1} NaCl observed the highest number of leaves (19.08) during the first week, and the lowest amount of leaves (6) was in 2 mg l^{-1} NaCl during the 2nd and 3rd weeks. Changes in shoot numbers were in the range from 1 to 2; most numbers were measured in 0.5 mg l^{-1} NaCl during the first and second weeks; however, fewer numbers resulted from 1 mg l^{-1} NaCl in the exact durations and also 2 mg l^{-1} for the first and second weeks. In addition, the longest shoots (16 cm) were caused by 0.5 mg l^{-1} NaCl in the first week; in contrast, the smallest shoots (7 cm) were observed with 1 mg l^{-1} NaCl in the third week. 0.5 mg l^{-1} NaCl × 1st week and 1 and 2 mg l^{-1} × 3rd week could also cause the highest and lowest FM (1.15 and 0.7 g), respectively. Moreover, the highest AP

Table 1: Variance analysis of BAP effect on the traits.

		Mean Square					
Source of Variation	df	Leaves Num	Shoot Num.	Shoot Length (cm)	FM (g)	AP (mg ml^{-1})	TPC (mg g^{-1})
Week	2	859**	285**	3678**	428**	69436**	124**
BAP	2	327**	69**	553**	34**	39652**	86.03**
BAP×Week	4	52**	12**	14**	2**	2913**	36**
Error	124	139.096	85	391	55	13048	211.07
Total	134	16.60	5.81	5.446	6.06	7.8452	6.41

*, ** significance in 5 % and 1 %, respectively

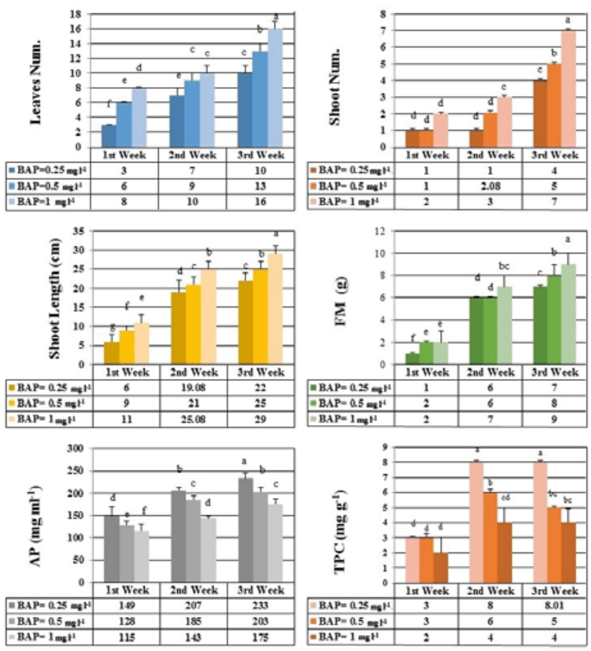

Figure 1: The means comparison of BAP effect on the traits

(150 mg ml^{-1}) was observed by 2 mg l^{-1} NaCl for 3 weeks, but the 0.5 mg l^{-1} NaCl caused the lowest AP (20 mg ml^{-1}) in the first week. Besides, the highest TPC (56 mg g^{-1}) was measured under a high range of salinity, caused by 2 mg l^{-1} NaCl, for 3 weeks; on the contrary, 0.5 mg l^{-1} NaCl could lead to the lowest TPC in the 1st week (6 mg g^{-1}). Generally, when NaCl concentration increased, morphological traits, including leaf number, shoot number and length, and FM, were raised, but AP and TPC decreased (Figure 2). Interestingly, there was a significant correlation among all traits, including leaf number, shoot number and length, FM, AP, and TPC, under NaCl treatment ($p < 0.01$; Table 4).

Salinity can decrease plant growth rates, shoot numbers, plant and shoot height, seed germination, and leaf numbers. For instance, an *in vitro* study indicated that high NaCl concentrations can significantly decrease morphological traits, including shoot length and number, FM, and dried mass, in *Paronychia argentea* Lam.. High salt concentration causes osmotic stress, leading to low water adsorption by plants. The osmotic stress eventually inhibits photosynthesis, impairs homeostasis, and peroxidises membrane lipids, causing ion stress to damage membrane permeability and accumulate reactive oxygen species (ROS), leading to oxidative stress (Hao et al., 2021; Osman et al., 2021; Li et al., 2021). In *in vitro Paulownia* culture, the addition of NaCl by reducing the water potential of the culture medium increased genetic diversity and selected lines resistant to salinity. The presence of NaCl led to the expression of specific molecular markers in resistant lines that were not seen in control plants. These results indicate the effect of NaCl in stimulating genetic pathways associated with resistance under laboratory culture conditions (Salem et al., 2022). In a study, the effect of different concentrations of NaCl (0 to 250 mM) on the *in vitro* growth of pepper (*Capsicum annuum* L.) was investigated. The results showed that increasing salinity significantly reduced germination, shoot and root growth, and physiological indices and increased visual damage. The high sensitivity of pepper to salinity stress was confirmed *in vitro* (Kara et al., 2025). Ghasemi-omran et al. (2021) reported that melatonin at concentrations of 5 and 10 μM improved growth, ionic balance, photosynthetic pigments, antioxidants, and increased steviol glycoside production in *Stevia rebaudiana* Bertoni. This effect was associated with a decrease in ROS and upregulation of kaurenoic acid hydroxylase and uridine diphosphate glycosyltransferase genes expression. In contrast, a higher concentration of melatonin (20 μM) had a negative effect on growth under salinity. In another *in vitro* study, Red pitaya (*Selenicereus polyrhizus* (F.A.C. Web.) Britton & Rose) showed greater tolerance to salinity stress (up to 150 mM NaCl) and less reduction in shoot and root length than white and hybrid pitaya. Increased salinity increased electrolyte leakage and decreased chlorophyll a and membrane

Table 2: Correlation among traits effected by BAP

	Leaves Num.	Shoot Num.	Shoot Length (cm)	FM (g)	AP (mg ml^{-1})	TPC (mg g^{-1})
Leaves Num.	1					
Shoot Num.	0.001**	1				
Shoot Length (cm)	0.001**	0.001**	1			
FM (g)	0.001**	0.001**	0.001**	1		
AP	0.001	0.001	0.001**	0.001**	1	
TPC	-0.023	-0.005	0.001*	0.001**	0.001**	1

*, ** significance in 5 % and 1 %, respectively

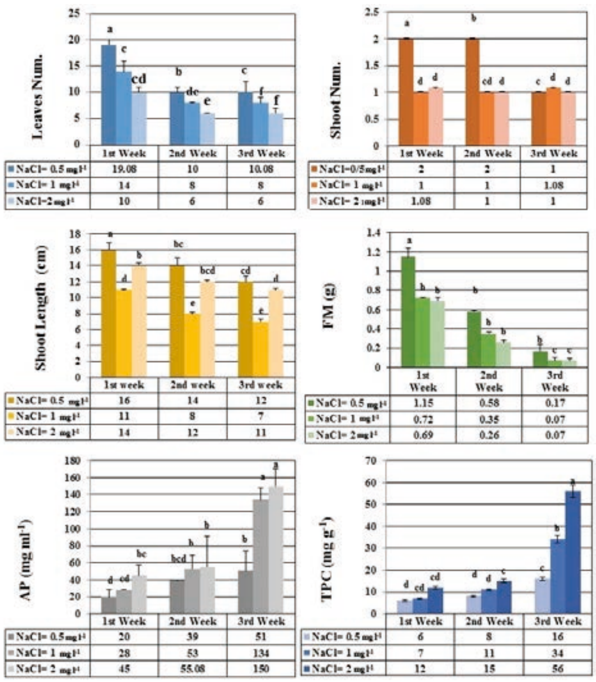

Figure 2. The means comparison of the traits effected by NaCl.

integrity in all species. The results introduce red pitaya as a resistant option for cultivation in saline areas and emphasize the efficiency of *in vitro* culture for screening of salinity tolerance (de Vasconcelos Dias et al., 2025).

In *H. perforatum*, low NaCl concentration increased growth, but high concentration reduced growth traits and increased AP and TPC. This pattern was similar to the results of plants such as *C. annuum* L. and *P. argentea* L., in which salinity reduced growth. In contrast, plants such as red pitaya and paulownia showed greater tolerance to salinity.

During oxidative stress, plants use enzymatic and non-enzymatic antioxidant mechanisms to cope with stress. The key antioxidant enzymes include peroxidase, superoxide dismutase, and catalase. Also, the non-enzymatic antioxidant system can provide several secondary metabolites containing many phenolic compounds (Kumar et al., 2023; Hassanuzzaman et al., 2021; Mohammadhassan et al., 2021; Barzin et al., 2016).

3.3 COLCHICINE

The findings showed that duration significantly affected leaf number, length, FM, AP, TPC ($p < 0.01$), and shoot number ($p < 0.05$). All traits could be significantly influenced by Col × duration ($p < 0.05$). Also, Col significantly changes all traits ($p < 0.01$), but TPC (Table 5).

Furthermore, the mean comparison indicated that

Table 3: Variance analysis of NaCl influence on the traits

		Mean Square					
Source of Variation	df	Leaves Num	Shoot Num.	Shoot Length (cm)	FM (g)	AP (mg ml^{-1})	TPC (mg g^{-1})
Week	2	454**	3**	189**	7**	148957**	19831**
NaCl	2	422**	18**	380**	1**	28851**	4794**
NaCl × Week	4	58**	4**	6**	0.0001**	38220**	6353**
Error	124	315	12	182	0.0001	53756	5298.045
Total	135	12.407	2.56	1.8507	1.9	12.98920	13.5801

*, ** significance in 5 % and 1 %, respectively

Table 4: Correlation among traits influenced by NaCl

	Leaves Num.	Shoot Num.	Shoot Length (cm)	FM (g)	AP (mg ml^{-1})	TPC (mg g^{-1})
Leaves Num.	1					
Shoot Num.	0.001**	1				
Shoot Length (cm)	0.001**	0.001**	1			
FM (g)	0.001**	0.001**	0.001**	1		
AP	0.001**	0.001**	0.001**	0.001**	1	
TPC	0.001**	0.001**	0.001*	0.001**	0.001**	1

*, ** significance in 5 % and 1 %, respectively

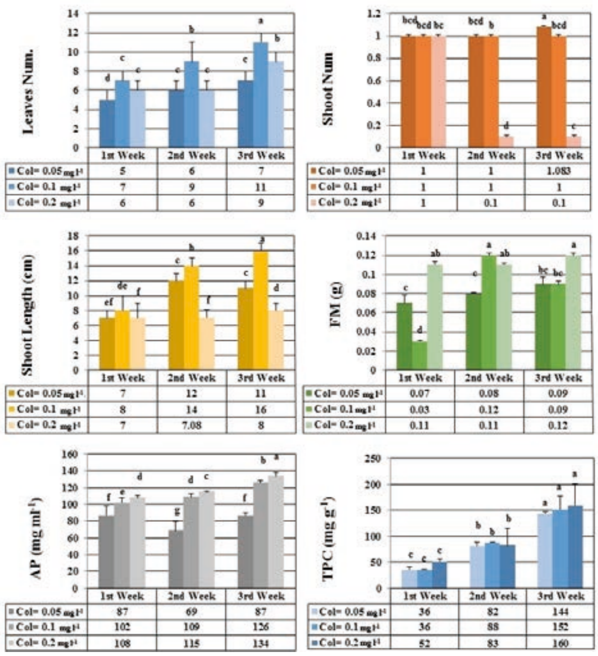

Figure 3: The means comparison of the traits treated by Col.

increasing Col concentrations could raise leaf number and shoot length, then decrease in higher concentrations. The highest and lowest leaf numbers and shoot length were 5 and 11, respectively, caused by 0.05 mg l^{-1} Col × 1st week and 0.1 mg l^{-1} Col × 3rd week, also 7 and 16 cm resulted from 0.05 and 0.2 mg l^{-1} Col ×1st week and 0.1 mg l^{-1} Col ×3rd week. Besides, the lowest shoot number (0.1) was observed in 0.2 mg l^{-1} Col in the 2nd and 3rd weeks. Also, the highest number (1.083) of shoots was measured in 0.05 mg l^{-1} Col×3rd week. There was no change for 0.1 mg l^{-1} Col for 3 weeks. In other measured traits, increasing colchicine concentration and treatment duration were also associated with an increase in FM; However, duration increased and then decreased FM in samples treated by 0.1 mg l^{-1} Col. Consequently, the lowest and highest FM was 0.03 and 0.12 g caused by 0.1 mg l^{-1} × Col 1st week, and 0.1 mg l^{-1} Col ×2nd week and 0.2 mg l^{-1} Col × 3rd week, respectively. At least, Col concentration can enhance AP and TPC over time and individually. Thus, the highest AP (134 mg l^{-1}) and TPC (160 mg g^{-1}) were observed in samples treated with 0.2 mg l^{-1} Col in the 3rd week. Also, the lowest AP (87 mg ml^{-1}) and TPC (36 mg g^{-1}) were caused by 0.5 mg l^{-1} Col × 1st week, as well as 0.1 mg l^{-1} Col in the same duration for TPC (Figure 3).

According to the results, there was a significant correlation between the number of leaves and shoots and other traits. Also, FM was correlated with AP and TPC. The correlations were observed between AP and TPC, as

Table 5: Variance analysis of Col effect on the traits

		Mean Square					
Source of Variation	df	Leaves Num	Shoot Num.	Shoot Length (cm)	FM (g)	AP (mg ml^{-1})	TPC (mg g^{-1})
Week	2	130**	1.08*	248**	0.013**	10034**	218999.024**
Col	2	116**	2**	546**	0.027**	20140**	1783ns
Col × Week	4	30**	2**	155.06**	0.023**	1347**	1910**
Error	124	10414	200.063	18592	1	1704305	212843
Total	134	6.56	6.095	16.97	0.0001	5.7671	3.9272

*, ** significance in 5 % and 1 %, respectively

Table 6: Correlation among Col-treated traits

	Leaves Num	Shoot Num.	Shoot Length (cm)	FM (g)	AP (mg ml^{-1})	TPC (mg g^{-1})
Leaves Num.	1					
Shoot Num.	0.001**	1				
Shoot Length (cm)	0.001**	0.001**	1			
FM (g)	0.001**	0.001**	-0.051	1		
AP	0.001**	0.001**	-0.021	0.001**	1	
TPC	0.001**	0.001**	0.001*	0.001**	0.001**	1

*, ** significance in 5 % and 1 %, respectively

well as shoot length and TPC ($p < 0.01$); however, shoot length was not correlated with FM and AP (Table 6).

Eng et al. (2021) demonstrated that Col can significantly increase shoot number and length, and leaf number in *Neolamarckia cadamba* (Roxb.) Bosser, although higher Col concentrations showed negative effects on these morphological traits. The same Col effects on FM, shoot length and number were observed in *Dracaena sanderiana* Mast. (Mujib et al., 2023) and *Glycin max* (L.) Merr. (Mangena, 2020).

Çömlekçioğlu and Özden (2020) studied the effects of the different Col concentrations on AP and TPC in *Physalis peruviana* L.. They found that TPC and AP can be enhanced when Col concentration increases. The same results were reported from *in vitro* research studying the effects of Col on AP and TPC in *Lavandula stricta* Delile (Nouri Dashlibroon et al., 2020) and *Nigella sativa* L. (Gupta et al., 2021).

There are many studies reporting growth regulatory function for Col, as well as phytohormones. Moreover, other studies indicated that low Col concentration could improve morphological traits (Abd El-Latif et al., 2018); however, it has not revealed how Col can influence AP, TPC, and secondary metabolites production (Mangena and Mushadu, 2023), although it might be related to the cytotoxicity, mutation, and tubulin-binding ability of high Col concentrations (Eng and Ho, 2019; Manzoor et al., 2019).

4 CONCLUSIONS

It could be concluded that 1 mg/l BAP can increase the studied morphological traits of *H. perforatum*, for 21 days, whilst 0.25 mg l^{-1} BAP can be more beneficial for higher AP and TPC levels in the same duration. Additionally, shoot length and leaves number, shoot number, and FM were respectively enhanced by 0.1, 0.05, and 0.2 mg l^{-1} Col. The production of the highest AP and TPC levels were induced by 0.2 mg l^{-1} Col. On the other hand, the studied morphological traits decrease when the NaCl concentration increases, whereas higher NaCl dosage can produce the highest levels of AP and TPC. The NaCl-treated traits showed a more significant correlation than other treatments.

5 REFERENCES

Abd El-Latif, F. M., El-Gioushy, S. F., Islam, S. E., & Zakry, T. A. (2018). Impact of papaya seed soaking in different BA, colchicine and EMS solutions on germination, growth and chromosomal behaviour. *Asian Journal of Biotechnology and Genetic Engineering*, *1*(1), 1-17. https://doi.org/10.9734/AJBGE/2018/40538.

Albuquerque, B. R., Heleno, S. A., Oliveira, M. B. P., Barros, L., & Ferreira, I. C. (2021). Phenolic compounds: Current industrial applications, limitations and future challenges. *Food & function*, *12*(1), 14-29. https://doi.org/10.1039/D0FO02324H.

Alexandri, S., Tsaktsira, M., Hatzilazarou, S., Kostas, S., Nianiou-Obeidat, I., Economou, A., ... & Tsoulpha, P. (2023). Selection for sustainable preservation through *in vitro* propagation of mature *Pyrus spinosa* genotypes rich in total phenolics and antioxidants. *Sustainability*, *15*(5), 4511. https://doi.org/10.3390/su15054511.

Aremu, A. O., Fawole, O. A., Makunga, N. P., Masondo, N. A., Moyo, M., Buthelezi, N. M., ... & Doležal, K. (2020). Applications of cytokinins in horticultural fruit crops: Trends and future prospects. *Biomolecules*, *10*(9), 1222. https://doi.org/10.3390/biom10091222.

Arumugam, G., Sinniah, U. R., Swamy, M. K., & Lynch, P. T. (2020). Micropropagation and essential oil characterization of *Plectranthus amboinicus* (Lour.) Sprengel, an aromatic medicinal plant. *In Vitro Cellular & Developmental Biology-Plant*, *56*, 491-503.https://doi.org/10.1007/s11627-020-10056-1.

Barzin, R., Abbaspour, H., Hajkazemian, M., Mahmoudi, A., & Hassan, R. M. (2016). Study of genetic diversity in oat seeds by using SSR molecular markers. *International Journal of Advanced Biotechnology and Research*, *7*(4), 1493-1497. http://www.bipublication.com/barzin2016/74-1493-97.

Budantsev, A. L., Prikhodko, V. A., Varganova, I. V., & Okovityi, S. V. (2021). Biological activity of *Hypericum perforatum* L.(Hypericaceae): a review. *Pharmacy & Pharmacology*, *9*(1), 17-31. https://doi.org/10.19163/2307-9266-2021-9-1-17-31.

Chatoui, K., Harhar, H., El Kamli, T., & Tabyaoui, M. (2020). Chemical composition and antioxidant capacity of Lepidium sativum seeds from four regions of Morocco. *Evidence-based Complementary and Alternative Medicine*, *2020*, 7302727. https://doi.org/10.1155/2020/7302727.

Çömlekçioğlu, N., & Özden, M. (2020). Effects of colchicine applications and ploidy level on fruit secondary metabolite profiles of goldenberry (*Physalis peruviana* L.). *Applied Ecology & Environmental Research*, *18*(1), 289-302. http://dx.doi.org/10.15666/aeer/1801_289302.

Cortleven, A., Leuendorf, J. E., Frank, M., Pezzetta, D., Bolt, S., & Schmülling, T. (2019). Cytokinin action in response to abiotic and biotic stresses in plants. *Plant, Cell & Environment*, *42*(3), 998-1018. https://doi.org/10.1111/pce.13494.

Cui, L., Liu, Z., Yin, Y., Zou, Y., Faizan, M., Alam, P., & Yu, F. (2023). Research progress of chromosome doubling and 2 n gametes of ornamental plants. *Horticulturae*, *9*(7), 752. https://doi.org/10.3390/horticulturae9070752.

de Vasconcelos Dias, M., Rodrigues, F. A., de Souza Ribeiro, M., Dambroz, C., Dória, J., & Pasqual, M. (2025). Physiological and morphological responses of *Selenicereus* species to salt stress *in vitro*. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *162*(2), 26. https://doi.org/10.1007/s11240-025-03082-7.

Delcheh, K. S., Kashefi, B., & Mohammadhassan, R. (2014). A review optimization of tissue culture medium medicinal plant: Thyme. *International Journal of Farming and Allied Sciences*, *3*(9), 1015-1019. https://ijfas.com/wp-content/uploads/2014/10/1015-1019.pdf.

Eng, W. H., & Ho, W. S. (2019). Polyploidization using colchicine in horticultural plants: A review. *Scientia Horticulturae*, *246*, 604-617. https://doi.org/10.3390/horticulturae9070752.

Eng, W. H., Ho, W. S., & Ling, K. H. (2021). Effects of colchicine treatment on morphological variations of *Neolamarckia cadamba*. *International Journal of Agricultural Technology*, *17*(1), 47-66. https://www.cabidigitallibrary.org/doi/full/10.5555/20210228445.

Etesami, H., Fatemi, H., & Rizwan, M. (2021). Interactions of nanoparticles and salinity stress at physiological, biochemical and molecular levels in plants: A review. *Ecotoxicology and Environmental Safety*, *225*, 112769. https://doi.org/10.1016/j.ecoenv.2021.112769.

Ghasemi-Omran, V. O., Ghorbani, A., & Sajjadi-Otaghsara, S. A. (2021). Melatonin alleviates NaCl-induced damage by regulating ionic homeostasis, antioxidant system, redox homeostasis, and expression of steviol glycosides-related biosynthetic genes in *in vitro* cultured *Stevia rebaudiana* Bertoni. *In Vitro Cellular & Developmental Biology-Plant*, *57*(2), 319-331. https://doi.org/10.1007/s11627-021-10161-9.

Giordano, M., Petropoulos, S. A., & Rouphael, Y. (2021). Response and defence mechanisms of vegetable crops against drought, heat and salinity stress. *Agriculture*, *11*(5), 463. https://doi.org/10.3390/agriculture11050463.

Gracheva, I. A., Shchegravina, E. S., Schmalz, H. G., Beletskaya, I. P., & Fedorov, A. Y. (2020). Colchicine alkaloids and synthetic analogues: current progress and perspectives. *Journal of Medicinal Chemistry*, *63*(19), 10618-10651. https://doi.org/10.1021/acs.jmedchem.0c00222.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, *94*(3), 651-715. https://doi.org/10.1007/s00204-020-02689-3.

Gupta, G., Memon, A. G., Pandey, B., Khan, M. S., Iqbal, M. S., & Srivastava, J. K. (2021). Colchicine induced mutation in plant for the assessment of morpho-physiological and biochemical parameter anti-inflammatory activity. *The Open Biotechnology Journal*, *15*(1), 173-182. http://dx.doi.org/10.2174/1874070702115010173.

Hallmark, H. T., & Rashotte, A. M. (2019). Review–cytokinin response factors: responding to more than cytokinin. *Plant Science*, *289*, 110251. https://doi.org/10.1016/j.tplants.2018.10.012.

Hameed, A., Ahmed, M. Z., Hussain, T., Aziz, I., Ahmad, N., Gul, B., & Nielsen, B. L. (2021). Effects of salinity stress on chloroplast structure and function. *Cells*, *10*(8), 2023. https://doi.org/10.3390/cells10082023.

Hao, S., Wang, Y., Yan, Y., Liu, Y., Wang, J., & Chen, S. (2021). A review on plant responses to salt stress and their mechanisms of salt resistance. *Horticulturae*, *7*(6), 132. https://doi.org/10.3390/horticulturae7060132.

Hasanuzzaman, M., Raihan, M. R. H., Masud, A. A. C., Rahman, K., Nowroz, F., Rahman, M., ... & Fujita, M. (2021). Regulation of reactive oxygen species and antioxidant defense in plants under salinity. *International Journal of Molecular Sciences*, *22*(17), 9326. https://doi.org/10.3390/ijms22179326.

Hemmati, N., Cheniany, M., & Ganjeali, A. (2020). Effect of plant growth regulators and explants on callus induction and study of antioxidant potentials and phenolic metabolites in *Salvia tebesana* Bunge. *Botanica Serbica*, *44*(2), 163-173. https://doi.org/10.2298/BOTSERB2002163H.

Imreova, P., Feruszova, J., Kyzek, S., Bodnarova, K., Zduriencikova, M., Kozics, K., ... & Chalupa, I. (2017). Hyperforin exhibits antigenotoxic activity on human and bacterial cells. *Molecules*, *22*(1), 167. https://doi.org/10.3390/molecules22010167.

Kara, E., Taşkın, H., Shimira, F., Karaköy, T., & Baktemur, G. (2025). Phenotypic responses of *Capsicum annuum* L. to salinity stress under *in vitro* conditions. *Vegetos*, 1-8. https://doi.org/10.1007/s42535-025-01412-w.

Karakas, F. P. (2020). Efficient plant regeneration and callus induction from nodal and hypocotyl explants of goji berry (*Lycium barbarum* L.) and comparison of phenolic profiles in calli formed under different combinations of plant growth regulators. *Plant Physiology and Biochemistry*, *146*, 384-391. https://doi.org/10.1016/j.plaphy.2019.11.009.

Khan, W. U. D., Tanveer, M., Shaukat, R., Ali, M., & Pirdad, F. (2020). An overview of salinity tolerance mechanism in plants. In M. Hassanuzzaman & M. Tanveer (Eds), *Salt and drought stress tolerance in plants: Signaling networks and adaptive mechanisms* (1-16). Cham: Springer. https://doi.org/10.1007/978-3-030-40277-8_1.

Kharel, P., Creech, M. R., Nguyen, C. D., Vendrame, W. A., Munoz, P. R., & Huo, H. (2022). Effect of explant type, culture medium, and BAP concentration on *in vitro* shoot development in highbush blueberry (*Vaccinium corymbosum* L.) cultivars. *In Vitro Cellular & Developmental Biology-Plant*, *58*(6), 1057-1065.https://doi.org/10.1007/s11627-022-10299-0.

Kozak, D., Parzymies, M., Swistowska, A., Marcinek, B., & Pogroszewska, E. (2021). The influence of growth regulators and explant position on the growth and development of *Mandevilla sanderi* (Hemsl.) woodson *in vitro*. *Acta Scientiarum Polonorum - Hortorum Cultus*, *20*(5), 127-138. https://www.cabidigitallibrary.org/doi/full/10.5555/20210502599.

Kulus, D. (2020). Influence of growth regulators on the development, quality, and physiological state of *in vitro*-propagated *Lamprocapnos spectabilis* (L.) Fukuhara. *In Vitro Cellular & Developmental Biology-Plant*, *56*(4), 447-457. https://doi.org/10.1007/s11627-020-10064-1.

Kumar, K., Debnath, P., Singh, S., & Kumar, N. (2023). An overview of plant phenolics and their involvement in abiotic stress tolerance. *Stresses*, *3*(3), 570-585. https://doi.org/10.3390/stresses3030040.

Leandro, V. Ã., de Oliveira Guerra, M., & Peters, V. M. (2017). Effect of the extract of *Hypericum perforatum* on neurodevelopment of regions related to pain control and convulsion. *Journal of Medicinal Plants Research*, *11*(6), 107-117. https://doi.org/10.5897/JMPR2016.6305.

Li, S. M., Zheng, H. X., Zhang, X. S., & Sui, N. (2021). Cytokinins as central regulators during plant growth and stress response. *Plant Cell Reports*, *40*, 271-282.https://doi.org/10.1007/s00299-020-02612-1.

Lima, E. M. F., Winans, S. C., & Pinto, U. M. (2023). Quorum sensing interference by phenolic compounds–A matter of bacterial misunderstanding. *Heliyon, 9*(7), e17657. https://doi.org/10.1016/j.heliyon.2023.e17657.

Liu, Y., Zhang, M., Meng, Z., Wang, B., & Chen, M. (2020). Research progress on the roles of cytokinin in plant response to stress. *International Journal of Molecular Sciences*, *21*(18), 6574. https://doi.org/10.3390/ijms21186574.

Mangena, P. (2020). Research article *in vivo* and *in vitro* application of colchicine on germination and shoot proliferation in soybean [*Glycine max* (L.) Merr.]. *Asian Journal Crop Science*, *12*, 34-42. https://doi.org/10.3923/ajcs.2020.34.42.

Mangena, P., & Mushadu, P. N. (2023). Colchicine-induced polyploidy in leguminous crops enhances morpho-physiological characteristics for drought stress tolerance. *Life*, *13*(10), 1966. https://doi.org/10.3390/life13101966.

Manjusa, A., & Pradeep, K. (2022). Herbal anthelmintic agents: a narrative review. *Journal of Traditional Chinese Medicine*, *42*(4), 641. https://doi.org/10.19852%2Fj.cnki.jtcm.2022.04.007.

Manzoor, A., Ahmad, T., Bashir, M. A., Hafiz, I. A., & Silvestri, C. (2019). Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants. *Plants*, *8*(7), 194. https://doi.org/10.3390/plants8070194.

Marawne, H., Mohammadhassan, R., Mohammadalipour, Z., & Ahmadpour, S. (2022). Valerian (*Valeriana officinalis*) extract inhibits TNF-α and iNOS gene expression in mouse LPS-activated microglial cells. *Traditional Medicine Research*, *7*(5), 47. https://doi.org/10.53388/TMR20220320003.

Mohammadhassan, R., Ferdosi, A., Seifalian, A. M., Seifalian, M., & Malmir, S. (2021). Nanoelicitors application promote antioxidant capacity of *Asparagus officinalis* (*in vitro*). *Journal of Tropical Life Science*, *11*(3), 259–265. http://dx.doi.org/10.11594/jtls.11.03.01.

Mok, M. C. (2019). Cytokinins and plant development—an overview. *Cytokinins*, 155-166. http://dx.doi.org/10.1201/9781351071284-12.

Mujib, A., Aslam, J., & Bansal, Y. (2023). Low colchicine doses improved callus induction, biomass growth, and shoot regeneration in *in vitro* culture of *Dracaena sanderiana* Sander ex Mast. *Propagation of Ornamental Plants*, *23*, 81-87. https://www.journal-pop.org/2023_23_3_81-87.html.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nazir, U., Gul, Z., Shah, G. M., & Khan, N. I. (2022). Interaction effect of auxin and cytokinin on *in vitro* shoot regeneration and rooting of endangered medicinal plant *Valeriana jatamansi* jones through tissue culture. *American Journal of Plant Sciences*, *13*(2), 223-240. https://doi.org/10.4236/ajps.2022.132014.

Nett, R. S., & Sattely, E. S. (2021). Total biosynthesis of the tubulin-binding alkaloid colchicine. *Journal of the American Chemical Society*, *143*(46), 19454-19465. https://doi.org/10.1021/jacs.1c08659.

Nouri Dashlibroon, H., Khorasaninejad, S., Mousavizadeh, S. J., & Mirjalili, M. H. (2020). Effects of colchicine treatment and polyploidy induction on yield components and some morphological and biochemical characteristics of *Lavandula stricta* Delile. *Iranian Journal of Medicinal and Aromatic Plants Research*, *36*(4), 572-589. https://doi.org/10.22092/ijmapr.2020.341489.2705.

Osman, N. A. E., Shatnawi, M., Shibli, R., Majdalawi, M., Al Tawaha, A. R. M., & Qudah, T. (2021). Salts induced salinity and *in vitro* multiplication of *Paronychia argentea*. *Ecological Engineering & Environmental Technology*, *22*(5), 55-64. http://dx.doi.org/10.12912/27197050/139408.

Oulahal, N., & Degraeve, P. (2022). Phenolic-rich plant extracts with antimicrobial activity: an alternative to food preservatives and biocides? *Frontiers in Microbiology*, *12*, 753518. https://doi.org/10.3389/fmicb.2021.753518.

Parcheta, M., Świsłocka, R., Orzechowska, S., Akimowicz, M., Choińska, R., & Lewandowski, W. (2021). Recent developments in effective antioxidants: The structure and antioxidant properties. *Materials*, *14*(8), 1984. https://doi.org/10.3390/ma14081984.

Ramedani, B., Akhavan, S., Mohammadhassan, R., Tutunchi, S., & Khazaei, A. (2015). Evaluation of DISC1 gene rs3738401 polymorphism in iranian parkinson patients affected by type 2 diabetes. *Bulletin of Environment, Pharmacology and Life Sciences*, *4*, 20-23. https://bepls.com/beplssept2015/5.pdf.

Saleh, B. (2023). GC/MS Analysis of *Hypericum perforatum* L.(Hypericaceae) Species. *Journal of Stress Physiology & Biochemistry*, *19*(2), 25-33. https://cyberleninka.ru/article/Saleh2023/192-2533.

Salem, J., Hassanein, A., El-Wakil, D. A., & Loutfy, N. (2022). Interaction between growth regulators controls *in vitro* shoot multiplication in *Paulownia* and selection of NaCl-tolerant variants. *Plants*, *11*(4), 498. https://doi.org/10.3390/plants11040498.

Shamilov, E. N., Abdullaev, A. S., Shamilli, V. E., & Azizov, I. V. (2019). Antiradiation properties of extracts from *Hypericum perforatum* L.. *Faktori Eksperimental'noi Evolucii Organizmiv*, (24), 313-316. https://doi.org/10.7124/FEEO.v24.1121.

Su, Y., Wei, M., Guo, Q., Huang, J., Zhao, K., & Huang, J. (2023). Investigating the relationships between callus browning in *Isatis indigotica* Fortune, total phenol content, and PPO and POD activities. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *155*(1), 175-182.https://doi.org/10.1007/s11240-023-02567-7.

Suryawanshi, M. V., Gujarathi, P. P., Mulla, T., & Bagban, I. (2024). *Hypericum perforatum*: a comprehensive review on pharmacognosy, preclinical studies, putative molecular mechanism, and clinical studies in neurodegenerative diseases. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 1-16. https://doi.org/10.1007/s00210-023-02915-6.

Tariq, S., Wani, S., Rasool, W., Shafi, K., Bhat, M. A., Prabhakar, A., ... & Rather, M. A. (2019). A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. *Microbial Pathogenesis*, *134*, 103580. https://doi.org/10.1016/j.micpath.2019.103580.

Velingkar, V. S., Gupta, G. L., & Hegde, N. B. (2017). A current update on phytochemistry, pharmacology and herb–drug interactions of *Hypericum perforatum*. *Phytochemistry Reviews*, *16*, 725-744. https://doi.org/10.1007/s11101-017-9503-7.

Verma, S. K., Gantait, S., Mukherjee, E., & Gurel, E. (2022). Enhanced somatic embryogenesis, plant regeneration and total phenolic content estimation *in Lycium barbarum* L.: a highly nutritive and medicinal plant. *Journal of Crop Science and Biotechnology*, *25*(5), 547-555.https://doi.org/10.1007/s12892-022-00150-8.

Wu, W., Du, K., Kang, X., & Wei, H. (2021). The diverse roles of cytokinins in regulating leaf development. *Horticulture Research*, *8*, 118. https://doi.org/10.1038/s41438-021-00558-3.

Wybouw, B., & De Rybel, B. (2019). Cytokinin–a developing story. *Trends in Plant Science*, *24*(2), 177-185. https://doi.org/10.1016/j.plantsci.2019.110251.

Yuling, L. I., Shaobo, Y. A. N., Xiuhong, M. A. O., Cuiyan, W. A. N. G., Jinna, W. A. N. G., Cuilan, L. I. U., ... & Yanhui, Q. I. A. O. (2022). Polyploidy induction by colchicine in forest trees: Research Progress. *Journal of Agriculture*, *12*(8), 55. https://doi.org/10.11923/j.issn.2095-4050.cjas2021-0077.

Zeb, A. (2020). Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry*, *44*(9), e13394. https://doi.org/10.1111/jfbc.13394.