

The efficacy of allelopathy of select lichens on antioxidant potential and multiplication of *Ceratophyllum demersum* L.

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Research Article

Keywords: allelopathic interactions, antioxidative properties, lichen extracts, plant tissue cultivation

Posted Date: April 30th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4298885/v1>

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Version of Record: A version of this preprint was published at Plant Cell, Tissue and Organ Culture (PCTOC) on October 15th, 2024. See the published version at <https://doi.org/10.1007/s11240-024-02894-3>.

Abstract

We aimed to investigate growth-promoting effect of *Dermatocarpon miniatum* and *Parmelia saxatilis* lichens on *Ceratophyllum demersum* propagated *in vitro*. Furthermore, we revealed antioxidant capacity variation in lichen-treated plant (allelopathic plant). *C. demersum* was subjected to methanol and water extracts from lichens, specifically *D. miniatum* and *P. saxatilis*. The most positive growth outcomes were witnessed when *C. demersum* was subjected to a methanol extract derived from *P. saxatilis*. We further examined DPPH scavenging and metal chelating activities, as well as total phenol and flavonoid contents, of the most robust allelopathic plants compared to control plants. Based on IC₅₀ values, allelopathic plants exhibited greater efficacy in terms of DPPH scavenging and metal chelation activities in comparison with control. When we analyzed total phenols and flavonoids, both allelopathic plant samples, treated with both extracts, exhibited significantly higher levels ($p < 0.05$) compared to control. Methanol extract from allelopathic sample had maximum total phenol content (73.93 µg gallic acid equivalent/mg extract) and flavonoid content (66.26 µg quercetin equivalent/mg extract). In conclusion, this study will provide valuable input for future investigation in domain of plant development control and augmentation of biologically active substance propagation in plants, with a particular focus on utilizing *P. saxatilis* lichen.

Introduction

In plant allelopathic interactions, chemical compounds secreted by a plant into its environment can affect the growth, germination or development of other plants. The strategy of providing competitive advantage or domination among plants occurs through these compounds called allelochemicals (Kato-Noguchi and Kato 2023). Plants can secrete allelochemicals from their roots, leaves or other organs. These chemicals can be toxins or growth stimulators that plants secrete into their environment (Mushtaq and Siddiqui 2018). Allelopathy, which is an important factor in the environmental interactions of plants, contributes to agriculture, horticulture and natural ecosystem processes (Ratnadass et al. 2012). At this point, the relationship between increasing the production yield of commercially important plants and allelopathy is important in terms of managing and optimizing inter-plant interactions (Aci et al. 2022; Khamare et al. 2022; Lin et al. 2022). Allelopathy can be integrated into plant breeding strategies and sustainable farming practices to increase the yield of commercially important plants. Allelopathy offers potential advantages in areas such as managing competition, disease and pest control, soil fertility and plant development (Ehrmann and Ritz 2013; Hussain et al. 2022).

One of the commercially important plants is *Ceratophyllum demersum* L., an aquatic plant. This plant is used in areas such as aquarium hobby and aquatic plant breeding. Its underwater floating leaves and branches can enhance the aquarium's appearance and aesthetics. It has the ability to improve water quality by absorbing nutrient salts and organic matter in aquatic environments (Lombardo and Dennis Cooke 2003; Hendriks et al. 2023). This feature is useful for maintaining the balance and health of aquatic ecosystems. It can absorb nutrients such as excess nitrate and phosphate in the water. It is important for cleaning the aquarium water and keeping algae growth under control (Kuntz et al. 2014;

Xing et al. 2020; Babur et al. 2021). Considering the pharmacological properties of *C. demersum*, its wound healing (Taranhalli et al. 2011), antioxidant (Karatas et al. 2015), acetylcholinesterase enzyme inhibitor (Murat et al. 2009), antimicrobial (Arya et al. 2022) and cytotoxic (Awati et al. 2021) effects come to the fore.

Thanks to the propagation of plants with tissue culture, mass production can be started. Plant growth regulators come to the fore in the tissue culture and propagation process of plants with pharmacological and commercial importance (Sanyal et al. 2022). At this point, organisms with allelopathic potential are noteworthy. Lichens, which have very different allelochemicals, are unique organisms that form a symbiotic relationship between a fungus and one or more photosynthetic organisms (Kalra et al. 2021). They can affect plants and other organisms by secreting chemicals into their environment. These chemicals can inhibit or stimulate the growth of plants (Gautam et al. 2021). In addition, lichens have various pharmacological activities such as antibacterial (James et al. 2023), antifungal (Rajendran et al. 2023), antioxidant (Emsen 2019), anti-inflammatory (Poulsen-Silva et al. 2023), antiviral (Makarevich et al. 2023) and antitumor (Emsen et al. 2019) in traditional medicine and modern drug development processes, thanks to the allelochemicals they carry. In addition, the activities of lichens such as herbicide (Gazo et al. 2019) and insecticide (Emsen et al. 2015) reveal their activities in the agricultural field. As far as we know, the efficacy of allelopathy of select lichens, which contain such effective chemicals, on *C. demersum*, an important commercial and medicinal plant, has not been investigated until now. In this research, our goal was to assess how two types of lichen, *Dermatocarpon miniatum* (L.) W. Mann and *Parmelia saxatilis* (L.) Ach., affect propagation of *C. demersum* by employing plant tissue culture methods. Furthermore, we explored how *P. saxatilis*, known for its stronger allelopathic influence, influences the antioxidant capacity of *C. demersum*.

Materials and methods

Initial extraction phase

Samples of *D. miniatum* and *P. saxatilis* lichens were initially finely pulverized using a high-speed centrifuge mill (Retsch ZM 200-Germany). Subsequently, they were obtained using methanol and water solvents in a Soxhlet apparatus (Thermal, Turkey). The obtained extracts were subsequently filtered via filter paper, and the outcome filtrates were densified using a rotary evaporator (IKA-Germany) prior to undergoing lyophilization. This sequence of procedures ultimately produced crude extracts.

Plant samples and explant selection

Sterile plant materials (*C. demersum*) utilized in the research were sourced from the Department of Biology, Karamanoğlu Mehmetbey University. Nodal explants were extracted from the plants under aseptic conditions. Subsequently, they were transferred to magenta containing growth medium.

Components of the nutrient media

Murashige and Skoog (MS) (Duchefa Biochemie) basal medium, 0.5 mg/L 6-benzylaminopurine (BAP) and 30 g/L saccharose were added to the culture medium. The experiments were conducted in liquid culture medium (without agar). The nutrient medium's pH was meticulously adjusted to 5.6–5.8 utilizing 1 N NaOH or 1 N HCl, followed via sterilization in an autoclave (121°C, 1.2 atm).

Second extraction phase

When *C. demersum* was subjected to various doses of methanol and water extracts from *D. miniatum* and *P. saxatilis*, it demonstrated its most vigorous development when a methanol extract from *P. saxatilis* was applied at 90 mg/L. Based on these findings, we processed samples of *C. demersum* that exhibited optimal development with the lichen treatment (*P. saxatilis* at 90 mg/L - referred to as the allelopathic plant) and those not subjected to lichen treatment (considered as the control plant). The plant specimens were finely pulverized using a high-speed centrifuge mil and subsequently underwent extraction using methanol and water solvents within a Soxhlet apparatus. Afterward, consequent extracts were passed by conventional filtration paper, and the resultant filtrates were densified using a rotary evaporating machine to generate raw extracts.

Inclusion of lichen extracts at various doses into the nutrient media

Lichen extracts were obtained through the utilization of methanol and water resolvers. To introduce lichen extracts into the media, a stock solution of lichen extract was initially prepared. Subsequently, they were introduced into the media at varying doses. After sterilization using filtration, the lichen extracts were incorporated into the medium. Control groups were also established in trials that omitted the use of lichen extracts. The aseptic nutrient media infused with lichen extracts at diverse doses were then transferred to the magentas and shoot regeneration trials were initiated from the explants.

Cultivation requirements and growth data

The magentas with explants were maintained in a growth chamber with a 16-hour lighting and 8-hour dark cycle. The thermal conditions were carefully maintained at $24 \pm 1^\circ\text{C}$. Growth datum was collected from shoots that developed from explants applied with lichen extracts over specific time intervals. These data encompassed parameters such as the frequency of regeneration, the count of shoots, and the mean length of shoots.

Free radical scavenging activity

To assess the ability of methanolic and aqueous extracts derived from both allelopathic and control plants to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, we conducted experiments with varying final extract concentrations: 12.5–400 mg/L. In accordance with the procedure, we introduced 20 μL of each extract into individual wells in a microplate, followed by the addition of 180 μL of DPPH solution (0.06 mM in methanol). The reduction in DPPH free radicals was determined by measuring the absorbance at 517 nm after 60 minutes, with the absence of light. We computed the percentage of

radical capturing efficacy for the extracts utilizing the subsequent formula: Radical capturing efficacy = $[(\text{Control absorbance} - \text{Extract absorbance}) / \text{Control absorbance}] \times 100$.

Chelation of metals

To assess the metal-binding capacity of methanolic and aqueous extracts from both allelopathic and control plants, we performed experiments using various end-stage extracts, specifically 12.5–400 mg/L. Following the established procedure, we introduced 50 μL of each extract into separate wells within a microplate. Afterward, we added 185 μL of water, 5 μL of FeCl_2 (2 mM), and 10 μL of ferrozine (5 mM) to these wells. The mixture was then kept at room temperature for 10 minutes, and spectrophotometric measurements were taken at 562 nm. The metal chelating activities of the extracts were calculated as a percentage using the formula: Metal chelating activity = $[(\text{Control absorbance} - \text{Extract absorbance}) / \text{Control absorbance}] \times 100$.

Determination of total phenol content

To determine the overall phenolic components in the extracts from both allelopathic and control plants, we employed gallic acid as a reference material. In this process, we placed 20 μL of the extracts (each at a concentration of 400 mg/L) and the gallic acid standard into separate microplate wells. Subsequently, we introduced 20 μL of 2N Folin solution and allowed the combination to incubate in darkness for a duration of 3 minutes. Subsequently, we introduced 20 μL of 35% (w/v) sodium carbonate and 140 μL of dH_2O to the wells and left them in the dark for 10 minutes. We then conducted spectrophotometric readings at 725 nm. The calculation of phenolic content in gallic acid equivalent (GAE) was performed using a standard calibration curve created with gallic acid.

Determination of total flavonoid content

For quantifying the overall flavonoid content in methanolic and aqueous extracts derived from both allelopathic and control plants, we employed quercetin as a benchmark. In this procedure, we introduced 50 μL of the extracts (each at a concentration of 400 mg/L) along with the quercetin standard into distinct microplate wells. Following this, we added 215 μL of 80% (v/v) ethyl alcohol, 5 μL of aluminum nitrate (at 10%, w/v), and 5 μL of 1M potassium acetate to the microtiter plates, and incubated them at room temperature for 40 minutes. Subsequently, we conducted spectrophotometric readings at 415 nm. The calculation of flavonoid content in quercetin equivalent (QE) was performed using a standard calibration curve created with quercetin.

Statistical analyses

Statistical analysis was performed for *in vitro* multiplication and antioxidant experiments using a one-way ANOVA test (Duncan). Probit model was employed to determine the values of the median inhibitor concentration (IC_{50}). Three-dimensional (3-D) density analysis was used to determine the correlations between data points. Heatmap and cluster analyses were used to group data points that share similar characteristics or are close to each other. All of these analytical procedures were conducted using SPSS software (version 21.0, IBM Corporation, Armonk, NY, USA).

Results

Methanol and water extracts applications of *D. miniatum*

The impact of *D. miniatum* on plant formation of *C. demersum* was examined by using methanol and water extracts (Fig. 1a). The highest regeneration frequency (100%) was obtained in the application of 70-90 mg/L methanol extract. The highest regeneration (100%) in MS nutrient medium containing water extract was determined in 50-70 and 100 mg/L applications. On the other hand, the lowest regeneration value (80%) in all lichen extract concentrations was observed in 100 mg/L methanol extract.

The effects of methanol extracts on the amount of shoot were examined and statistically significant differences were found at $p < 0.05$ (Fig. 1b). The maximum number of shoot (150.67) was determined in 80 mg/L methanol concentration (Fig. 2a and b). While the count of shoots increased in culture media containing methanol extract up to 80 mg/L, the use of methanol extract more than this value decreased the number of shoots. The least number of shoots was identified at 100 mg/L methanol extract. The maximum shoot number (127.75 shoots/explants) was obtained at 90 mg/L in culture medium treated with water extract (Fig. 2c and d). As a result of lower rate water extract applications, few shoots were formed. The concentration of 50 mg/L resulted in the lowest number of shoots (88.47 shoots per explant).

Shoot lengths were affected by different methanol extracts ($p < 0.05$) (Fig. 1c). While the longest shoot (4.52 cm) was obtained at 90 mg/L methanol extract, The most compact shoot was identified in the control group applications (1.35 cm). Water extracts were investigated on plant length values. The longest shoot was obtained in the application of 100 mg/L water extract (4.88 cm). In general, the application of water extract at high concentrations had a positive effect on shoot length values.

Methanol and water extracts applications of *P. saxatilis*

The influences of methanol and water extracts of *P. saxatilis* at different concentrations on micropropagation of *C. demersum* were examined (Fig. 3a). Shoot regeneration percentages were significant at the $p < 0.05$ level. In methanol extract applications, 100% shoot regeneration percentage was recorded at 80-100 mg/L. In water-based applications, shoot regeneration values were found to be high.

The impact of methanol extracts from *P. saxatilis* on the count of shoots was assessed (Fig. 3b) At a lichen concentration of 90 mg/L, the highest shoot count per individual plant segment (189.13 shoots/explant) was observed (Fig. 4a and b). Limited shoot growth was achieved with low methanol extract concentrations. The lowest shoot count was recorded in the cultures without lichen extract at 112.73 shoots/explant, subsequently the application of 50 mg/L lichen, resulting in 124.70 shoots/explant.

Different water extracts had significant effects on plant number ($p < 0.05$). The highest shoot number was recorded in plants in the MS media without any water extract applied. In addition, as the water

extract concentration in the culture increased, the number of shoots decreased. When the cultures with water extracts were compared, the maximum shoot number (94.45 shoot/explant) was observed at 50 mg/L (Fig. 4c and d), while the minimum shoot count (45.20 shoot/explant) was found at 100 mg/L.

The study delved into the influences of methanol and water extracts of *P. saxatilis* on shoot length data, as depicted in Fig. 3c. The utmost shoot length of 4.86 cm was achieved using a 50 mg/L water extract. As water extract concentrations increased, there was a subsequent decline in shoot length. Regarding methanol extract applications, the most extended shoot emerged at 90 mg/L (1.54 cm), while the shortest shoot length was discerned at a concentration of 50 mg/L (1.27 cm). Overall, water extract applications tended to yield longer shoots compared to methanol extract applications.

Assessing DPPH scavenging abilities of the extracts

When evaluating the DPPH scavenging capabilities within methanol and water extracts from both allelopathic and control plants, it was observed that the activity levels of these extracts increased as their concentrations rose. Notably, in the experiments involving the methanol extract, it was found that the allelopathic plants exhibited superior activity in the context of the control. Within the allelopathic plants, tests conducted at concentrations of 200 and 400 mg/L showed DPPH scavenging percentages of 85.18% and 86.96%, respectively. While these two data did not differ statistically ($p > 0.05$) compared to each other, they had a significantly ($p < 0.05$) higher potential than all other applications in the control group. Considering the trials with water extract, the maximum concentration treatments in the allelopathic and control plant trials showed 92.77% and 91.93% activity, respectively, and these two values were statistically indifferent ($p > 0.05$). The DPPH scavenging activity (89.59%) resulting from the 200 mg/L trial of the allelopathic plant was significantly higher ($p < 0.05$) compared to the control treatment (Fig. 5a). When taking into account the IC_{50} values, which were used to gauge the effectiveness of various concentration applications, it was found that the water extract from the allelopathic plant demonstrated the highest efficacy, registering a data of 53.60 mg/L (Table 1).

Chelation of metals by the extracts

Evaluating the metal-binding capabilities of methanol and water extracts from both allelopathic and control samples indicated that all applications of these extracts demonstrated increased activity as their concentration levels rose. In particular, upon analyzing the experiments with the methanol extract, it was ascertained that the allelopathic samples demonstrated greater activity when contrasted with control. Within the allelopathic plants, the 400 mg/L trial attracted attention as the most effective chelator in the entire experimental group with its metal chelating activity of 70.27%. Again, the applications of 100 and 200 mg/L of the same group revealed high levels in the metal chelation experimental group with their activities of 63.36% and 64.56%, respectively. Furthermore, it's worth noting that there was no statistically significant difference between these two sets of data ($p > 0.05$). In summary, every concentration of allelopathic samples displayed a notably increased capacity for metal chelation activity in comparison to the control ($p < 0.05$). The 400 mg/L sample of the allelopathic plant showed the highest activity (74.80%) in water extract applications. Among allelopathic samples,

applications of 50-400 mg/L contained significantly ($p < 0.05$) higher data compared to the control group, while the activities elicited by the 12.5 and 25 mg/L concentrations of the allelopathic and control groups were not statistically ($p > 0.05$) different (Fig. 5b). When taking into account the calculated IC_{50} values, it was determined that the lowest value, specifically 48.20 mg/L, was associated with the water extract derived from the allelopathic plant (Table 2).

Analysis of antioxidant compounds of the extracts

In terms of total phenol and total flavonoid contents, when methanol and water extracts of allelopathic and control plants were compared within themselves, allelopathic samples had higher data than the control. When the total phenol content was examined, the highest ratio (73.93 $\mu\text{g GAE/mg extract}$) belonged to the methanol extract of the allelopathic plant. The difference between the phenolic compound ratios of the samples was significant ($p < 0.05$).

Considering the total flavonoid content, the highest rate (66.26 $\mu\text{g QE/mg extract}$) was in the methanol extract obtained from the allelopathic sample, similar to the phenol content analysis, and all data were statistically ($p < 0.05$) different from each other (Table 3).

Analysis of heatmap, cluster, and 3-D density

We used heatmap, cluster, and 3-D density assays to determine the relationships between the antioxidant activities (DPPH scavenging and metal chelating) elicited by control and allelopathic plants. When evaluated at different concentrations, the DPPH scavenging and metal chelating activities at 200 and 400 mg/L concentrations were closely related and exhibited a steep gradient. Similarly, a close but less pronounced gradient was observed in the data at 12.5 and 25 mg/L concentrations (Fig. 6a).

When comparing the concentrations of control and allelopathic plants, it was noted that the methanol-extracted allelopathic plant exhibited a steeper gradient in DPPH scavenging activity at 100 and 200 mg/L concentrations. In the water extract, it was evident that the allelopathic plant at the 100 mg/L concentration exhibited significantly higher DPPH scavenging activity (Fig. 6f). When similar evaluations were performed for metal chelating activity, the most noticeable gradient difference was observed in the experiments conducted with 50 and 100 mg/L concentrations with the methanol extract. In these experiments, the allelopathic plant demonstrated higher activity compared to the control (Fig. 6g).

Based on the IC_{50} values of the extracts, when a general assessment was made using heatmap and cluster analyses, it was observed that the methanol extract of the control plant formed a distinct cluster for both DPPH scavenging and metal chelating activities (Fig. 6b-e).

We employed 3-D density analysis to ascertain correlations between data points in DPPH scavenging and metal chelating activities. Specifically, this is how we identified correlations in regions with dense data points. Accordingly, while a significant relationship ($p < 0.01$) was observed in all treatments, the

strongest correlations were found in water extracts. The correlation coefficients for water extracts of allelopathic and control plants were 0.98 and 0.96, respectively (Fig. 6h-k).

Discussion

Plant tissue culture holds significant importance in agriculture, crop cultivation, genetic engineering, medical applications, and scientific investigations. Thanks to this method, control over the growth and characteristics of plants can be achieved, genetic variations can be produced, and a deeper understanding of the genetic structure of plants can be obtained. In this context, the production of medicinal and commercial plants has important effects in terms of health, economy, environmental sustainability and social welfare (Suman 2017; Gairola et al. 2018; Al-Qudah et al. 2022).

C. demersum is a plant species that plays an important role in aquatic ecosystems and improves water quality. It can also be grown as an ornamental plant in some regions, as it has various uses both ecologically and aesthetically (Wang and Pei 2012; Dai et al. 2014). Reproduction of this plant, which is known for its pharmacological effects as well as its commercial importance, with tissue culture techniques at the desired rate will both increase the yield level and save time (Ozyigit et al. 2023).

Among the different activities of *C. demersum*, its antibacterial effect is an example. Within a research investigation, the antimicrobial potential of silver nanoparticles derived from *C. demersum* was assessed. These silver nanoparticles were tested for their effectiveness against two distinct types of bacteria responsible for spoiling food and water. Notably, the nanoparticles exhibited a significant capability to impede the growth of *Escherichia coli*, a common bacterial strain, as well as *Staphylococcus aureus* (Abdulqahar et al. 2021). Another study on the antimicrobial activity of *C. demersum* was carried out on seventeen different organisms. In the study where chloroform, ethanol, and methanol extracts of *C. demersum* were tested, ethanol extracts were the most effective (Fareed et al. 2008). In a separate study, a group of researchers conducted an investigation. Their focus was on identifying the major components that can be found in the essential oil of *C. demersum*. It was noted that *C. demersum* had the highest number of major components among the studied aquatic plants. During the research, the scientists also predicted the biological activities of major components found in *C. demersum*, including their potential as antineoplastic, anti-inflammatory, antifungal, and antibacterial agents. This abundance of major components suggests that *C. demersum* possesses a remarkable ecological adaptability and potential activity in various ecological interactions, particularly in allelopathic interactions within aquatic ecosystems (Kurashov et al. 2016). There is also evidence showing the cytotoxic potential of *C. demersum*. Awati et al. (2021) demonstrated the anticancer activity of this plant on the HT-29 human colon malignant cell line. In addition to the pharmacological activities of *C. demersum*, its antioxidant capacity also has an important place. The antioxidant capacity of plants is important because antioxidants are essential components that protect the body against damage from free radicals (Akbari et al. 2022; Kok et al. 2023). Free radicals, which are reactive molecules formed during normal cellular activities, are neutralized by antioxidants. Antioxidants can reduce the risk of chronic diseases such as cancer, heart disease, diabetes, and neurodegenerative diseases because

oxidative stress caused by free radicals plays a significant role in the development of these diseases (Sharifi-Rad et al. 2020; Emsen et al. 2023). Moreover, antioxidants that help the immune system function properly can delay skin aging and the signs of aging by reducing cellular damage caused by oxidative stress (Varesi et al. 2022). Studies on the change of antioxidant capacity in the plant are available in the literature. Mishra et al. (2006) reported that when exposed to lead (Pb) for a shorter duration, *C. demersum* exhibited a positive response, leading to the increased production of various antioxidant enzymes. However, when exposed to Pb for longer periods, the activity of these antioxidant enzymes decreased. Similarly, a study conducted by Monferran et al. (2007) demonstrated that 1,2- and 1,4-dichlorobenzenes can trigger the activation of the antioxidant system in *C. demersum*. This activation displays a specific pattern depending on the isomer, indicating that the plant's defense mechanism plays a crucial role in neutralizing reactive oxygen species. This, in turn, aids in safeguarding the organism against the detrimental oxidative effects caused by the tested xenobiotics' prooxidant actions. In a separate research study, scientists examined how the growth and physiological reactions of *C. demersum* were influenced by sodium dodecyl sulfate, phenol, and a combination of both substances. The findings indicated that in response to oxidative stress, *C. demersum* has the capability to trigger its antioxidant defense mechanisms, including the enhancement of superoxide dismutase and peroxidase. Furthermore, when facing simultaneous stressors, the activities of catalase, superoxide dismutase, and peroxidase showed a notable increase compared to exposure to a single stress factor (Liu and Wu 2018).

In this current research, we unveiled the abilities of methanol and water extracts acquired from *C. demersum* to scavenge DPPH radicals and chelate metals. In addition, we analyzed the total phenol and flavonoid contents of the aforementioned extracts. One of our primary objectives was to enhance the plant's antioxidant capacity by leveraging allelopathic interactions. We achieved this aim with the application of *P. saxatilis*, which is a lichen species. Initially, our research revealed that methanol extract of this lichen species had the most significant impact on the development of *C. demersum*. The allelopathic potential of lichens on different plants is known. In a research study, scientists utilized extracts obtained from six different lichens, which contained secondary metabolites. They conducted experiments involving the cultivation of the moss *Physcomitrella patens*, which was treated with these lichen extracts. Notably, usnic acid exhibited a significant impact on cell division in protonemata, suggesting a potent influence on the early stages of bryophyte development due to the presence of allelochemicals found in lichen secondary metabolites (Goga et al. 2017). In a study conducted within a natural ecosystem, it was observed that in challenging environments such as alpine dwarf shrubs and lichen heaths, the presence of lichens and the subsequent enhancement of the local microclimate played a more crucial role in facilitating the gathering of vascular plants. Depending on the specific plant species under investigation, many lichens appeared to promote rather than hinder the initial stages of plant establishment in arctic-alpine heathland regions dominated by lichen growth (Nystuen et al. 2019). Lokajová et al. (2014) examined the possible harmful effects of separate secondary metabolites and combinations of these compounds on the lichen photobiont *Trebouxia erici*, which was cultivated without a symbiotic partner. This evaluation was based on specific physiological and biochemical

criteria. It seems that a majority of the secondary metabolites investigated in this study may act as allelochemicals, regulating the growth of the algal partner within the lichen thallus.

In our study, the antioxidant activities in allelopathic plant samples treated with lichen, *P. saxatilis* were higher than those in control plants. The antioxidant activity of this lichen and the diversity of its bioactive components may contribute to the development and antioxidant capacity of *C. demersum*. Because upon analyzing the overall levels of phenols and flavonoids, higher proportions were observed in allelopathic plants. In a study, it was discovered that the methanol extract from *P. saxatilis* contained larger amounts of phenolic compounds. Similarly, methanol extracts raised the total antioxidant capacity levels in human lymphocytes to a greater extent than water extracts (Emsen and Kolukisa 2021). Likewise, in our investigation, methanol extracts exhibited higher levels of antioxidant compounds when compared to water extracts. In a conducted study that focused on phenolic compounds, the methanol extracts from *P. saxatilis* were found to contain 1.0% (w/w) of total phenolic constituents, expressed as gallic acid equivalent (Gulluce et al. 2006). Again, a study showing that methanol extract of *P. saxatilis* had a high antioxidant potential was carried out by Özen and Kinalioğlu (2008). The methanol extract displayed strong antioxidant activity, which was statistically similar to that of α -tocopherol. In contrast, the aqueous extracts exhibited weak antioxidant activity. In another research, the acetone extract from *P. saxatilis* demonstrated the most significant activity in scavenging free radicals, with an inhibition rate of 55.3%. Additionally, the examined extracts exhibited notable abilities to reduce oxidative stress and scavenge superoxide anion radicals. There were strong correlations observed between the total phenolic and flavonoid contents and the antioxidant effects of the tested extracts (Kosanić et al. 2012). In a research study involving the use of ethanol extracts from *P. saxatilis*, scientists examined the antioxidative properties of these extracts. The results revealed that these extracts effectively countered DPPH radicals, showing a wide range of antioxidative capacities, spanning from 14.76–79.76% in *P. saxatilis*. Furthermore, in *P. saxatilis*, the ability to neutralize DPPH radicals was found to be strongly correlated with the phenol content, with a high correlation coefficient of 0.9981 (Ćilerdžić et al. 2016).

Conclusions

In our research, we discovered that the *D. miniatum* and *P. saxatilis* demonstrated the ability to enhance the growth of the *C. demersum*. Specifically, we observed that *P. saxatilis*, in particular, led to a substantial increase in yield. Additionally, the antioxidant activity and the total phenol and flavonoid content in *C. demersum* plants treated with *P. saxatilis* significantly increased when compared to the control groups. Briefly, this study showed that the allelopathic potential of lichens is a significant factor that influences the functioning of natural ecosystems. A better understanding of this potential can help us comprehend ecosystem health and plant-organism interactions and can aid in the development of sustainable environmental management strategies.

Declarations

Funding This work was financially supported by the Karamanoğlu Mehmetbey University Scientific Research Projects Commission (Project No. 19-YL-20).

Data availability The whole datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

Ethical responsibility The submitted work is original and has not been published elsewhere in any form or language.

Competing interests The authors have no relevant financial or nonfinancial interests to disclose.

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Tables

Table 1 IC₅₀ values (mg/L) of extracts obtained from control and allelopathic plants for scavenging on DPPH radicals

Extract	Treatment	IC ₅₀ (Limits)	Slope ± Standard error (Limits)
Methanol	Control plant	234.05 (203.05–275.39)	1.29 ± 0.07 (1.15–1.43)
	Allelopathic plant	86.43 (79.85– 93.64)	2.01 ± 0.08 (1.85–2.16)
Water	Control plant	100.65 (92.81–109.39)	1.96 ± 0.08 (1.80–2.12)
	Allelopathic plant	53.60 (49.13–58.38)	1.81 ± 0.07 (1.66–1.96)

Table 2 IC₅₀ values (mg/L) of extracts derived from both allelopathic and control plants in relation to their ability to chelate ferrous ions

Extract	Treatment	IC ₅₀ (Limits)	Slope ± Standard error (Limits)
Methanol	Control plant	209.37 (172.28–264.49)	0.86 ± 0.06 (0.74–0.98)
	Allelopathic plant	72.49 (62.44–84.16)	0.93 ± 0.06 (0.81–1.06)
Water	Control plant	80.16 (63.41–102.31)	0.58 ± 0.05 (0.46–0.69)
	Allelopathic plant	48.20 (40.30–56.87)	0.84 ± 0.06 (0.72–0.96)

Table 3 The total phenolic and total flavonoid concentrations (µg/mg) in extracts acquired from allelopathic and control plants.

Extract	Treatment	Total phenol (Gallic acid equivalent)	Total flavonoid (Quercetin equivalent)
Methanol	Control plant	52.40 ± 0.35 b	38.80 ± 0.28 b
	Allelopathic plant	73.93 ± 0.77 a	66.26 ± 0.17 a
Water	Control plant	37.65 ± 0.19 d	2.78 ± 0.17 d
	Allelopathic plant	41.97 ± 0.67 c	5.56 ± 0.06 c

Values that have distinct letters within the same column are significantly different at a confidence level of $p < 0.05$.

Figures

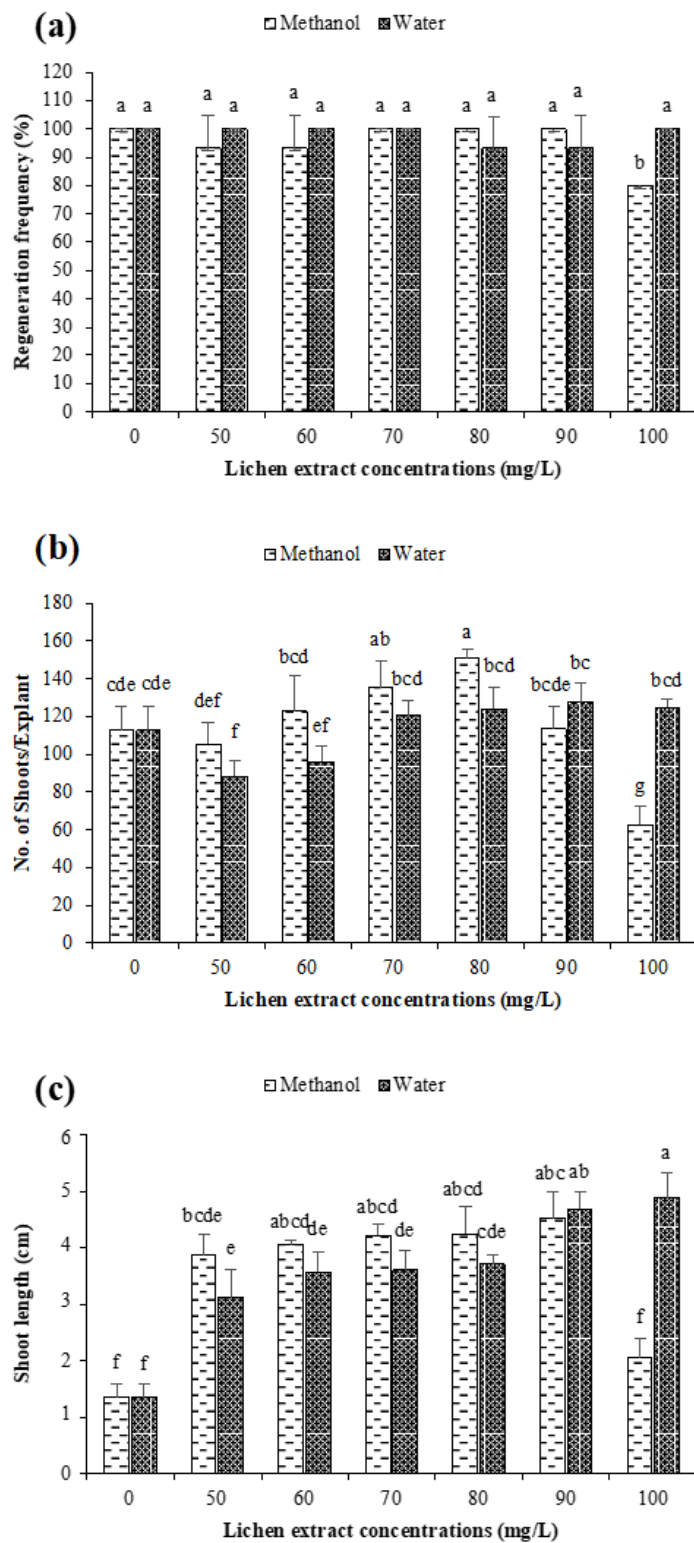


Figure 1

Influence of methanol and water extracts of *D. miniatum* on multiple plant proliferation of *C. demersum*. The impact of applying various extracts on (a) shoot formation frequency, (b) mean shoot count and (c) shoot length. Vertical bars depict the standard deviation observed across three separate experiments. Dissimilar letters are used to indicate statistically significant distinctions ($p < 0.05$).

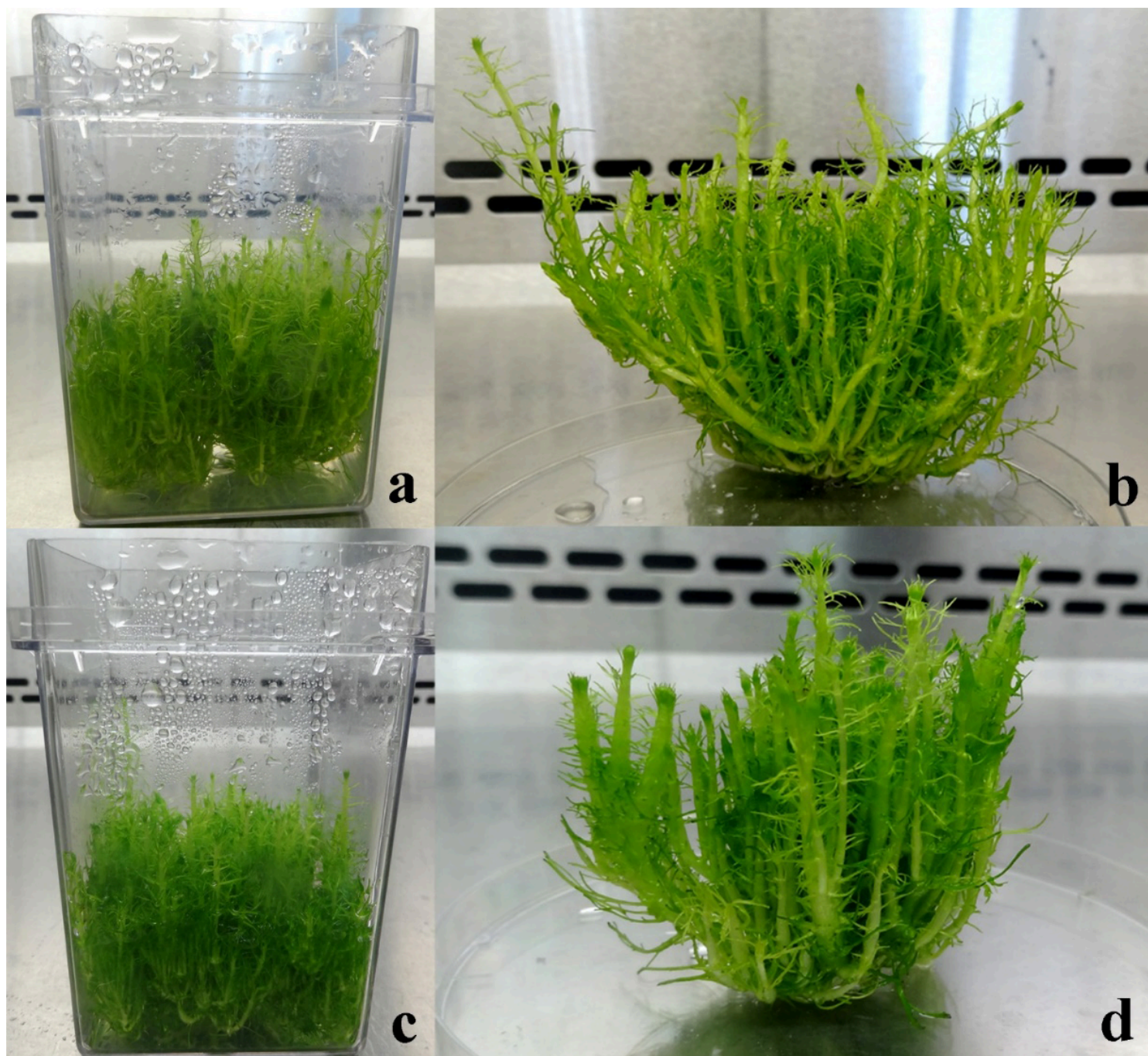


Figure 2

In vitro shoot proliferation of *C. demersum* treated with methanol and water extracts of *D. miniatum*. New-multiple shoot formations in MS medium including **(a, b)** 80 mg/L methanol and **(c, d)** 90 mg/L water extract.

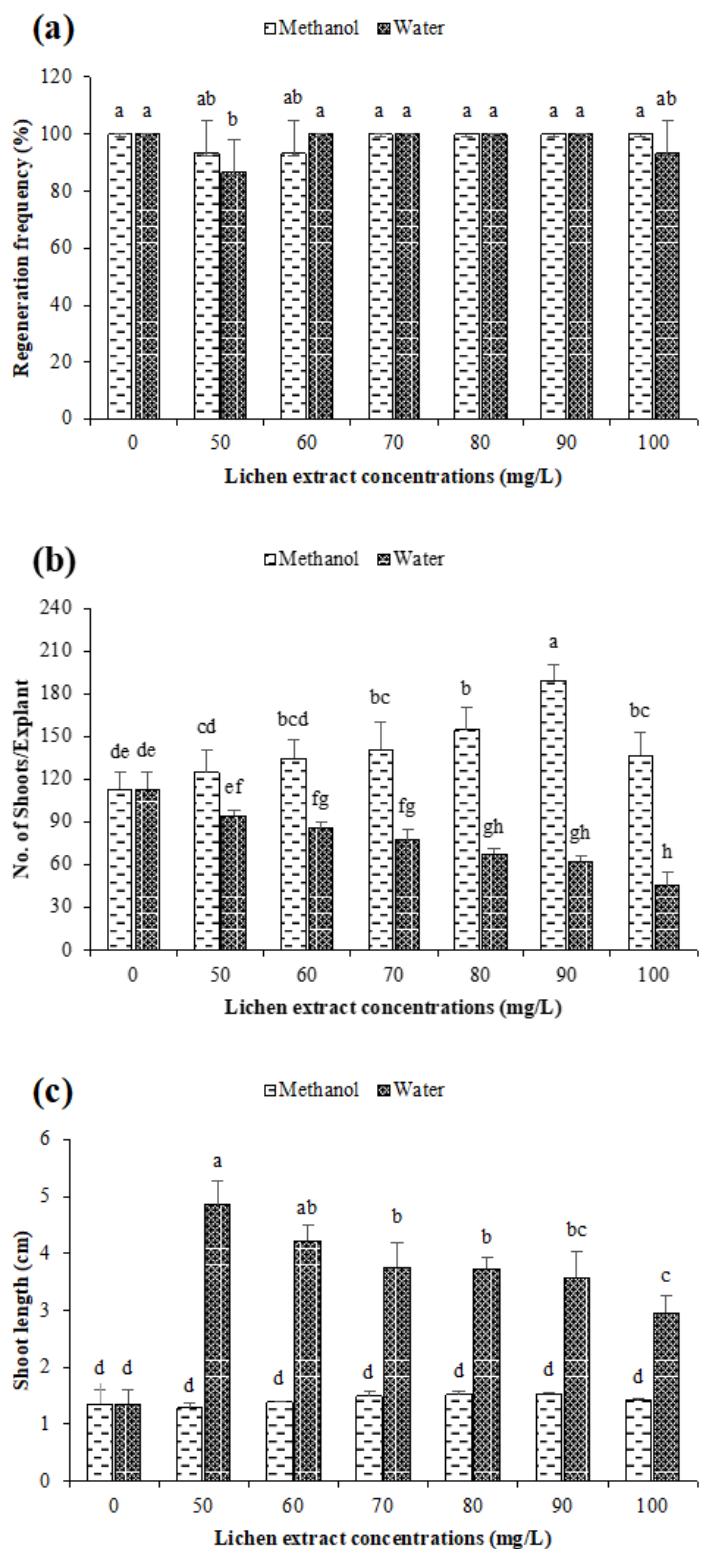


Figure 3

Influence of methanol and water extracts of *P. saxatilis* on multiple plant proliferation of *C. demersum*. The impact of applying various extracts on **(a)** shoot formation frequency, **(b)** mean shoot count and **(c)** shoot length. Vertical bars depict the standard deviation observed across three separate experiments. Dissimilar letters are used to indicate statistically significant distinctions ($p < 0.05$).

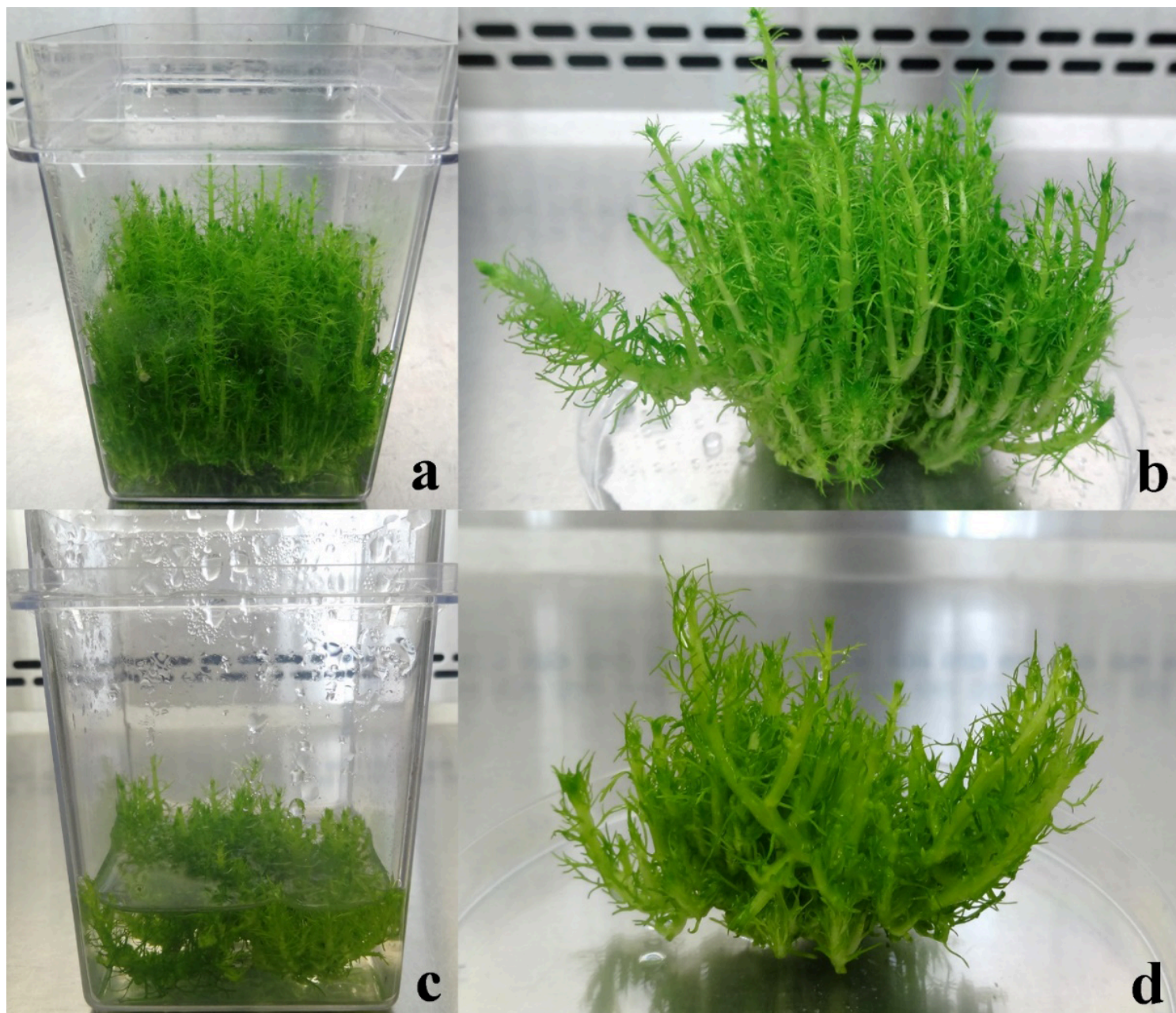


Figure 4

In vitro shoot proliferation of *C. demersum* treated with methanol and water extracts of *P. saxatilis*. New-multiple shoot formations in MS medium including **(a, b)** 90 mg/L methanol and **(c, d)** 50 mg/L water extract.

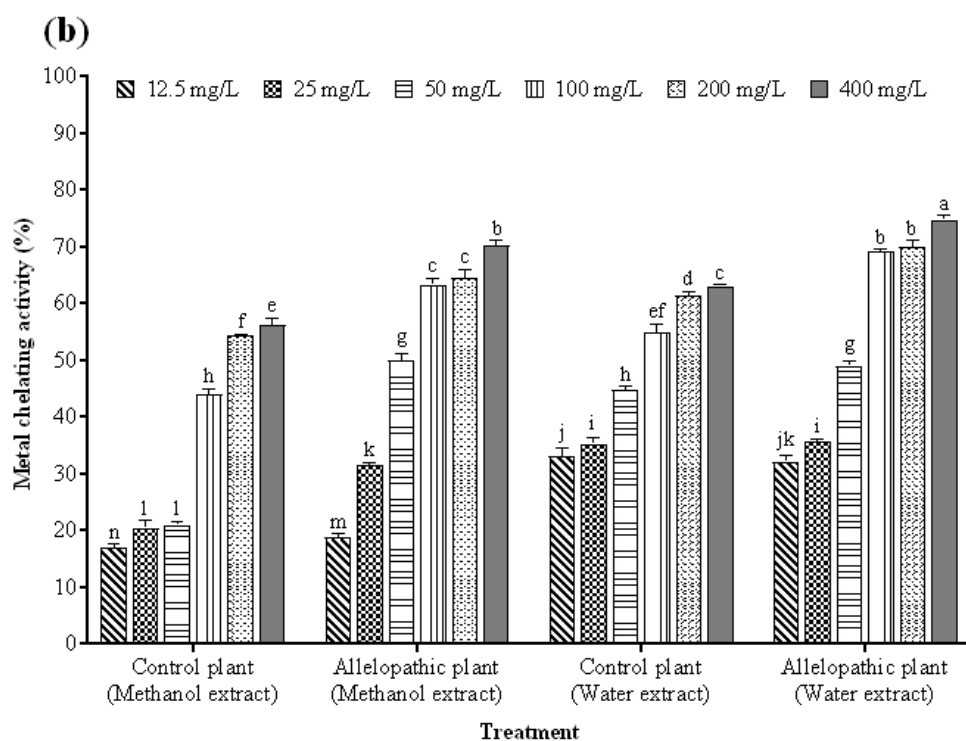
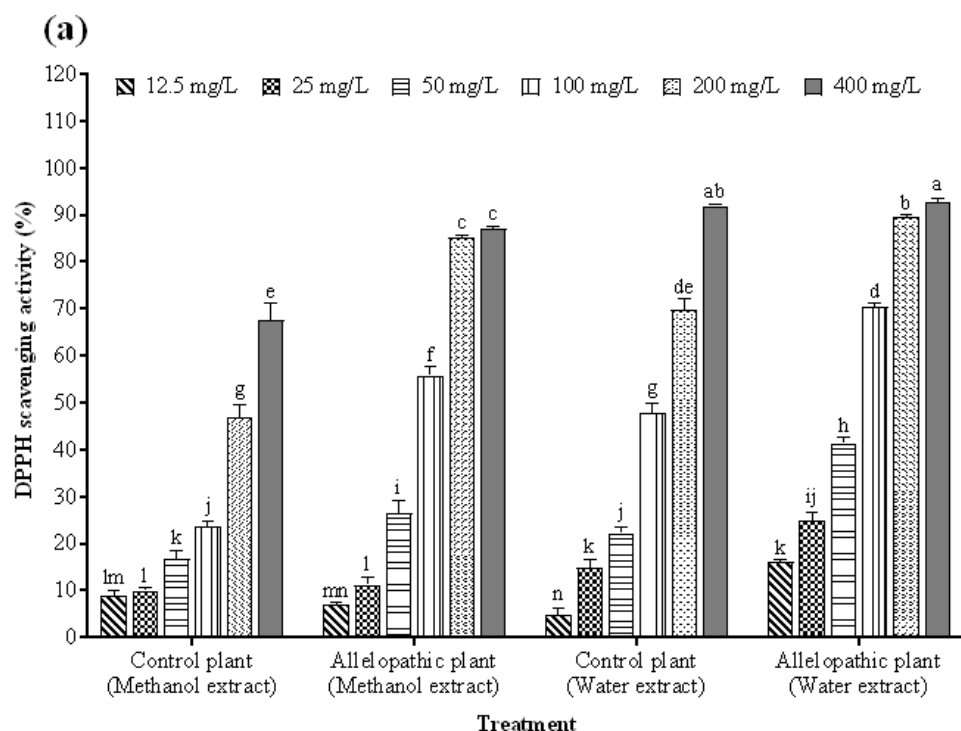


Figure 5

(a) Evaluation of the ability of various extracts from allelopathic and control plants to neutralize DPPH radicals, and **(b)** assessment of their capacity to chelate metals. Vertical bars depict the standard deviation observed across three separate experiments. Dissimilar letters are used to indicate statistically significant distinctions ($p < 0.05$).

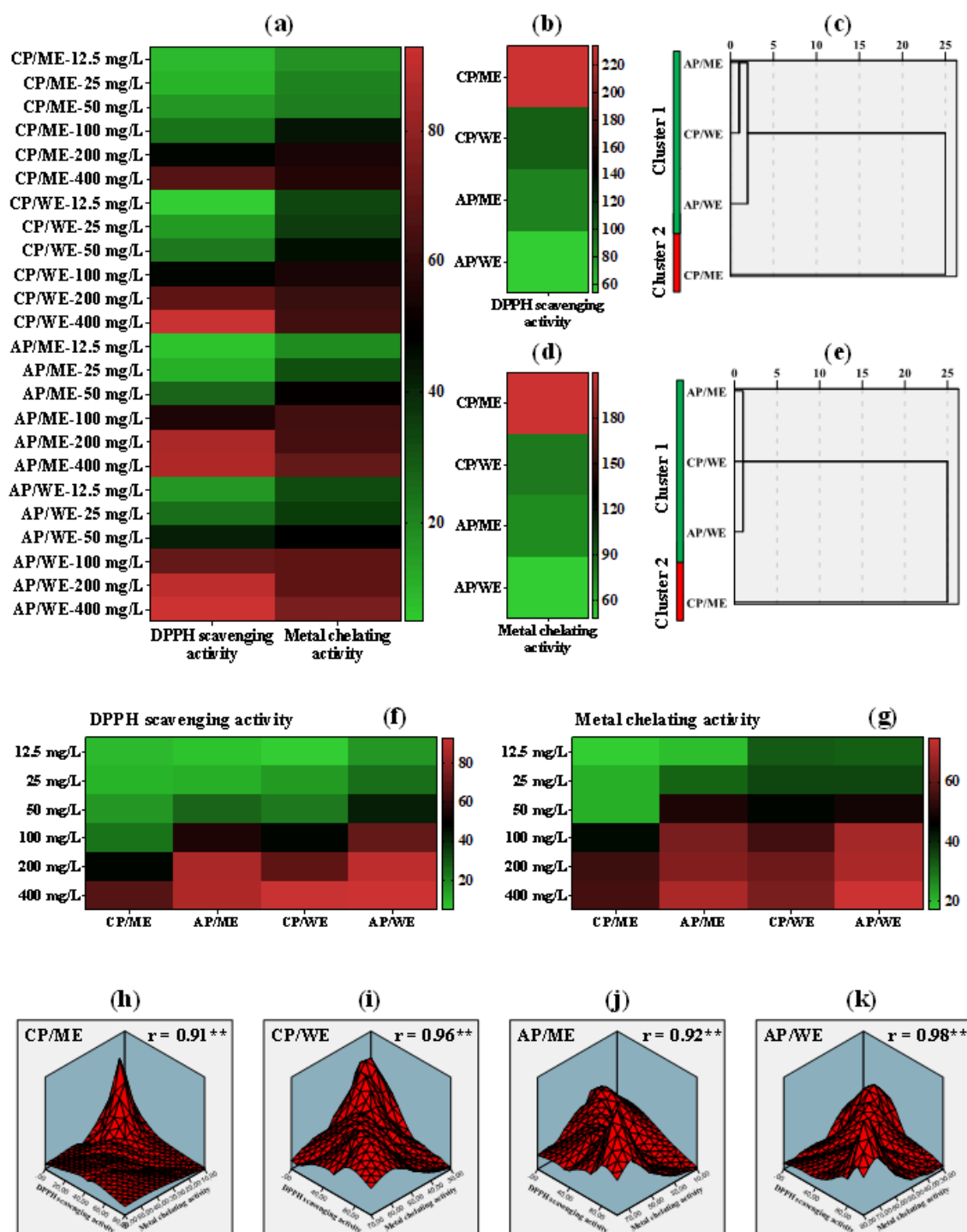


Figure 6

(a, f, g) Heatmaps depicting the percentage of DPPH scavenging and metal chelating activities of different concentrations of the extracts are presented. The heatmaps are based on IC_{50} values for (b) DPPH scavenging and (d) metal chelating activities of the extracts. In the heatmap analyses, high and low activities are indicated by red and green colors, respectively. Dendrograms based on IC_{50} values for (c) DPPH scavenging and (e) metal chelating activities of the extracts are shown. (h, i, j, k) Three-

dimensional density analysis was conducted for DPPH scavenging and metal chelating activities of the extracts. In the 3-D density analysis, correlation coefficients (r) were calculated. The double asterisks (**) emphasize the significance level of 0.01. CP/ME refers to the methanol extract of the control plant, CP/WE refers to the water extract of the control plant, AP/ME refers to the methanol extract of the allelopathic plant, and AP/WE refers to the water extract of the allelopathic plant.