



Machine learning-assisted evaluation of antioxidant and metal chelating capacities in in vitro propagated *Ceratophyllum demersum* L. under different LED light conditions

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Abstract

The aim of this study is to explore the effects of different LED light spectra on the antioxidant capacity of *Ceratophyllum demersum* L. under in vitro culture conditions, using machine learning techniques to predict and analyze the plant's metabolic responses. Both White and Red LEDs achieved 100% shoot regeneration, with Red LED producing the highest shoot count (85.27) and longest shoots (3.16 cm). Additionally, antioxidant analysis showed significant variations in phenolic and flavonoid content based on light and extraction methods. Red LED acetone extracts had the highest phenolic content (63.99 µg GAE/mg), while Blue LED acetone extracts yielded the highest flavonoid content (167.58 µg QE/mg). White LED acetone extracts showed the strongest DPPH scavenging activity (90.14% at 400 µg/mL), indicating broad-spectrum light enhances antioxidants. Metal chelation was highest in White LED water extracts. Numerous machine learning techniques were employed to predict DPPH radical scavenging and metal chelation activities. XGBoost emerged as the top-performing algorithm for DPPH activity prediction, achieving the lowest *MAE* (3.754) and the highest *R*² (0.887), along with one of the lowest *RMSE* values (5.027). MLP (Multilayer Perceptron) also showed strong performance with relatively low *RMSE* (5.528) and *MAE* (4.200) on the test set. For metal chelation activity, Cubist demonstrated the best performance, with the lowest test *RMSE* (5.129) and *MAE* (4.141) values, along with one of the highest *R*² values (0.899). This study highlights the potential of machine learning algorithms in predicting antioxidant activities and the significant impact of light conditions on these activities.

Key message

Machine learning enhances precision in assessing antioxidant capacity of *Ceratophyllum demersum* L., revealing LED light-driven optimization strategies for in vitro culture conditions.

Keywords Artificial intelligence · Antioxidant potential · DPPH activity · *In vitro* culture · Machine learning

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Introduction

Ceratophyllum demersum L. is a widely distributed aquatic plant found in freshwater ecosystems, known for its high resistance to environmental stress conditions (Luengluetham et al. 2023; Zhao et al. 2024). Commonly referred to as coontail (Ali et al. 2023), this plant is recognized for its contributions to ecological balance and holds potential for bioremediation (pollution cleanup) applications (Ugur and Dogan 2024). However, the significance of *C. demersum* extends beyond its ecological role; it also has notable medicinal and pharmacological value (Tokgoz et al. 2024). This plant exhibits antioxidant properties due to the bioactive compounds it contains, playing a protective role against cellular damage caused by oxidative stress (Eliašová et al. 2021; Emsen and Dogan 2018). Oxidative stress refers to the damage caused by free radicals in cells, which can lead to serious health issues over time, such as cancer, diabetes, and cardiovascular diseases (Jomova et al. 2023; Lim et al. 2023). The Fe^{2+} chelation assay measures a compound's ability to bind free iron ions, which is a crucial mechanism contributing to antioxidant activity (Valko et al. 2005). Free Fe^{2+} ions can participate in the Fenton reaction, producing highly reactive hydroxyl radicals ($\bullet\text{OH}$) that cause significant oxidative damage to biomolecules such as lipids, proteins, and DNA. By chelating Fe^{2+} , certain compounds prevent this reaction, thereby reducing oxidative stress. This protective effect helps maintain cellular integrity and prevents damage associated with oxidative stress-related diseases (Zhao 2019; Wang et al. 2021). Additionally, metal-chelating compounds can work synergistically with other antioxidants, enhancing overall antioxidant capacity. For example, polyphenols and flavonoids not only scavenge free radicals but also chelate metal ions, further strengthening the body's defense against oxidative damage. Thus, the Fe^{2+} metal chelation ability of a compound plays a vital role in its overall antioxidant potential (Cherrak et al. 2016; Pan et al. 2022). Antioxidants help combat these free radicals, contributing to the prevention of such diseases (Muscolo et al. 2024). *C. demersum* is rich in antioxidants like phenolic compounds and flavonoids (Awati et al. 2020; Masłyk et al. 2024), making it a natural source with potential health benefits.

Plant tissue culture is a widely used method in the fields of biotechnology and plant physiology, allowing for the cultivation of plant cells or tissues under controlled conditions in a laboratory setting (Loyola-Vargas and Ochoa-Alejo 2024). This technique takes advantage of the totipotent properties of plant cells, enabling them to produce a complete plant (Kose et al. 2021; Loyola-Vargas and Ochoa-Alejo 2024). Tissue culture offers significant advantages, particularly in the production and rapid propagation of

bioactive compounds in medicinal plants (Bapat et al. 2023). Moreover, it is well known that light plays a crucial role in plant development (Miao et al. 2023), and the effects of light sources are increasingly being studied in tissue culture experiments (Cavallaro et al. 2022; Shang et al. 2023).

In recent years, LED (Light Emitting Diode) lights have been frequently used in plant cultivation and tissue culture studies (Ekin and Dogan 2024; Grzyb et al. 2024). The features of LEDs, such as low energy consumption, long lifespan, and the ability to easily adjust different wavelengths, offer significant opportunities to optimize plant growth conditions (Trivellini et al. 2023). Different wavelengths can have varying effects on plant metabolism (Klimek-Szczykutowicz et al. 2022). The use of LED lights in plant tissue culture has the potential to increase the production of bioactive compounds in plants (Klimek-Szczykutowicz et al. 2022; Rocha et al. 2022). Therefore, investigating the effects of LED lights on the antioxidant capacity of *C. demersum* can provide valuable insights into the production of medicinal plants.

Previous studies have investigated the antioxidant properties of *C. demersum* (Awati et al. 2021; Emsen and Dogan 2018; Tokgoz et al. 2024), highlighting its pharmacological significance (Awati et al. 2020, 2021). However, there is no comprehensive study in the literature examining the effects of different LED lights on the antioxidant capacity of *C. demersum* under tissue culture conditions. This gap presents an important research opportunity to better understand the potential effects of controlled light sources like LED lights on this plant and to optimize its antioxidant capacity.

Light plays a vital role in regulating plant metabolism, significantly influencing the production of antioxidant compounds (Hernández-Adasme et al. 2023; Lingwan et al. 2023). The wavelength and intensity of light can activate signaling pathways and defense mechanisms within plant cells, which shapes their responses to oxidative stress (Breen et al. 2023; Babla et al. 2023). LED technology has emerged as an effective means to precisely control these processes. Different LED light conditions can stimulate plant cells to produce a variety of defense compounds, thereby enhancing their ability to combat oxidative stress (Loi et al. 2020; Shi et al. 2023; Livadariu et al. 2023). Consequently, understanding the effects of LED lighting on the antioxidant capacity of plants is crucial for advancements in biotechnology and agricultural practices (Loi et al. 2020; Lazzarin et al. 2021).

Machine learning techniques have gained prominence as powerful tools in plant biotechnology, providing robust capabilities for analyzing intricate biological data and predicting various plant traits (Eftekhari et al. 2024; Aasim et al. 2024; Zhang et al. 2024). These methods facilitate the examination of complex datasets, enabling the identification of patterns and interactions that traditional statistical

methods may overlook (Makridakis et al. 2018; Rajula et al. 2020). While previous research has established various micropropagation techniques for *C. demersum* (Karatas et al. 2014; Dogan 2019), there is a significant opportunity to leverage machine learning-assisted approaches to enhance our understanding of this species. By utilizing *C. demersum* as an in vitro model organism, we can apply advanced machine learning algorithms to analyze complex datasets generated from antioxidant activity assessments under different LED light conditions. This integration allows for more accurate predictions of antioxidant capacities and uncovers intricate patterns and interactions that traditional methods may overlook (Karydas et al. 2020; Kim et al. 2024; Liu 2024).

By focusing on machine learning in the context of *C. demersum* micropropagation, we can optimize cultivation protocols and improve the pharmacological efficacy of its bioactive compounds. The application of machine learning in plant research not only deepens our understanding of how light affects plant antioxidant production but also fosters the development of innovative strategies to enhance the pharmacological potential of medicinal plants. Overall, this approach represents a significant advancement for both agricultural practices and the biotechnological use of plant-derived bioactive compounds (Mahto and Mahato 2024; Alloun and Calvio 2024).

Understanding how different LED light spectra (white, red, and blue) influence the antioxidant capacity of *C. demersum* in vitro can reveal new opportunities to enhance the production of bioactive compounds, ultimately improving their pharmacological efficacy. However, a systematic and quantitative approach is required to elucidate the metabolic responses of *C. demersum* under controlled light conditions. Therefore, this study aims to experimentally assess the impact of different LED spectra on antioxidant capacity using biochemical assays and subsequently apply machine learning techniques to analyze and predict metabolic responses. By combining experimental and computational approaches, we seek to establish a more comprehensive understanding of the relationship between light spectra and antioxidant activity, providing valuable insights for optimizing in vitro cultivation strategies.

Materials and methods

Experimental location and plant material

The experiments were conducted in the Research Laboratories of the Biology Department at Karamanoğlu Mehmetbey University. The LED light experiments were carried out in a temperature-controlled growth chamber. The *Ceratophyllum*

demersum L. used in this study was obtained sterile from the Biology Department of Karamanoğlu Mehmetbey University. The sterilization procedures were performed according to the method previously described by Karatas et al. (2014).

Tissue culture conditions and LED light applications

In the propagation studies, shoot tips were used as the explant source. The experiments utilized a basic nutrient medium solidified with 0.65% agar (Duchefa Biochemie), containing Murashige and Skoog (MS-Duchefa Biochemie) mineral salts and vitamins (Murashige and Skoog 1962) and 3% sucrose (Merck). As a plant growth regulator, 1.0 mg/L Benzylaminopurine (BAP) was added to the nutrient medium. The pH of the medium was adjusted to 5.7 ± 1 using 1 N NaOH or 1 N HCl, and then sterilized at 121 °C under 1.2 atm pressure for 20 min. Shoot tip explants isolated from sterile plants were placed in MS nutrient media containing plant growth regulators for shoot regeneration.

Cultures containing shoot tip explants, prepared in sterile cabinets, were exposed to different LED lights, including white (550 nm, 1500 lx), red (660 nm, 98 lx), and blue (450 nm, 270 lx) LEDs. Additionally, control experiments were conducted under white fluorescent light. The light intensities applied in the experiments were standardized at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ across all light trials. There was a distance of 50 cm between the LED lights and the plants, with the lights fixed to a special shelving system to optimize the setup (Sheibani et al. 2023; Ugur and Dogan 2024). At the end of eight weeks, the experiments were concluded, and data were collected on shoot regeneration percentage, average number of shoots per explant, and shoot length.

Extraction process of plants

After *C. demersum* was exposed to different LED and fluorescent light samples, the relevant plants were ground into powder with an ultracentrifugal grinder (Retsch ZM 200, Germany) and then subjected to extraction with acetone and water solvent in a Soxhlet (Termal, Turkey) device. The extracts were filtered on ordinary filter paper. The obtained filtrates were concentrated with a rotary evaporator (IKA, Germany). At the end of this process, crude extracts were obtained. The percent yield was calculated as follows: $(\text{w/w}) \times 100$. The yield rates of the obtained extracts are given in Table 1.

Determination of total phenol content

To determine the overall phenolic components in acetone and water extracts obtained from plant exposed to different lights, we employed gallic acid as a reference material.

Table 1 Yield values (% w/w) of acetone and water extracts obtained from plant samples exposed to different light sources (white LED, red LED, blue LED, and fluorescent light)

Extract	Extract abbreviation	Yield (% w/w)
Acetone extract obtained from plant exposed to white LED light	WL-AE	12.80
Water extract obtained from plant exposed to white LED light	WL-WE	11.60
Acetone extract obtained from plant exposed to red LED light	RL-AE	6.00
Water extract obtained from plant exposed to red LED light	RL-WE	12.40
Acetone extract obtained from plant exposed to blue LED light	BL-AE	15.60
Water extract obtained from plant exposed to blue LED light	BL-WE	19.40
Acetone extract obtained from plant exposed to fluorescent light	F-AE	8.60
Water extract obtained from plant exposed to fluorescent light	F-WE	10.40

In this process, we placed 20 μ L of the extracts (each at a concentration of 400 mg/L) and the gallic acid standard into separate microplate wells. Subsequently, we introduced 20 μ L of 2 N Folin solution and allowed the combination to incubate in darkness for a duration of 3 min. Subsequently, we introduced 20 μ L of 35% (w/v) sodium carbonate and 140 μ L of dH₂O to the wells and left them in the dark for 10 min. 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 acetone and water extracts obtained from plant exposed to different lights, we employed quercetin as a benchmark. In this procedure, we introduced 50 μ L 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 μ L of 80% (v/v) ethyl alcohol, 5 μ L of aluminum nitrate (at 10%, w/v), and 5 μ L of 1 M potassium acetate to the microtiter plates, and incubated them at room temperature for 40 min. 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.

Free radical scavenging activity

To assess the ability of acetone and water extracts obtained from plant exposed to different lights 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 μ L of each extract into individual wells in a microplate, followed by the addition of 180 μ L 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 min, with the absence of light. We computed the percentage of radical capturing efficacy for the extracts utilizing the subsequent formula: Radical capturing efficacy = [(Control absorbance– Extract absorbance) / Control absorbance] $\times$ 100.

Chelation of metals

To assess the metal-binding capacity of acetone and water extracts obtained from plant exposed to different lights, we performed experiments using various end-stage extracts, specifically 12.5–400 mg/L. Following the established procedure, we introduced 50 μ L of each extract into separate wells within a microplate. Afterward, we added 185 μ L phosphate buffer (0.05 M, pH: 6.2), 5 μ L of FeCl₂ (2 mM), and 10 μ L of ferrozine (5 mM) to these wells. The mixture was then kept at room temperature for 10 min, 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 = [(Control absorbance– Extract absorbance) / Control absorbance] $\times$ 100.

Machine learning algorithms: a brief overview

Within the scope of the study, twelve different machine learning algorithms were performed for the prediction of DPPH radical scavenging and metal chelating activities, separately. These algorithms can be classified as follows: (i) tree-based: classification and regression trees (CART) and Cubist, (ii) kernel-based: support vector machines (SVMs), (iii) ensemble-based: bootstrap aggregating (bagging), random forests (RF), boosting (extreme gradient boosting), (iv) regularization-based: lasso and elastic net regression, (v) splines-based: multivariate adaptive splines (MARS), (vi) neural networks-based: multilayer perceptron (MLP) and (vii) instance-based: k-nearest neighbors (KNN). The main details of each algorithm are outlined in Table 2.

Machine learning: experimental setup and evaluation

Prior to model development, comprehensive data preprocessing steps were implemented to ensure optimal model performance. The dataset underwent normalization to

Table 2 Comparative characteristics, advantages, and limitations of machine learning algorithms evaluated in this study

Algorithm	Key Features	Pros	Cons
CART (Li et al. 1984)	Constructs a decision tree by recursively partitioning the feature space into rectangular regions. Handles both classification and regression tasks through binary splitting	• Highly interpretable and can handle both numerical and categorical variables • Automatically performs feature selection and captures non-linear relationships	• Prone to overfitting, especially with deep trees • Can be unstable as small changes in data can result in very different tree structures
Cubist (Quinlan 1992)	An enhanced model trees that combines rule-based models with linear regression. Creates a set of rules with associated multivariate linear models	• Provides explicit rules that are easy to interpret and implement • Combines the advantages of both rule-based systems and linear regression	• Limited availability in software packages and implementations. • Can be computationally intensive for large datasets
SVM (Cortes and Vapnik 1995)	Maps input data into high-dimensional feature space using kernel functions to find optimal hyperplanes. Transforms complex non-linear problems into simpler linear separation tasks	• Effective in high-dimensional spaces and works well with clear margin of separation • Robust against overfitting due to maximum margin property	• Computationally intensive for large datasets • Sensitive to kernel choice and parameter tuning
Bagging (Breiman 1996)	Creates multiple training datasets through bootstrap sampling and trains independent models on each sample. Aggregates predictions through averaging or voting	• Reduces overfitting and variance through model averaging • Parallel processing capability as models are independent	• May not perform as well as boosting for reducing bias
Random Forests (Breiman 2001)	Combines multiple decision trees with random feature selection at each split. Aggregates predictions from all trees through majority voting or averaging	• Excellent performance out of the box and handles high-dimensional data well • Provides feature importance measures and is less prone to overfitting	• Limited interpretability due to multiple models • Models can be computationally expensive and memory-intensive • Less interpretable than single decision trees
XGBoost (Freund and Schapire 1996)	Implements gradient boosting with advanced regularization and tree pruning techniques. Builds trees sequentially, focusing on correcting errors of previous models	• Superior performance in many machine learning competitions and handles missing values well • Includes built-in regularization and is highly scalable	• Requires careful tuning of multiple hyperparameters • Can be prone to overfitting if not properly configured
Lasso (Tibshirani 1996) & Elastic Net (Zou and Hastie 2005)	Implements L1-norm (Lasso) and combined L1/L2-norms(Elastic Net) regularization to perform feature selection and prevent overfitting. Automatically shrinks coefficients towards zero	• Performs automatic feature selection and handles multicollinearity well • Combines the benefits of both ridge and lasso regression in Elastic Net	• May not perform well with highly correlated features for Lasso • Requires careful tuning of regularization parameters
MARS (Friedman 1991)	Constructs piecewise linear models using hinge functions to capture non-linear relationships. Automatically determines optimal locations for splitting predictor variables	• Handles non-linear relationships without requiring polynomial terms • Automatically identifies important variables and interactions	• Can be computationally intensive for high-dimensional data • May not perform well with highly categorical predictors
MLP (Hornik et al. 1989)	Consists of multiple layers of interconnected neurons that transform input features through non-linear activation functions. Learns complex patterns through backpropagation and gradient descent	• Capable of learning highly complex non-linear relationships • Versatile and can be applied to various types of data and problems	• Requires significant amount of training data and computational resources • Prone to overfitting and sensitive to hyperparameter choices
KNN (Altman 1992)	Makes predictions based on the majority vote or average of k nearest neighbors in the feature space. Stores all training instances for comparison during prediction	• Simple to implement and understand with no training phase required • Naturally handles multi-class problems and can be used for both classification and regression	• Computationally expensive during prediction phase • Sensitive to irrelevant features and the curse of dimensionality

standardize the numerical features, while categorical variables were transformed using one-hot encoding to convert them into a suitable format for regression analysis. The preprocessed dataset was then partitioned into training and test sets, with two-thirds allocated for model training and the remaining one-third reserved for final model evaluation. To ensure robust model development and parameter optimization, a five-times repeated 10-fold cross-validation approach was employed on the training dataset. For hyperparameter tuning, an extensive grid search was conducted across a parameter space comprising 50 units for each possible parameter, enabling thorough exploration of the optimal parameter combinations for each algorithm.

The model development process was executed using the identified optimal parameters, and the resulting models were subsequently evaluated on the held-out test dataset. Model performance assessment was conducted using multiple metrics, including Root Mean Squared Error (*RMSE*) to quantify prediction accuracy, Mean Absolute Error (*MAE*) to measure the average magnitude of errors, and the coefficient of determination (R^2) to assess the proportion of variance explained by the model. For the best-performing model, as determined by these metrics on the test data, additional analyses were performed to enhance model interpretability. These included the generation of variable importance plots to identify the most influential features in the model's predictions, as well as scatter plots comparing predicted versus actual response values to visualize the model's predictive accuracy and any potential systematic biases. The modelling of machine learning algorithms was conducted using R (R Core Team, 2024), with the tidymodels framework (Kuhn and Wickham 2020) serving as the primary tool for model development and evaluation. The tidymodels ecosystem facilitated a streamlined workflow for data preprocessing, cross-validation, and model tuning, ensuring reproducible and systematic analysis procedures.

Statistical analyses

A one-way ANOVA test (Duncan) was applied to identify differences among the extracts. The probit model was selected to calculate the median inhibitory concentration (IC_{50}) values for DPPH and metal chelating activities of

the extracts. Three-dimensional (3-D) density analysis measured correlation levels between DPPH scavenging and metal chelating activities across various extracts. Cluster analyses were employed to explore proximity and distance relationships among different extract concentrations based on their DPPH and metal chelating activities. All analyses were performed using SPSS software (version 21.0, IBM Corporation, Armonk, NY, USA).

Results

In vitro production of plants under LED lights

When examining the shoot regeneration percentages, 100% regeneration was achieved in cultures under both White LED and Red LED lighting (Table 3). The lowest regeneration rates were observed in cultures exposed to fluorescent light. The highest average number of shoots, recorded as 85.27 shoots per explant, was obtained from the MS nutrient medium under Red LED lighting (Fig. 1). This was followed by an average of 78.53 shoots per explant in cultures under White LED lighting. Conversely, the fewest shoots were identified in cultures under white fluorescent light, with an average of 71.17 shoots per explant.

In terms of shoot length, the best results were observed in the Red LED treatments, where the average length was 3.16 cm. The shortest shoots were recorded in the cultures exposed to white fluorescent light, with an average length of 2.48 cm. These findings indicate that the type of light conditions significantly influences both shoot regeneration rates and growth metrics in *C. demersum* explants.

Antioxidant content analysis of extracts

After exposing *C. demersum* to different LED and fluorescent light samples, the total phenol content of the acetone and water extracts obtained from the plants was calculated based on the GAE (Fig. 2a), which we used as a standard, and the total flavonoid content was calculated based on the QE, also used as a standard (Fig. 2b).

When the total phenol content was examined, the highest value was observed in RL-AE, with 63.99 μg GAE/mg

Table 3 Effects of different light conditions on shoot regeneration parameters in *C. demersum*

Light conditions	Shoot regeneration (%)	Average number of shoots per explant	Shoot length (cm)
White LED	100 $\pm$ 0.00 a	78.53 $\pm$ 7.30 ab	2.95 $\pm$ 0.09 b
Red LED	100 $\pm$ 0.00 a	85.27 $\pm$ 5.20 a	3.16 $\pm$ 0.05 a
Blue LED	93.33 $\pm$ 11.54 a	73.42 $\pm$ 6.10 b	2.52 $\pm$ 0.12 c
White fluorescent light	86.67 $\pm$ 11.54 b	71.17 $\pm$ 3.12 b	2.48 $\pm$ 0.06 c

Data represent mean $\pm$ standard deviation (SD). Different letters within each column indicate statistically significant differences among light conditions ($p < 0.05$, ANOVA followed by Duncan's post-hoc test)

Fig. 1 Shoot regeneration from shoot tip explants of *C. demersum*. (a, b, c) Regenerated shoots produced under red LED light after eight weeks



extract. The general ranking among the extracts was as follows: RL-WE < F-WE < WL-WE < F-AE < BL-AE < BL-WE < WL-AE < RL-AE. When comparing the acetone and water extracts of the same plant, it was observed that, except for the blue LED light applications, the acetone extracts had higher total phenol content compared to the water extracts. In addition, the difference between the total phenol content of BL-AE and BL-WE was found to be insignificant ($p < 0.05$), while the total phenol content in all the other extracts differed significantly ($p < 0.05$) (Table 4).

When the total flavonoid content in the plant extracts was considered, BL-AE showed the highest value (167.58 μg QE/mg extract). The ranking for total flavonoid content in the extracts was as follows: F-WE < WL-WE < BL-WE < R

L-WE < F-AE < RL-AE < WL-AE < BL-AE. When comparing the acetone and water extracts of the same plant, it was determined that the acetone extracts had higher total flavonoid content than the water extracts (Table 4).

The network analyses for total phenol (Fig. 2c) and flavonoid contents (Fig. 2d) illustrate the relationships between different treatments, highlighting the influence of both extraction solvents and light conditions. In the phenol network (Fig. 2c), acetone extract appears to be a central node, indicating its significant role in maximizing phenolic content across all light treatments. Blue, red, and white LEDs are closely connected to AE, suggesting these light sources enhance phenolic compound biosynthesis when paired with acetone extraction. Conversely, water extract

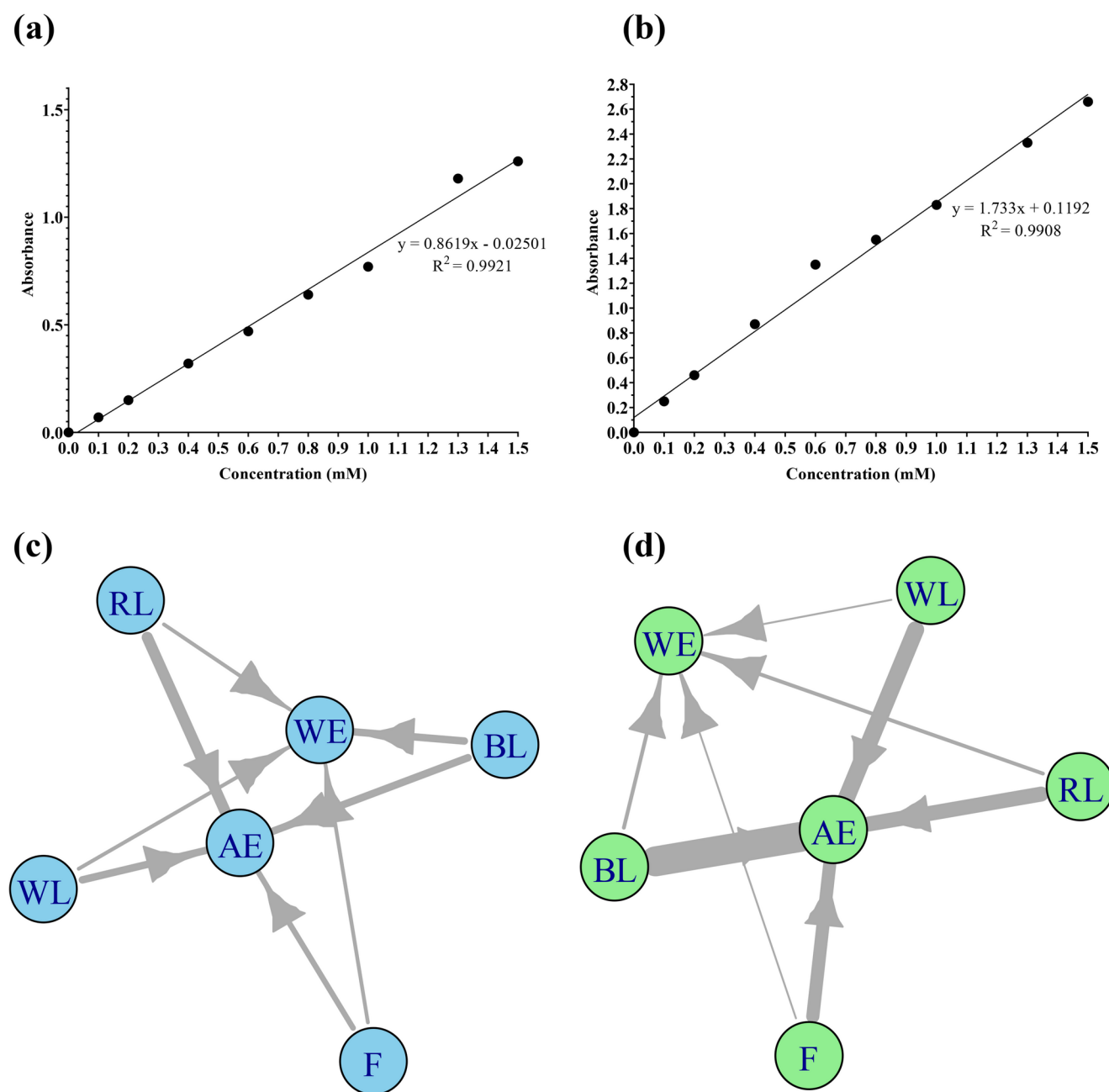


Fig. 2 (a) Linear regression graphs of gallic acid used as a standard in the calculation of total phenol content and (b) quercetin used as a standard in the calculation of total flavonoid content. Network analyses for (c) total phenol and (d) flavonoid contents of different treatments. AE: Acetone extract, WE: Water extract, BL: Blue led light, RL: Red led light, WL: White led light, F: Fluorescent light. WL-AE: White led

light acetone extract, WL-WE: White led light water extract, RL-AE: Red led light acetone extract, RL-WE: Red led light water extract, BL-AE: Blue led light acetone extract, BL-WE: Blue led light water extract, F-AE: Fluorescent light acetone extract, F-WE: Fluorescent light water extract

shows weaker associations, reflecting its lower efficacy in phenolic extraction.

In the flavonoid network (Fig. 2d), acetone extract again serves as a pivotal node, particularly in combination with blue LED light, which exhibits the strongest association. This aligns with the known role of blue light in stimulating flavonoid biosynthesis. Fluorescent light and water

extract demonstrate weaker connections, further confirming their limited influence on flavonoid production. These findings underline the synergistic effects of specific light wavelengths and organic solvents in optimizing antioxidant compound yields.

Table 4 Total phenolic and flavonoid contents ($\mu\text{g}/\text{mg}$) of acetone and water extracts obtained under different light conditions

Extract	Total phenol (Gallic acid equivalent)	Total flavonoids (Quercetin equivalent)
WL-AE	48.10 $\pm$ 0.82 b	92.03 $\pm$ 1.04 b
WL-WE	29.70 $\pm$ 1.92 e	15.71 $\pm$ 0.57 g
RL-AE	63.99 $\pm$ 2.04 a	83.69 $\pm$ 0.68 c
RL-WE	22.74 $\pm$ 0.65 f	23.01 $\pm$ 0.04 e
BL-AE	44.71 $\pm$ 1.32 c	167.58 $\pm$ 0.83 a
BL-WE	47.13 $\pm$ 1.50 bc	20.57 $\pm$ 1.10 f
F-AE	38.19 $\pm$ 1.47 d	75.03 $\pm$ 1.51 d
F-WE	24.58 $\pm$ 0.85 f	13.61 $\pm$ 1.02 h

Data are presented as mean $\pm$ standard deviation (SD), $n=3$. Different letters within each column indicate statistically significant differences among extracts ($p<0.05$, ANOVA followed by Duncan's post-hoc test). Total phenolic content is expressed as gallic acid equivalent (GAE), and total flavonoid content is expressed as quercetin equivalent (QE). WL-AE: White LED light acetone extract, WL-WE: White LED light water extract, RL-AE: Red LED light acetone extract, RL-WE: Red LED light water extract, BL-AE: Blue LED light acetone extract, BL-WE: Blue LED light water extract, F-AE: Fluorescent light acetone extract, F-WE: Fluorescent light water extract

DPPH scavenging activities of extracts

After exposing *C. demersum* to different LED and fluorescent light sources, the DPPH radical scavenging activities of acetone and water extracts obtained from the plants were evaluated across a range of concentrations: 12.5–400 $\mu\text{g}/\text{mL}$ (Fig. 3). The results revealed that the highest DPPH scavenging activity, measured at 90.14%, was observed in the acetone extract obtained under white LED light (WL-AE) at the maximum tested concentration of 400 $\mu\text{g}/\text{mL}$. This finding underscores the enhanced antioxidant potential of WL-AE at higher concentrations compared to the other tested extracts.

Although the 400 $\mu\text{g}/\text{mL}$ concentration of other extracts, including those obtained under different light sources (except for BL-AE and fluorescent light applications), also demonstrated relatively high scavenging activities, statistical analysis indicated that the differences in DPPH scavenging activities between these extracts were not significant ($p>0.05$). This suggests that while various extracts exhibit notable antioxidant properties at high concentrations, their efficacy does not vary substantially across these conditions.

Conversely, the lowest DPPH scavenging activity, recorded at 15.52%, was observed in the water extract from plants exposed to white LED light (WL-WE) at the lowest tested concentration of 12.5 $\mu\text{g}/\text{mL}$. This value was significantly different ($p<0.05$) from all other experimental conditions, highlighting a pronounced concentration-dependent effect on antioxidant activity, particularly in water extracts. These findings collectively emphasize the role of both

extraction solvent and light source in modulating the antioxidant potential of *C. demersum* extracts (Fig. 3).

When the different extracts of each light application were evaluated based on IC_{50} values, an important parameter for determining activity levels, it was found that acetone extracts generally had lower IC_{50} values, indicating higher activity, except in blue LED light applications. This suggests that acetone extracts were more effective at DPPH scavenging, except in blue LED light applications. RL-AE had the lowest IC_{50} value (5.33 $\mu\text{g}/\text{mL}$), making it the most effective extract (Table 5).

Metal chelation activities of extracts

After *C. demersum* was exposed to different LED and fluorescent light samples, the metal chelating activities of the acetone and water extracts obtained from the plants were determined at concentrations of 12.5, 25, 50, 100, 200, and 400 $\mu\text{g}/\text{mL}$ (Fig. 3).

The highest metal chelating activity (61.32%) in the entire experimental group was observed at the maximum concentration (400 $\mu\text{g}/\text{mL}$) of WL-WE, and this value was significantly different ($p<0.05$) from the data obtained from all other applications (Fig. 3). The lowest activity (4.04%) was found in the 12.5 $\mu\text{g}/\text{mL}$ application of BL-WE, and this value did not show any statistical difference ($p>0.05$) compared to the 12.5 $\mu\text{g}/\text{mL}$ applications of WL-WE and RL-AE (Fig. 3).

When the different extracts of each light application were evaluated based on their IC_{50} values, which are important for determining activity levels, it was found that acetone extracts generally had lower IC_{50} values, indicating higher activity, except for the blue LED light applications. This trend is similar to the DPPH scavenging activity results, suggesting that acetone extracts generally had more effective metal chelating capacity, except under blue LED light conditions. BL-WE was determined to have the lowest IC_{50} value (171.30 $\mu\text{g}/\text{mL}$), making it the most effective extract (Table 5).

Examination of 3-D density distributions and clustering methods

We employed 3-D density analysis and clustering techniques to examine the relationships between antioxidant activities—specifically DPPH radical scavenging and metal chelating activities—induced by various plant extracts. Correlation levels between DPPH scavenging and metal chelation activities were assessed using 3D graphical analysis, revealing statistically significant positive correlations at the $p<0.01$ level across all tested extracts. Among these, the highest correlation coefficients were observed for BL-WE

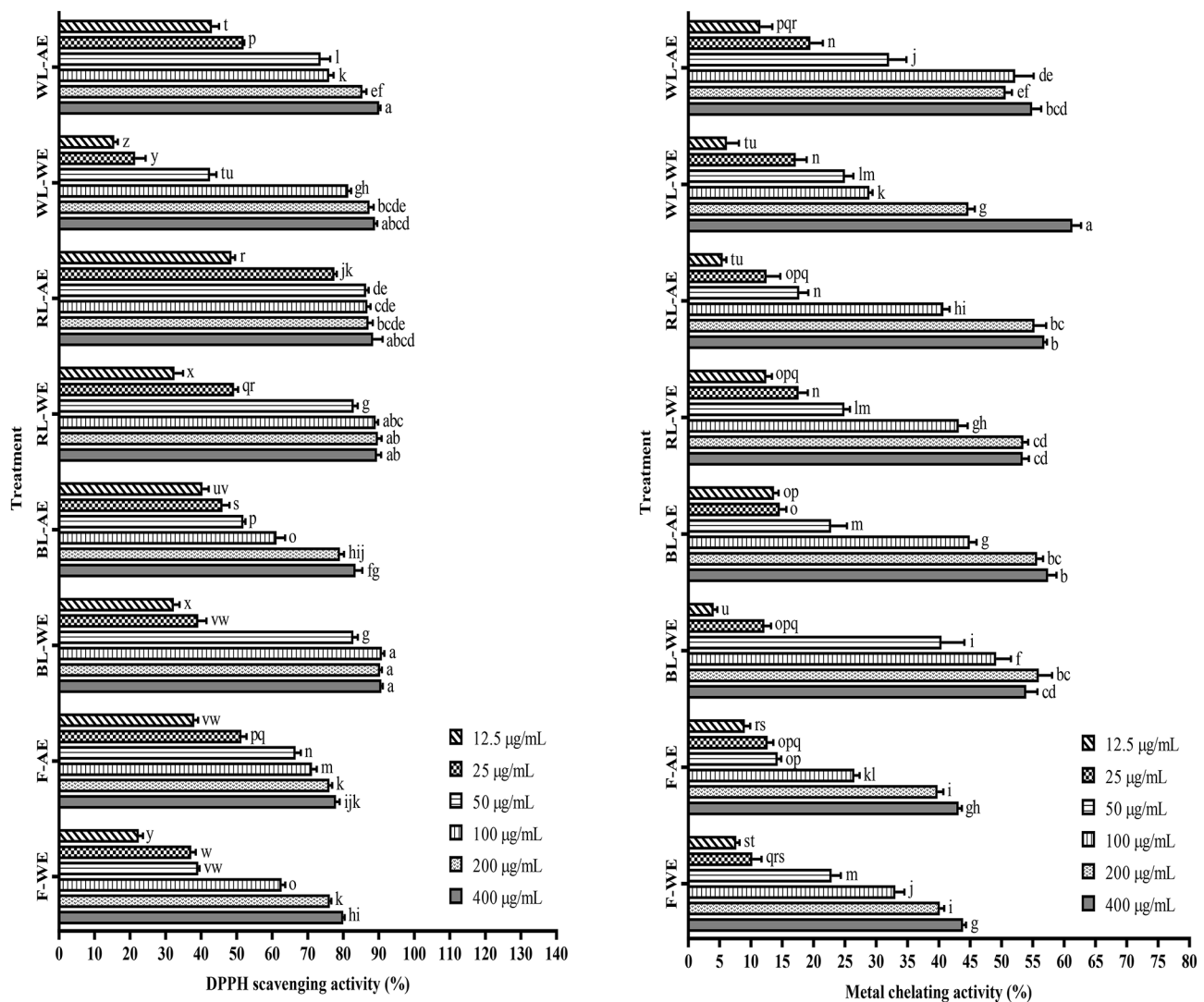


Fig. 3 (a) DPPH scavenging and (b) metal chelating activities (%) of the obtained extracts (Mean±Standard Deviation, $n=3$) (Differences between means indicated with different letters are significant at $p<0.05$ level). WL-AE: White led light acetone extract, WL-WE:

White led light water extract, RL-AE: Red led light acetone extract, RL-WE: Red led light water extract, BL-AE: Blue led light acetone extract, BL-WE: Blue led light water extract, F-AE: Fluorescent light acetone extract, F-WE: Fluorescent light water extract

($r=0.98$), F-WE ($r=0.97$), and BL-AE ($r=0.96$), respectively. The 3-D density plots indicated that DPPH scavenging and metal chelating activities were positively associated within specific activity ranges, with particularly high intensities at certain levels of both activities (Fig. 4a).

Hierarchical clustering delineated three distinct clusters for DPPH scavenging activity. RL-AE was segregated into an individual cluster, WL-WE and F-WE grouped into a separate cluster, and all other extracts were consolidated within Cluster 1. In terms of metal chelating activity, two main clusters were identified: F-AE and F-WE clustered together, while the remaining extracts formed the second cluster (Fig. 4b).

The results of benchmarking machine learning algorithms

The performance results of twelve different machine learning algorithms for the prediction of DPPH radical scavenging and metal chelating activities are presented in Tables 6 and 7, respectively. The following results are revealed in Table 6, which includes the DPPH radical scavenging results: The bold text highlights the key finding of our model comparison, indicating that XGBoost demonstrated the best performance in predicting DPPH activity, achieving the lowest MAE (3.754) and highest R^2 (0.887) on the test set, along with one of the lowest $RMSE$ values (5.027). MLP also shows strong performance with relatively low $RMSE$ (5.528) and MAE (4.200) on the test set. In contrast, Elastic

Table 5 IC₅₀ values (μg/mL) for DPPH scavenging and metal chelating activities of acetone and water extracts obtained under different light conditions

Extract	IC ₅₀ (Limits)	Slope±Standard error (Limits)
<i>DPPH scavenging activities</i>		
WL-AE	18.07 (14.37–21.86)	1.00±0.06 (0.87–1.13)
WL-WE	53.29 (48.76–58.13)	1.78±0.07 (1.16–1.92)
RL-AE	5.33 (3.13–7.86)	0.77±0.06 (0.64–0.91)
RL-WE	20.21 (29.51–36.87)	1.31±0.07 (1.17–1.45)
BL-AE	32.21 (17.14–23.33)	0.84±0.06 (0.72–0.96)
BL-WE	23.30 (20.29–26.37)	1.46±0.07 (1.31–1.60)
F-AE	21.98 (16.36–27.83)	0.72±0.06 (0.60–0.84)
F-WE	59.15 (51.93–67.14)	1.11±0.06 (0.99–1.24)
<i>Metal chelating activities</i>		
WL-AE	186.08 (155.33–230.13)	0.89±0.06 (0.77–1.02)
WL-WE	245.65 (207.45–299.76)	1.08±0.06 (0.95–1.22)
RL-AE	209.10 (181.89–245.02)	1.24±0.07 (1.11–1.38)
RL-WE	221.24 (183.29–276.98)	0.91±0.06 (0.79–1.04)
BL-AE	193.78 (164.44–234.39)	1.01±0.06 (0.88–1.13)
BL-WE	171.30 (148.40–201.40)	1.11±0.06 (0.98–1.24)
F-AE	550.43 (411.80–803.40)	0.86±0.06 (0.72–0.99)
F-WE	434.38 (336.43–601.93)	0.89±0.06 (0.75–1.02)

IC₅₀ values are presented as mean with 95% confidence limits. Slope values are expressed as mean±standard error with 95% confidence limits. WL-AE: White LED light acetone extract, WL-WE: White LED light water extract, RL-AE: Red LED light acetone extract, RL-WE: Red LED light water extract, BL-AE: Blue LED light acetone extract, BL-WE: Blue LED light water extract, F-AE: Fluorescent light acetone extract, F-WE: Fluorescent light water extract

Net Regression, Lasso Regression, and Ridge Regression demonstrate relatively poor performance with higher error rates ($RMSE > 12$ and $MAE > 10$ on test sets) and lower R^2 values (around 0.57–0.58). Consequently, XGBoost demonstrates relatively consistent performance across both data splits, indicating its robust generalization capability. This consistency suggests that the model effectively captures the underlying relationships within the data, enabling it to perform well on unseen samples. As illustrated in Fig. 5a, which depicts the correlation between the predicted and actual values of DPPH radical scavenging activity, the XGBoost algorithm exhibits a strong agreement between these values. This strong alignment reflects the model's ability to produce accurate and reliable predictions. The results highlight XGBoost's potential as a dependable tool for modeling DPPH radical scavenging activity, especially in scenarios requiring high predictive accuracy and generalizability across diverse datasets.

Among the test performances of machine learning algorithms for metal chelating activity given in Table 7, the bold text highlights the key finding of our model comparison, indicating that Cubist performs the best overall performance with the lowest test $RMSE$ (5.129) and MAE (4.141) values, along with one of the highest RSQ values (0.899). Similar to DPPH radical scavenging activity

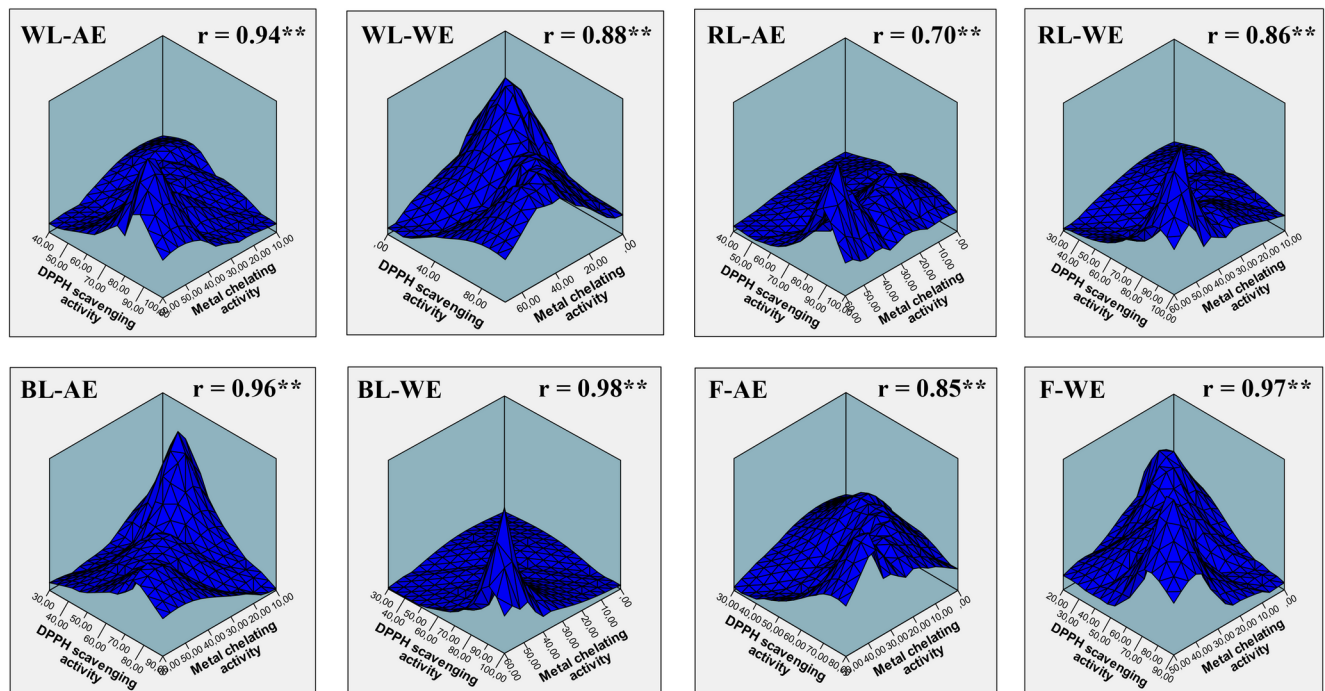
results, MLP also performs notably well with strong R^2 values (0.896) and relatively low error metrics. In contrast, KNN and Lasso Regression show relatively poor performance with higher error rates ($RMSE > 10$ for test sets) and lower R^2 values (0.401 for Lasso Regression test). The ensemble methods, such as Random Forests and XGBoost, exhibit moderate predictive performance, achieving RSQ values in the vicinity of 0.87. This positions them within the mid-range of the overall performance spectrum when compared to other models analyzed. On the other hand, the Cubist model demonstrates a superior ability to generalize predictions, as evidenced by the high degree of agreement between the predicted and actual values for metal chelating activity, shown in Fig. 5b. This figure highlights the Cubist model's capacity to capture the underlying patterns in the data effectively, leading to highly accurate and reliable predictions. Such performance underscores the robustness and suitability of the Cubist model for modeling metal chelating activity.

Discussion

Plant tissue culture techniques serve as an important tool in plant physiology research, while evaluating the effects of different light sources on plants constitutes a significant area of study in this field (Palka et al. 2023; Sarropoulou et al. 2023; Shang et al. 2023). The results of this study demonstrate the significant impact of different light conditions on the shoot regeneration of *C. demersum* from shoot tip explants. Notably, 100% shoot regeneration was achieved under both White LED and Red LED lighting, indicating that these light conditions are highly conducive to the successful propagation of this aquatic plant. In contrast, the fluorescent light conditions yielded the lowest regeneration rates, suggesting that not all light sources are equally effective in promoting plant tissue regeneration. This finding aligns with previous research that highlights the positive effects of specific light wavelengths on plant growth and development (Dogan 2020; Lim et al. 2023; Wai et al. 2024).

The highest average number of shoots per explant was recorded at 85.27 shoots under Red LED conditions. This suggests that Red LED light not only enhances the overall regeneration percentage but also promotes prolific shoot formation, which is crucial for successful propagation and cultivation of *C. demersum*. The efficacy of red light in stimulating shoot proliferation can be attributed to its ability to promote photosynthesis and enhance the synthesis of growth-promoting hormones, such as auxins and cytokinins, which are vital for cell division and differentiation in plant tissues (Cavallaro et al. 2022; Zhu et al. 2022).

(a)



(b)

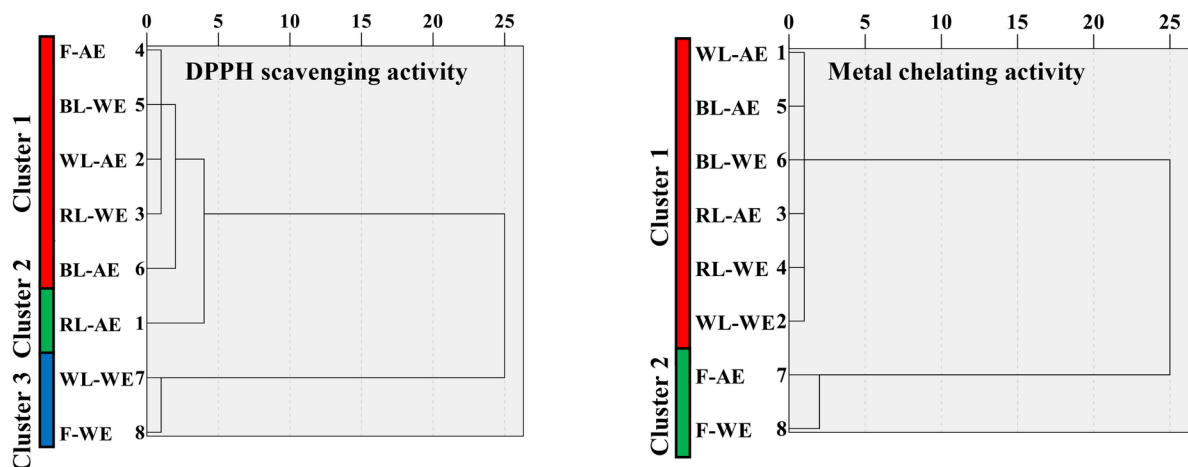


Fig. 4 (a) 3-D density analysis was conducted to evaluate the DPPH scavenging and metal chelating activities of the extracts. In this analysis, correlation coefficients (r) were calculated, with double asterisks (**) indicating a significance level of 0.01. (b) Cluster analysis was carried out based on the average DPPH scavenging and metal chelat-

ing activities of the extracts. WL-AE: White led light acetone extract, WL-WE: White led light water extract, RL-AE: Red led light acetone extract, RL-WE: Red led light water extract, BL-AE: Blue led light acetone extract, BL-WE: Blue led light water extract, F-AE: Fluorescent light acetone extract, F-WE: Fluorescent light water extract

Similarly, the cultures under White LED light also demonstrated a robust regeneration capacity with an average of 78.53 shoots per explant. White light, which is a combination of various wavelengths, may provide a balanced environment for plant growth, supporting not only shoot regeneration but also the overall health of the plant tissues. However, the marginally lower shoot count compared to the Red LED treatment indicates that specific wavelengths, such as red light, may have a more pronounced effect on

stimulating shoot proliferation in *C. demersum*. Additionally, the successful effects of red LED lights on plant production and development have been documented by many researchers previously (Saleem et al. 2020; Gu et al. 2020; Jin et al. 2023). Conversely, the cultures exposed to white fluorescent light yielded the lowest average shoot count of 71.17 shoots per explant. This outcome suggests that while fluorescent light can support basic plant growth, it may

Table 6 Performance comparison of machine learning algorithms for DPPH radical scavenging activity: *RMSE*, *MAE*, and *R*² metrics (Bold values indicate the best-performing model)

Algorithm	Split	<i>RMSE</i>	<i>MAE</i>	<i>R</i> ²
Bagging (CART)	Train	4.987	5.096	0.783
	Test	5.260	5.408	0.764
CART	Train	6.946	6.120	0.792
	Test	8.043	7.127	0.700
Cubist	Train	5.140	4.771	0.863
	Test	5.943	4.835	0.850
Elastic Net Regression	Train	10.962	9.896	0.786
	Test	12.349	10.901	0.585
KNN	Train	9.465	8.019	0.803
	Test	10.377	8.364	0.702
Lasso Regression	Train	10.360	10.255	0.774
	Test	12.363	10.915	0.583
MARS	Train	7.552	6.635	0.752
	Test	7.793	6.398	0.725
MLP	Train	3.901	5.418	0.849
	Test	5.528	4.200	0.860
Random Forests	Train	6.663	4.900	0.829
	Test	7.261	5.367	0.769
Ridge Regression	Train	10.220	10.698	0.747
	Test	12.362	10.895	0.570
SVM (Polynomial Kernel)	Train	7.013	6.903	0.768
	Test	8.746	7.217	0.714
SVM (Radial Kernel)	Train	8.990	7.556	0.706
	Test	9.682	7.820	0.655
XGBoost	Train	4.635	3.603	0.869
	Test	5.027	3.754	0.887

Table 7 Performance comparison of machine learning algorithms for metal chelating activity: *RMSE*, *MAE*, and *R*² metrics (Bold values indicate the best-performing model)

Algorithm	Split	<i>RMSE</i>	<i>MAE</i>	RSQ
Bagging (CART)	Train	5.219	4.488	0.873
	Test	6.046	4.561	0.862
CART	Train	5.264	4.278	0.872
	Test	7.136	5.486	0.793
Cubist	Train	4.732	4.016	0.888
	Test	5.129	4.141	0.899
Elastic Net Regression	Train	8.805	7.918	0.775
	Test	10.195	8.153	0.579
KNN	Train	10.769	9.940	0.793
	Test	12.406	9.819	0.401
Lasso Regression	Train	8.811	7.919	0.773
	Test	10.220	8.178	0.578
MARS	Train	6.105	5.179	0.879
	Test	5.282	4.277	0.898
MLP	Train	4.425	3.792	0.896
	Test	7.311	4.543	0.811
Random Forests	Train	4.863	4.143	0.866
	Test	5.639	4.223	0.873
Ridge Regression	Train	9.009	8.025	0.750
	Test	10.319	8.295	0.571
SVM (Polynomial Kernel)	Train	7.651	6.472	0.772
	Test	8.218	5.878	0.787
SVM (Radial Kernel)	Train	8.258	7.164	0.737
	Test	6.341	4.730	0.856
XGBoost	Train	4.731	4.152	0.876
	Test	6.156	4.878	0.869

lack the specific wavelengths necessary for optimal shoot regeneration.

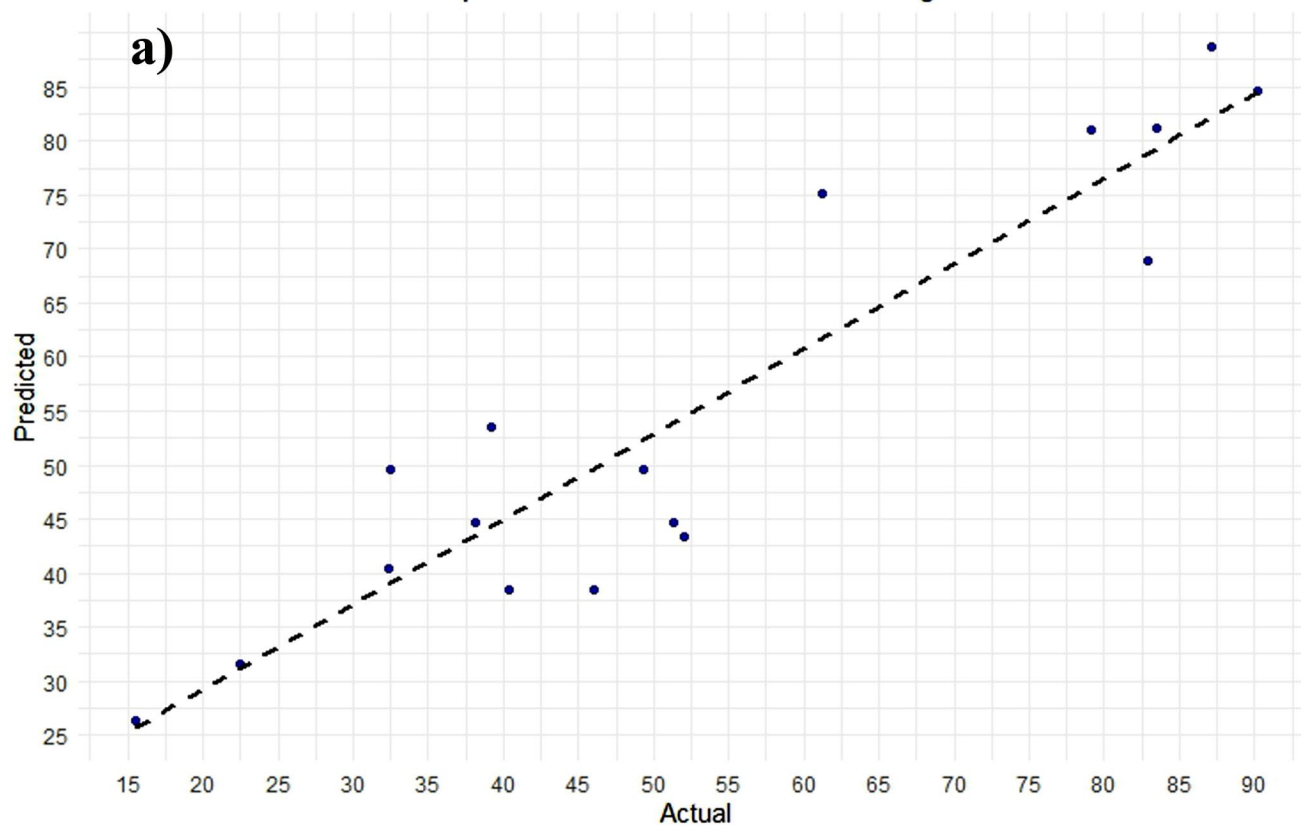
In terms of shoot length, the most favorable results were observed under Red LED light, with an average length of 3.16 cm. This finding underscores the importance of light conditions not only in enhancing shoot regeneration rates but also in promoting the elongation of the regenerated shoots, which is critical for the successful establishment of the plant. In contrast, the shortest shoots, measuring an average of 2.48 cm, were found in cultures under white fluorescent light. The implications of shorter shoot lengths can impact the subsequent growth and establishment of the plants, potentially limiting their adaptability in natural or controlled environments. The lower efficiency of fluorescent lighting can also be linked to its limited spectrum, which may not adequately fulfill the light requirements of *C. demersum* during the regeneration process. In line with our study, it has been reported in research comparing LED lights to fluorescent lights that lower results were observed in plants under fluorescent light (Gam et al. 2020; Hamedalla et al. 2022).

This study evaluated the total phenolic and flavonoid content, DPPH scavenging activity, and metal chelation

capacity of acetone and water extracts obtained from *C. demersum* exposed to various LED and fluorescent light sources. The results demonstrate that the antioxidant properties of the plant extracts vary significantly depending on light conditions and the extraction solvent used, consistent with previous studies that explored the influence of light exposure on secondary metabolite production in plants.

The total phenolic content of the extracts revealed that the highest phenolic production occurred in the RL-AE, with a value of 63.99 µg GAE/mg extract. Previous studies have shown that red light enhances phenolic biosynthesis by activating key enzymes in the phenylpropanoid pathway (Xie et al. 2020; Lan et al. 2024). Phenolic compounds are well known for their ability to neutralize free radicals and reduce oxidative stress (Karatas et al. 2015). The higher total phenolic content observed in acetone extracts compared to water extracts, except for blue LED light treatments, can be attributed to acetone's superior ability to dissolve hydrophobic compounds (Jin et al. 2019). The unique behavior observed under blue LED light, where no significant difference was found between BL-AE and BL-WE, suggests that blue light may stimulate the production of different phenolic compounds, which are less hydrophobic (Shi et al. 2022).

The fit between actual and predicted values based on testing data



The fit between actual and predicted values based on testing data

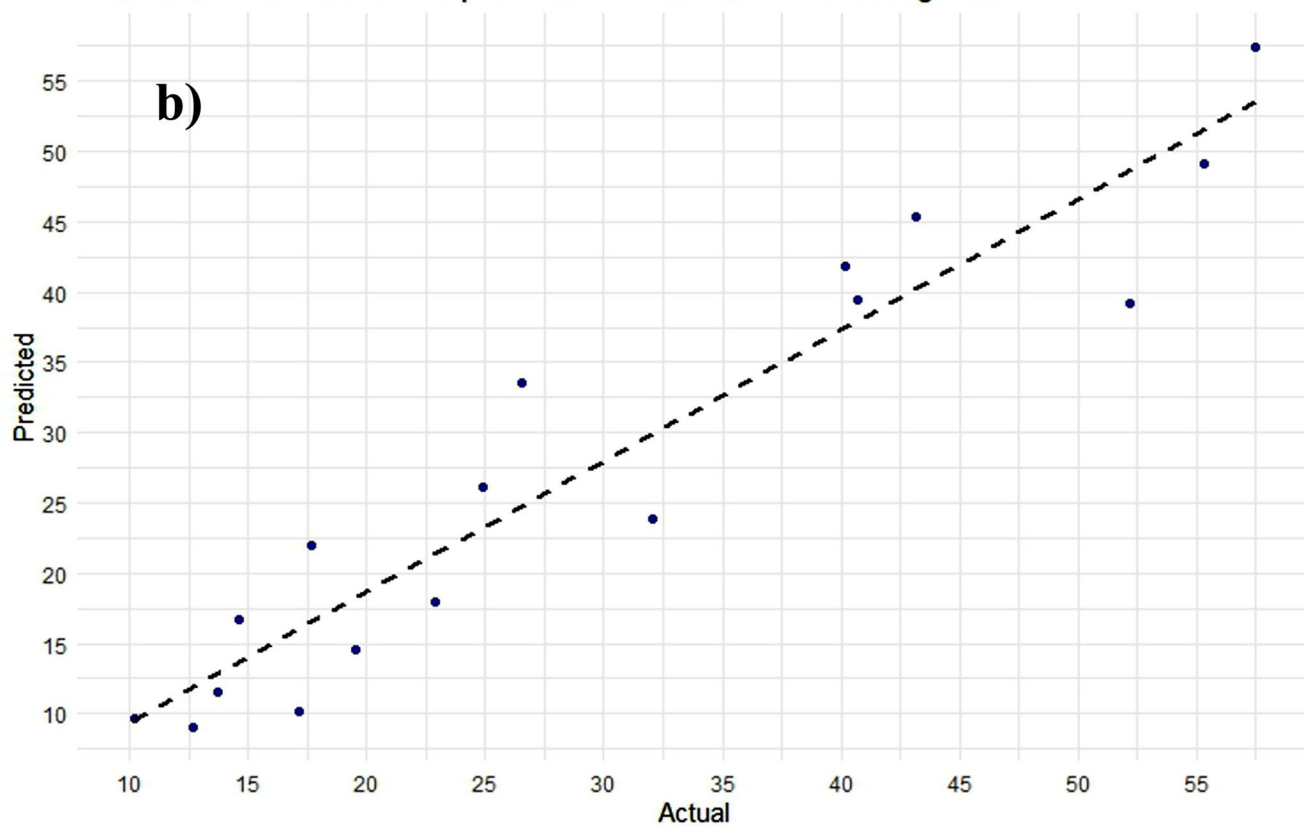


Fig. 5 Comparison of actual and predicted antioxidant activities using machine learning algorithms. **(a)** The comparison of actual and predicted DPPH radical scavenging activity for testing data based on XGBoost algorithm. **(b)** The comparison of actual and predicted metal chelating activity for testing data based on Cubist algorithm

Regarding total flavonoid content, the BL-AE showed the highest value. This is in line with previous findings indicating that blue light enhances flavonoid production in plants (Taulavuori et al. 2016; Le et al. 2021). Flavonoids are crucial compounds that protect plants from environmental stressors such as UV radiation and oxidative stress (Ferreira et al. 2021), which explains the high production under blue light, which mimics high-energy light exposure. The generally higher flavonoid content in acetone extracts further supports the idea that acetone is more efficient in extracting flavonoid compounds (Bui et al. 2021).

The DPPH scavenging activity results revealed that the highest activity (90.14%) was observed in the WL-AE at a concentration of 400 $\mu\text{g/mL}$. White light may promote the balanced production of various antioxidant compounds due to its broad wavelength (Klimek-Szczykutowicz et al. 2022; Singh and Mishra 2015). The lower IC_{50} values of acetone extracts compared to water extracts, indicating higher scavenging activity, have been observed in other plant studies as well (Adedapo et al. 2011; Emsen et al. 2017). This suggests that acetone extracts contain stronger antioxidant compounds, with phenolics being a major contributor to DPPH scavenging activity (Sowunmi and Afolayan 2015).

The lowest IC_{50} value (5.33 $\mu\text{g/mL}$) was observed in RL-AE, making it the most effective extract in terms of DPPH scavenging. This is likely related to its high phenolic content. Interestingly, BL-AE did not show similarly high scavenging activity despite its high flavonoid content, which may suggest that the specific flavonoids produced under blue light contribute more to other antioxidant mechanisms such as metal chelation (Andrabi et al. 2024).

The metal chelation tests revealed that the WL-WE had the highest chelation activity (61.32% at 400 $\mu\text{g/mL}$). This indicates that white light may enhance the production of compounds with chelating properties. Metal chelation is a significant antioxidant mechanism, as it prevents harmful reactions caused by transition metals (Gulcin and Alwasel 2022). The higher metal chelation activity of water extracts under white light suggests that this light condition may stimulate the production of more hydrophilic chelators (Imbert et al. 2002).

Acetone extracts generally showed lower IC_{50} values and thus higher chelation activity, except under blue LED light conditions. This finding supports the idea that acetone is a more effective solvent for extracting compounds with high chelation capacity (Zhang et al. 2020). Although the BL-WE showed lower chelation activity at lower concentrations, it

had the lowest IC_{50} value (171.30 $\mu\text{g/mL}$) at higher concentrations, indicating that the flavonoid and phenolic compounds induced by blue light become effective chelators at higher doses (Ekiert et al. 2022).

This study highlights the utility of different machine learning algorithms in predicting antioxidant activities, specifically DPPH radical scavenging and metal chelation, showcasing their ability to model complex biological interactions under varying LED light conditions. Performance comparisons indicate significant variation among algorithms, with ensemble methods and neural networks consistently outperforming linear models.

For predicting DPPH radical scavenging activity, XGBoost demonstrated the best performance, achieving the lowest *MAE*, among the lowest *RMSE*, and the highest coefficient of determination ($R^2 = 0.887$). These findings illustrate XGBoost's capability to capture nonlinear relationships and intricate variable interactions. Neural networks, such as the *MLP*, also showed strong predictive accuracy, further emphasizing their effectiveness in handling complex data patterns. On the other hand, linear algorithms like Elastic Net, Lasso Regression, and Ridge Regression underperformed, with higher error metrics and lower R^2 values, indicating their limited ability to model the nonlinear and multifactorial characteristics of DPPH activity data.

For the prediction of metal chelating activity, the Cubist algorithm emerged as the most effective, delivering the lowest test *RMSE* and *MAE* values while achieving the highest coefficient of determination ($R^2 = 0.899$). This superior performance can be attributed to Cubist's hybrid architecture, which combines regression trees with rule-based learning, allowing it to effectively model complex, nonlinear relationships and interactions within the dataset. The ability of the algorithm to capture these intricate patterns demonstrates its suitability for applications requiring high precision in predictive modeling.

Similarly, the *MLP* algorithm also performed well, further affirming its robustness across different datasets and tasks. Its capacity to adapt to diverse biological datasets underscores the flexibility and power of neural network models in handling multifactorial data with nonlinear dynamics. In contrast, algorithms such as *KNN* and Lasso Regression showed significantly lower predictive accuracy. Lasso Regression, in particular, yielded the lowest R^2 among all tested models, indicating its limited capacity to account for the inherent complexity and nonlinear characteristics of the metal chelating activity data. These results emphasize the need for more advanced algorithms, like ensemble methods and neural networks, to address the challenges posed by highly intricate biological datasets. The findings also underscore the importance of selecting appropriate machine learning models tailored to the specific nature of the data

for achieving accurate predictions in predictive biology and related fields.

Interestingly, while ensemble methods such as Random Forest and XGBoost perform exceptionally well for DPPH radical scavenging activity, their performance for metal chelating activity is more moderate, with R^2 values around 0.87. This discrepancy suggests that the specific characteristics of the dataset and the underlying chemical interactions may influence the suitability of different algorithms.

The variable importance analysis offers significant insights into how different LED light treatments influence antioxidant activities, providing a deeper understanding of the underlying biological processes. For DPPH radical scavenging activity, the analysis underscores the pivotal role of specific LED light spectra in driving the observed antioxidant responses, as highlighted by the variable importance plot. These findings suggest that certain wavelengths may have a more pronounced effect on the production of bioactive compounds involved in oxidative stress mitigation.

In the case of metal chelating activity, the Cubist model exhibits a strong alignment between predicted and actual values, demonstrating its high accuracy, generalizability, and robustness in modeling complex biological interactions. This consistency across datasets highlights the model's capacity to capture intricate relationships between variables and its suitability for predictive applications in plant biology and biotechnology. The demonstrated effectiveness of the Cubist model positions it as a valuable tool for future research aimed at optimizing antioxidant production and understanding the multifactorial influences of environmental conditions on plant metabolism.

Conclusions

This study demonstrates that the antioxidant capacity of *C. demersum* is significantly influenced by light conditions and extraction solvents. Red and white LED light combined with acetone extraction were the most effective for enhancing phenolic content, flavonoid content, DPPH scavenging activity, and metal chelation activity. These findings support previous research on the impact of light spectra on secondary metabolite production. Future studies should identify specific phenolic and flavonoid compounds produced under different light conditions and their roles in antioxidant mechanisms. Additionally, factors like light duration, intensity, and nutrient availability should be explored to optimize antioxidant capacity. The use of machine learning techniques provided valuable insights into predicting the antioxidant capacities of *C. demersum*. Algorithms such as XGBoost and Cubist effectively modeled the complex interactions between light treatments, solvents, and antioxidant

activities, offering superior predictive accuracy over traditional models. This research highlights the critical role of light conditions and extraction solvents in shaping the antioxidant properties of *C. demersum*.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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