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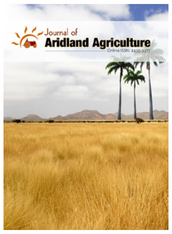
Research Article



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Effect of heavy metals on phenylpropanoid biosynthesis in *Euonymus alatus*

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ABSTRACT

The productivity of the phenylpropanoid biosynthesis pathway in plants varies depending on the type of stress. In this work, we looked into how different phenylpropanoid chemicals accumulated in *Euonymus alatus* following exposure to different concentrations of $CuCl_2$ (0.1, 0.5, and 1 mM), $HgCl_2$ (0.1, 0.5, and 1 mM), and $NiSO_4$ (10, 50, and 100 mM). We analyzed some of the individual phenolic chemicals by high-performance liquid chromatography (HPLC). In nearly all cases, rutin showed the largest concentration among the phenylpropanoid chemicals, followed by epicatechin, sinapic acid, *p*-coumaric acid, *trans*-cinnamic acid, ferulic acid, and caffeic acid. However, due to the change in the concentration of the heavy metals, the amount of phenylpropanoid changed. The highest accumulation of phenylpropanoid was documented in 0.1 mM $CuCl_2$, whereas it was reduced in 1 mM $HgCl_2$ exposed plants. These findings unequivocally demonstrate that the phenylpropanoid metabolic pathway took part in the heavy metal tolerance process, which shielded *E. alatus* from the oxidative damage brought on by heavy metals. Thus, under a variety of environmental stress situations, this species with a high tolerance to heavy metals may survive.

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INTRODUCTION

Euonymus alatus, commonly identified as winged spindle or burning bush, is a deciduous tree species of flowering plant belonging to the Celastraceae family. It is disseminated mainly in China, Japan, and Korea. Because of its eye-catching fall color and bright pink or orange fruit, this plant is a favorite ornamental in gardens and parks (Qin *et al.*, 2011; Ning *et al.*, 2022). In traditional Chinese and Korean medicine, corky-winged stems are used. This plant can treat cancer, hyperglycemia, and complications from diabetes (Fan *et al.*, 2020; He *et al.*, 2022). The following secondary metabolites i.e., flavonoids, terpenoids, steroids, lignans, cardenolides, phenolic acids, and alkaloids have been documented from this plant in different experiments (Zhai *et al.*, 2016).

A class of long-lasting, non-biodegradable inorganic chemical substances known as heavy metals causes harm to both people and plants as well as animals. They are extremely harmful to plant tissue at greater doses (Lombardi & Sebastiani, 2005), which has a substantial impact on the plastoquinone, carotenoid level, electron transport system, and chloroplast (Kisa *et al.*, 2016). Large tracts of land are contaminated with heavy metals, particularly by inorganic pollutants brought on by urbanization, industrial waste, and agriculture (Demirezen & Aksoy, 2006; Kisa *et al.*, 2016). It is becoming more and more crucial to develop heavy metal-tolerant plants, which can detoxify heavy metals, in order to solve this issue. Because it is inexpensive and simple to operate and maintain, plant-based phytoremediation of heavy metals is one of the most successful methods (Kivaisi, 2001; Madera-Parra *et al.*, 2015). Most plants accumulate secondary metabolites as a result of abiotic stressors (Zhao *et al.*, 2005). Among such metabolites, the most significant and often studied metabolic pathway is the phenylpropanoid pathway (Biala & Jasinski, 2018).

Phenylpropanoids compounds have anti-allergenic, anti-bacterial, anti-inflammatory, antioxidant, and anti-viral activities, which are beneficial to the health of humans (Cevallos-Casals & Cisneros-Zevallos, 2010; Korkina *et al.*, 2011; Panda *et al.*, 2011). Furthermore, these substances have anti-diabetic and anti-cancer properties, aiding in the prevention of cardiovascular illnesses (Yang *et al.*, 2001; Yao *et al.*, 2004). Additionally, they are essential mediators for the interactions between plants and other creatures and play a critical role in responding to stressful circumstances like fluctuations in light intensity and mineral deficits (Clemens & Weber, 2016). Kisa *et al*. (2016) studied how heavy metals affect the metabolic profile of phenolic compounds in plants. Nevertheless, it is unknown how heavy metals affect *E. alatus*'s phenylpropanoid profile and how the phenylpropanoid pathway contributes to the species' ability to survive in adverse environments. It is therefore likely that the phenylpropanoid accumulation is responsible for this tolerance capacity. Gaining insight into the process that allows heavy metals to be tolerated might improve the effectiveness of reducing heavy metals effect.

MATERIALS AND METHODS

Plant Materials

The studied plating material of *Euonymus alatus* (one-year-old) grown from a cutting was procured from Xplant (Seoul, Korea) and arranged into individual pots 11 cm× 11 cm in size. For five months, the one-year-old *E. alatus* trees were raised in a greenhouse at Chungnam National University's experimental farm in Daejeon, South Korea. After reaching a height of 70 cm, the trees were treated with different concentrations of heavy metals.

Heavy Metal Stress Treatments

Different concentrations of $CuCl_2$ (0.1, 0.5, and 1 mM), $HgCl_2$ (0.1, 0.5, and 1 mM), and $NiSO_4$ (10, 50, and 100 mM) were tested, in order to know the impact of Cu, Hg, and Ni toxicity on *E. alatus*. The standard stock solution was used to create all the working concentrations, whereas all of the heavy metals were acquired from a commercial source (Sigma, St. Louis, MO, USA). Three duplicates were carried out for each experimental dosage.

Analysis of Phenylpropanoid Content and High-Performance Liquid Chromatography (HPLC)

The leaf samples of *E. alatus* were harvested weekly following heavy metal treatments. HPLC was used to determine the phenylpropanoid content of the samples after they were freeze-dried for 72 hours. A small modification was made to the previously described methodology of Kim *et al.* (2020) for the analysis of the soluble phenylpropanoid compounds. Using a mortar and pestle, the stored samples were taken and ground into a fine powder. Each sample was weighed out at 100 mg and put into 3 mL of 80% aqueous MeOH for HPLC analysis. After one minute of vortexing, the mixtures were immediately subjected to a vigorous 60-minute sonication at 35 °C. After centrifuging sonicated samples at 10,000 × g for 15 minutes, the supernatants were gathered, filtered, and sterilized using 0.45 μm filters in preparation for HPLC analysis. Reversed-phase chromatography was used to perform HPLC separation on an Agilent 1260 Infinity Quaternary LC (Agilent Technologies, Inc., Germany) using a C18 column (250 × 4.6 mm, 5 μm, RStech, Daejeon, South Korea). The mobile phase, gradient programs, HPLC conditions, identification, and quantification of phenylpropanoid compounds were similar to the protocol described by Kim *et al*. (2022).

Statistical Analysis

The results were analyzed using IBM SPPS Statistics 24 software. Duncan Multiple Range Test with one-way ANOVA at the 5% significance level was used for the analyses. For each treatment, all experiments were done in triplicate.

RESULTS

Effect of $CuCl_2$ on Phenylpropanoid Contents

Depending on the concentration of $CuCl_2$, *E. alatus* exhibited a wide variety of responses related to phenylpropanoid biosynthesis. The total phenylpropanoid contents reached 2.16, 2.05, and 2.07 mg/g dry weight at exposure to 0.1, 0.5, and 1 mM $CuCl_2$, respectively. At all the concentrations, the highest phenylpropanoid content (mg/g dry weight) was obtained for rutin followed by epicatechin, sinapic acid, *p*-coumaric acid, ferulic acid, trans-cinnamic acid, and caffeic acid. However, when compared to the control, *trans*-cinnamic acid showed a decreased accumulation at 0.1 mM $CuCl_2$. Most of the phenylpropanoid contents were higher in the plant exposed to 0.5 mM $CuCl_2$, except for caffeic acid and epicatechin (Figure 1).

Effect of $HgCl_2$ on Phenylpropanoid Contents

There was a minor impact of $HgCl_2$ on the phenylpropanoids' accumulation. The total phenylpropanoid production (mg/g dry weight) was slightly higher at control followed by 0.5, 0.1, and 1.0 mM of $HgCl_2$. The phenylpropanoid level decreased at higher doses. In contrast, most of the phenylpropanoid contents showed slight or little accumulation in the plant exposed to $HgCl_2$. From the overall dose, the highest phenylpropanoid contents (mg/g dry weight) were observed for rutin followed by epicatechin, sinapic acid, *trans*-cinnamic acid, *p*-coumaric acid, ferulic acid and caffeic acid (Figure 2).

Effect of $NiSO_4$ on Phenylpropanoid Contents

The plant exposed to different concentrations of NiSO4 showed that overall phenylpropanoid concentration rose significantly. The plants subjected to 100 mM $NiSO_4$ exhibited greater accumulations than those exposed to 10 mM and 50 mM. The total phenylpropanoid accumulation increased with increasing the concentration of heavy metals. Rutin had the

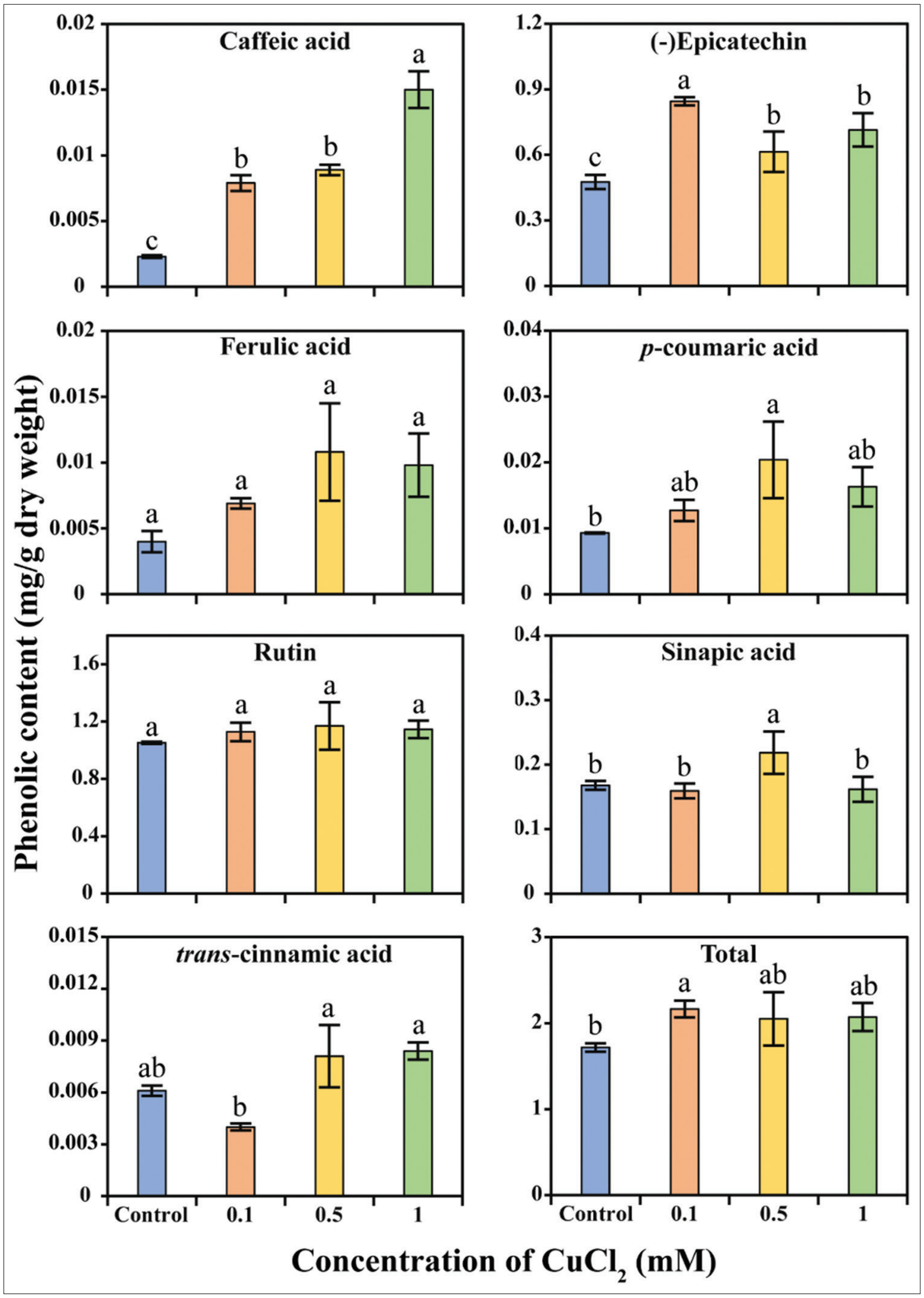

Figure 1: Effect of different concentrations of $CuCl_2$ on phenylpropanoid compounds in *Euonymus alatus*

highest concentration (mg/g dry weight), whereas epicatechin, sinapic acid, p-coumaric acid, trans-cinnamic acid, ferulic acid, and caffeic acid showed close concentrations as that of the rutin. On the other hand, the levels of trans-cinnamic acid and caffeic acid were considerably lower than in the control (Figure 3).

DISCUSSION

The way that plants react to stress may be influenced by phenylpropanoid chemicals. The productivity of the phenylpropanoid biosynthesis pathway in plants varies depending on the type of stress. In this work, we examined the accumulation of several chemicals involved in the phenylpropanoid pathway following exposure to different heavy metals. Using varying doses of $CuCl_2$, $HgCl_2$, and $NiSO_4$, the impact of Cu, Hg, and Ni toxicity on the phenylpropanoid biosynthesis of *E. alatus* was evaluated in this work.

According to our findings, the amount of phenylpropanoid compounds varied depending on the heavy metal employed. In this study, we examined some of the specific phenolic compounds. Among the phenylpropanoid compounds, the

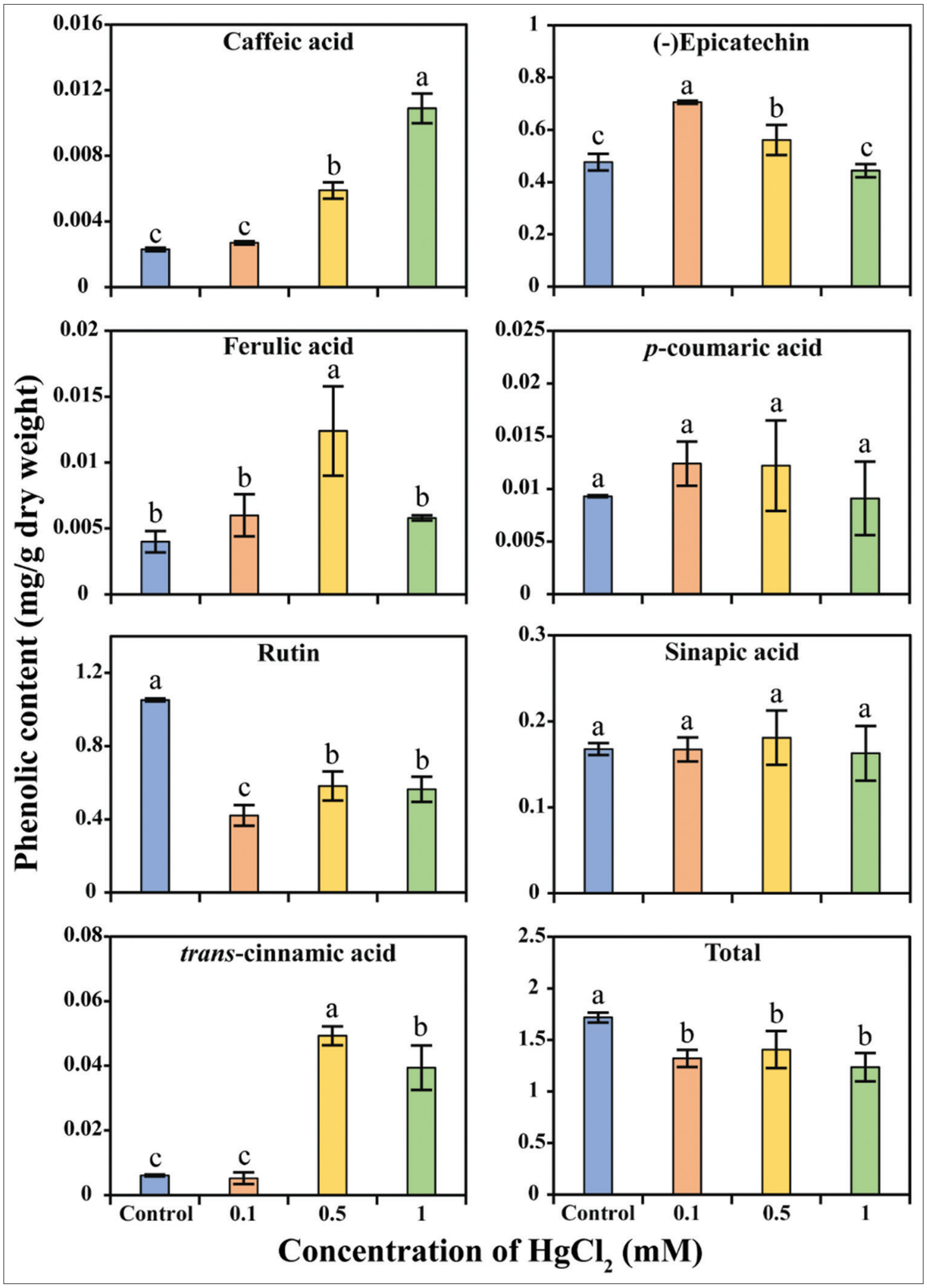

Figure 2: Effect of different concentrations of $HgCl_2$ on phenylpropanoid compounds in *Euonymus alatus*

highest accumulation was noticed for rutin followed by epicatechin, sinapic acid, p-coumaric acid, trans-cinnamic acid, ferulic acid, and caffeic acid in all the exposed heavy metals. However, their concentration varied due to the variation of heavy metals and their concentration. This result was consistent with the previous study that the exposure of *Robinia pseudoacacia* to various heavy metals, the result showed that the concentration of the phenylpropanoid varied depending on the concentration of the heavy metals (Kim *et al.*, 2020). According to earlier research, the phenylpropanoid molecules mentioned above have potent antioxidant properties (Mehra *et al.*, 2013; Enogieru *et al.*, 2018; Grzesik *et al.*, 2018; Shen *et al.*, 2019; Xu *et al.*, 2019), and exposure of plants to heavy metals boosted their accumulation. This indicates a relationship between the concentration of heavy metals in the plant's organs and the accumulation of different phenylpropanoid chemicals (Kisa *et al.*, 2016). It was noticed that comparatively the use of different concentrations of $CuCl_2$ showed the maximum accumulation of total phenylpropanoid biosynthesis than $HgCl_2$ and $NiSO_4$. A similar result was obtained in the previous study that *R. pseudoacacia* was exposed to various heavy metals, the $CuCl_2$ exposure showed the highest accumulation of phenylpropanoid compounds (Kim *et al.*, 2020). In this study, the maximum amount of phenylpropanoid was found

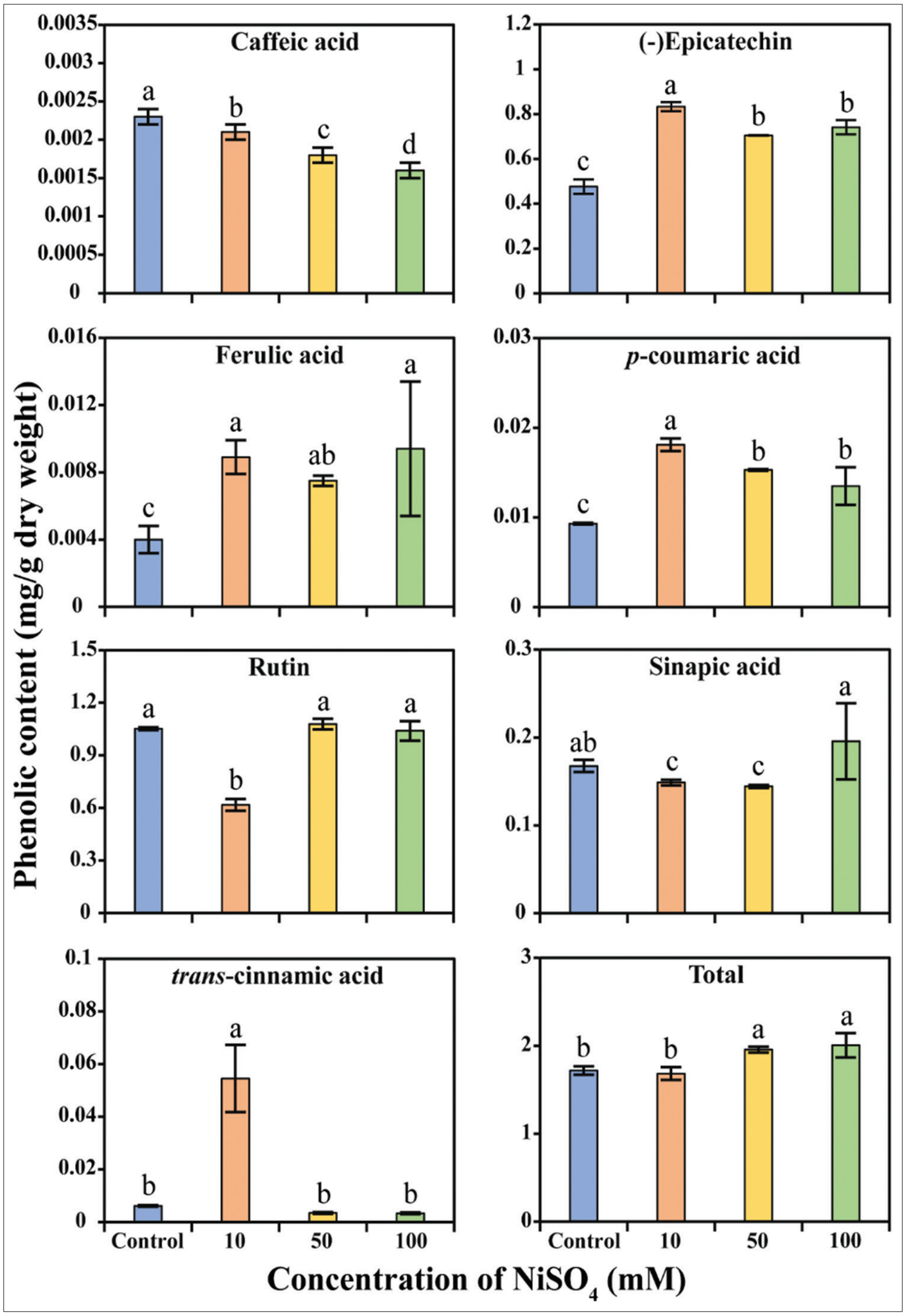

Figure 3: Effect of different concentrations of $NiSO_4$ on phenylpropanoid biosynthesis in *Euonymus alatus*

in 0.1 mM $CuCl_2$, however, when *E. alatus* was exposed to 1 mM $HgCl_2$, the amount of phenylpropanoid decreased. In fact, with the use of $HgCl_2$, the accumulation of phenylpropanoid decreased compared to control. This finding suggests that a decline in important enzyme activity associated with the phenylpropanoid biosynthesis pathway may be the cause of the drop in phenylpropanoid concentrations (Chung *et al.*, 2006; Kisa *et al.*, 2016). This suggested that a significant amount of different phenylpropanoid compounds accumulated in *E. alatus* to shield them against a variety of heavy metal stressors.

CONCLUSION

The present investigation revealed that the plant's total phenylpropanoid compound levels were progressively elevated in *E. alatus* upon exposure to heavy metals. In all the heavy metal exposure the highest individual phenylpropanoid content was achieved in rutin and epicatechin, which leads to the highest accumulation of total phenolic content. In light of this, the synthesis of phenylpropanoid chemicals may help to lessen the formation of ROS generated by stress brought on by heavy metals and let *E. alatus* thrive in heavily polluted environments. This partially elucidates why *E. alatus* is tolerant to various heavy metals, making it a useful species for the bioremediation of heavy metals from the environment. This helps to explain that *E. alatus* is a valuable species for the bioremediation of heavy metals from the environment due to its tolerance to different heavy metals.

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AUTHOR'S CONTRIBUTION

J.K.K. and S.U.P. designed the experiments. R.S., H.-H.K., B.V.N., J.Y. and B.B.P. performed the experiments and analyzed the data. R.S. wrote the manuscript. J.K.K. and S.U.P. revised the manuscript. All authors read and approved the final manuscript.

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