Effect of Elicitors Application on Phenolics Content, Antioxidant Properties and Curcumin Content of *in vitro* Propagated Leaves and Rhizomes of *Curcuma caesia*

Zainol Haida [a,b] and Mansor Hakiman [a,b,*]

Curcuma caesia Roxb., commonly known as black turmeric, has high medicinal and economic value, primarily due to its wide range of bioactive compounds. Due to overharvesting and habitat destruction, *C. caesia* populations have been reduced drastically. Conventional propagation through rhizomes is inefficient due to susceptibility to microbial infections, long maturation periods, and unstable bioactive compounds content. This study aimed to enhance phenolics content, antioxidant properties, and curcumin content in *in vitro* propagated *C. caesia* through application of elicitors. *C. caesia* plantlets were treated with different concentrations of methyl jasmonate, salicylic acid, silver nitrate, chitosan, and yeast extract. The total phenolics, flavonoids, tannins, antioxidants, and curcumin content in leaves and rhizomes were assessed. At 200 µM, methyl jasmonate significantly enhanced total phenolic, flavonoid, and tannin content and antioxidant properties in leaves and rhizomes. Silver nitrate (200 µM) and methyl jasmonate (100 to 200 µM) yielded the highest total curcumin content. Overall, methyl jasmonate was the most effective elicitor for improving phenolics content, antioxidant activities and curcumin accumulation. These findings highlight the potential of elicitor-based strategies, particularly methyl jasmonate, as an effective and sustainable approach to enhance the yield and quality of pharmaceutically important bioactive compounds in *C. caesia,* offering promising prospects for its conservation, commercial cultivation and medicinal applications.

DOI: 10.15376/biores.20.3.7574-7591

Keywords: Phenolics; Antioxidant; Curcumin; Biotic elicitor; Abiotic elicitor

*Contact information: a: Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; b: Laboratory of Sustainable Resources Management, Institute of Tropical Forestry and Forest Products, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; *Corresponding author: mhakiman@upm.edu.my*

INTRODUCTION

The Zingiberaceae is a rhizomatous perennial herb family comprising 52 genera with over 1,500 species (Jana *et al.* 2019). This family is well known for its aromatic rhizomes. Several popular species within this family are *Zingiber, Kaempferia, Etlingera* and *Curcuma*, which are frequently studied due to high economic value, particularly in Asian region countries including China, India, Indonesia, Thailand, Vietnam, and Malaysia (Pham *et al.* 2021; Van *et al.* 2021). The genus *Curcuma* includes more than 100 species, and it has been extensively used in traditional medicine and food industry as a flavoring and coloring agents (Dosoky and Setzer 2018; Sultana *et al.* 2021).

Curcuma species have high market demand due to their numerous bioactive compounds. One of the valuable species is *Curcuma caesia* Roxb., which is commonly known either as black turmeric or as Kali Haldi in India. *C. caesia* is indigenous to India, where it has been traditionally used to treat fever, asthma, leukoderma, stomach aches, and bronchitis (Fozia *et al.* 2012; Das *et al.* 2013; Amalraj *et al.* 2017). Meanwhile, pharmacological studies have demonstrated that *C. caesia* extract from rhizomes or leaves possesses various biological activities such as anti-acne, anticancer, anti-asthmatic, antimicrobial, anti-inflammatory and antidiabetic (Kaur *et al.* 2018; Borah *et al.* 2019; Jain *et al.* 2019; Rai *et al.* 2020). The documented pharmacological properties of *C. caesia* and supporting scientific evidence have led to overharvesting of its raw materials. In addition, rapid human population growth has resulted in habitat destruction and threatening many plant species including *C. caesia* (Yadav *et al.* 2021). Recent statistics indicate a severe decline in *C. caesia* populations, leading to its classification as an endangered species (Borah *et al.* 2019). Therefore, plant tissue culture offers a viable alternative for propagation and secondary metabolites of *C. caesia.* Plant tissue culture, also known as *in vitro* or micropropagation, is an advanced biotechnological tool widely used for various purposes (Kataria *et al.* 2022). The key advantages of plant tissue culture are rapid multiplication, production of disease-free plantlets, and ability to produce and manipulate secondary metabolites (Chandana *et al.* 2018).

In plant tissue culture, manipulation of secondary metabolites can be done *via* elicitation processes. Although plants have the genetic potential to produce vast array of secondary metabolites, only a limited portion is typically synthesized under natural conditions. The biosynthesis of these compounds is highly influenced by internal developmental and external environmental stimuli (Thakur *et al.* 2019). To overcome this limitation, elicitors are supplemented into the basal medium to stimulate secondary metabolites production. Elicitors can be divided into biotic and abiotic elicitors. Biotic elicitors originate from biological sources such as plant hormones (example: methyl jasmonate, salicylic acid and gibberellic acid), plant cell fragments (example: chitin), and bacterial strains (Bhaskar *et al.* 2021). In contrast, abiotic elicitors include chemicals and physical agents such as heavy metals, osmotic stress and UV radiation (Gupta and Jain 2022). The concentration and type of elicitors used varies depending on the plant species, exposure duration, and plant developmental stage.

C. caesia is conventionally propagated through rhizomes. However, conventional propagation of *C. caesia* is lengthy, requiring approximately nine months for the plant to mature (Borah *et al.* 2019). To promote sustainable utilization of *C. caesia*, it is important to consider strategies that reduce the need for repeated planting. *C. caesia* is a perennial rhizomatous herb, and partial harvesting of rhizomes can allow the remaining plant to continue growing and regenerate under favorable conditions and adequate care. Large-scale harvesting to fulfill the industry demands can lead to depletion of natural populations. Additionally, the production of secondary metabolites in conventionally propagated plants is highly unstable due to environmental factors such as fertilizer application, plant maintenance, and climate. These limitations make conventional propagation unsuitable for large-scale production of high-quality raw materials with stable secondary metabolites content. To address these challenges, *in vitro* propagation was employed in this work to produce uniform plant material. Elicitation was applied as complementary strategy to enhance the accumulation of bioactive compounds under controlled environments. The objective of this study was to determine the phenolics

content, antioxidant properties and curcumin content of micropropagated leaves and rhizomes of *C. caesia* as influenced by supplementation of elicitors.

EXPERIMENTAL

Planting Materials

Twelve-week-old plantlets from the stock plants were cut to approximately 1 ± 0.5 cm in size. The microshoots were inoculated on the MSB5 basal medium supplemented with 15 µM BAP, 6 µM IBA and 50 g/L sucrose for ten weeks (Haida *et al.* 2022). After ten weeks, the plantlets were transferred onto the same basal medium formulation with an addition of different types and concentrations of elicitor. The exposure of plantlets to elicitor was conducted for two weeks. The analysis on phenolics content, antioxidant properties and total curcumin content were carried out after the end of elicitors exposure.

Preparation of Elicitors

Salicylic acid

The stock solution of salicylic acid (R&M Chemicals, Selangor, Malaysia) was prepared at a concentration of 1000 mg/L (7.24 mM). Briefly, a total of 0.1 g of salicylic acid powder was placed in a beaker. A few drops of 1 M of sodium hydroxide were added. After the powder was fully dissolved, the volume was made up to 100 mL using distilled water. The salicylic acid stock solution was further diluted to the concentration of 100, 150 and 200 µM for the experiments (Bayraktar *et al.* 2016).

Methyl jasmonate

The 95% of methyl jasmonate liquid solution (4.36 M) was purchased for Sigma-Aldrich (St. Louis, USA). For the experimentation, the stock solution of methyl jasmonate was diluted to the concentration of 100, 150 and 200 µM by using 99% of ethanol (Bayraktar *et al.* 2016).

Silver nitrate

The silver nitrate stock solution (R&M Chemicals, Selangor, Malaysia) was prepared at a concentration of 1000 mg/L (5.89 mM). A total of 0.1 g of silver nitrate powder was placed in a beaker and 100 mL of distilled water was added. The solution was stirred until the powder was fully dissolved. For the experimentation, the stock solution was diluted to the concentration of 100, 150, and 200 µM during basal medium preparation (Paeizi *et al.* 2018).

Chitosan

The chitosan powder (≥75%, deacetylated from shrimp shells) was purchased from Sigma-Aldrich (St. Louis, USA). The chitosan stock solution was prepared at a concentration of 1000 mg/L. A total of 0.1 g of chitosan powder was put in each beaker and placed on a hot plate. Then, the chitosan powder was dissolved using 0.1 M of acetic acid on a medium heat with a constant stirred on a hot plate. After all the chitosan powder was dissolved, the volume was made up to 100 mL using distilled water. The stock solution of chitosan was diluted to the concentration of 100, 150 and 200 mg/L and supplemented into the basal medium (Vanda *et al.* 2019).

Yeast

The yeast powder (R&M Chemicals, Selangor, Malaysia) at the concentration of 100, 150 and 200 mg/L were added during the preparation of basal medium (Rasouli *et al.* 2021). The basal medium was stirred on the hot plate until all the powder was fully dissolved.

Analysis of Phenolics Content and Antioxidant Properties

Sample preparation and extraction

The plantlets were taken out from the culture vessels after two weeks exposure to elicitors. The plantlets were cut, and the leaves and rhizomes were separated. The roots were removes and the samples were cleaned under running tap water to remove the attached basal medium. The leaves and rhizomes samples were dried in an oven at a temperature of 50 °C for 24 h.

After drying process, the leaves and rhizomes samples were subjected to an extraction process. The extraction of leaves sample was conducted with the following extraction conditions; 54.02 mL/g solvent-solid ratio, 70% methanol concentration, 70 °C of extraction temperature, and 105 min of extraction time. Meanwhile, extraction of rhizomes sample was conducted as follows; 60 mL/g solvent-solid ratio, 57.38% acetone concentration, 50 °C of extraction temperature and 195 min of extraction time. All the extraction samples were kept in the refrigerator at a temperature of 4 °C and used within five days. The extraction conditions for leaves and rhizomes were optimized using Response Surface Methodology prior to this experiment.

Quantification of phenolics content and antioxidant activities

The phenolics content and antioxidant activities were determined using the protocol as described by Haida *et al*. (2020).

Analysis of Total Curcumin Content

The quantification of total curcumin content was carried out by adding 0.05 g of leaves or rhizomes crude powder in 25 mL of 95% of ethanol. The solution was sonicated for 10 min and 50 mL of ethanol was added. The solution was filtered, and 2 mL of sample extract was diluted using 95% of ethanol (25 mL). The absorbance of extract was measured at 425 nm using UV-vis spectrophotometry. The calibration curve was constructed using different concentration of curcumin (Pawar *et al.* 2018).

Statistical Analysis

The experiment was set up in Completely Randomized Design (CRD) with four replications and each replication consisted of 10 samples. The statistical analysis was conducted using analysis of variance (ANOVA) and the means separation was evaluated using Duncan's Multiple Range Test (DMRT) with $P<0.05$. The statistical analysis was performed using Statistical Analysis System (SAS) version 9.4.

RESULTS AND DISCUSSION

Phenolics Content and Antioxidant Properties in *In Vitro* Propagated Leaves of *C. caesia* as Affected by Supplementation of Elicitors

Various types of elicitors were supplemented in the basal medium to determine its effect on phenolic content and antioxidant properties in leaves of *C. caesia*. The results in Table 1 show that the supplementation of elicitors at various concentrations were significantly affected the total phenolic, flavonoid and tannin content in *in vitro* propagated leaves of *C. caesia*. The total phenolic content produced in this study ranged from 17.65 to 25.51 mg GAE/g DW. The highest total phenolic content was significantly produced from the treatment of 200 µM methyl jasmonate with 25.51 mg GAE/g DW, followed by the treatment of 150 µM silver nitrate and methyl jasmonate with 24.36 and 23.66 mg GAE/g DW, respectively. The lowest total phenolic content was recorded from the control treatment with 18.39 mg GAE/g DW.

The total flavonoid content recorded ranged between 15.04 and 23.60 mg RE/g DW (Table 1). The highest total flavonoid content was significantly produced from the treatment of 200 µM methyl jasmonate with 23.60 mg RE/g DW. Meanwhile, there was no significant difference between the treatment of 150 µM silver nitrate and 200 mg/L of yeast on total flavonoid content with 21.35 and 20.81 mg RE/g DW, respectively. Among all the treatments, there was no significant difference between the control, 100, 150, 200 µM salicylic acid, 200 µM silver nitrate and 100 mg/L yeast on total flavonoid content in *in vitro* propagated leaves of *C. caesia.* The values recoded were 15.04, 15.40, 15.67, 14.50, 15.22, and 14.40 mg RE/g DW, respectively.

The total tannin content accumulated in the *in vitro* propagated leaves of *C. caesia* ranged between 1.52 and 2.48 mg TAE/g DW (Table 1). All the treatments were able to produce more than 2 mg TAE/g DW except the control, 200 µM silver nitrate, 100 and 150 mg/L chitosan, and 100 mg/L yeast with 1.89, 1.91, 1.92, 1.52 and 1.81 mg TAE/g DW, respectively. The highest total tannin content was significantly produced from the treatment of 200 µM methyl jasmonate with 2.48 mg TAE/g DW. The second highest total tannin content was accumulated from the treatment of 150 µM silver nitrate and 200 mg/L yeast with 2.36 and 2.35 mg TAE/g DW, respectively. The lowest accumulation of total tannin content was obtained from the treatment of control and 200 µM silver nitrate with 1.89 and 1.91 mg TAE/g DW, respectively.

The antioxidant properties including DPPH free radical scavenging activity, ferrous ion chelating activity, and superoxide anion radical scavenging activity in *in vitro* propagated leaves of *C. caesia* were significantly affected by supplementation of elicitors. Based on the results in Table 1, the highest DPPH free radical scavenging activity was significantly exhibited from the treatment of 100 µM methyl jasmonate with 20.44 mg TE/g DW. Meanwhile, the treatment of 200 µM silver nitrate, 100 mg/L chitosan and 200 mg/L of yeast exhibited slightly lowered DPPH free radical scavenging activity than 100 µM methyl jasmonate with 20.00, 20.11 and 20.01 mg TE/g DW, respectively. The lowest DPPH free radical scavenging activity exhibited from the treatment of 100 µM salicylic acid with 17.97 mg TE/g DW.

In the ferrous ion chelating activity analysis, all the treatments except 100 µM silver nitrate successfully exhibited more than 80% of chelating activity (Table 1). The highest ferrous ion chelating activity was recorded from the basal medium fortified with 150 mg/L chitosan with 86.2% of chelating activity, followed by the basal medium

fortified with 200 µM silver nitrate and 100 mg/L yeast with 85.0 and 85.1% of chelating activity, respectively. The lowest ferrous ion chelating activity was significantly produced from the basal medium supplemented with 100 µM silver nitrate with 79.41% of chelating activity.

The analysis of superoxide anion radical scavenging activity also was conducted on *in vitro* propagated leaves of *C. caesia*. The superoxide anion radical scavenging activity recorded ranged between 17.0 and 41.2% of inhibition (Table 1). As observed in Table 2, the control treatment only exhibited 22.2% of inhibition.

By exposing the plantlets to the various elicitors, the superoxide anion radical scavenging activity was enhanced, except for the treatment of 200 µM salicylic acid, 100 µM silver nitrate, 100 and 200 mg/L chitosan, and 100 mg/L yeast with 17.0, 19.5, 19.1, 19.4, and 21.4% of inhibition, respectively. The highest superoxide anion radical scavenging activity was recorded from the treatment of 200 µM methyl jasmonate with 41.2% of inhibition and the treatment of 200 µM salicylic acid significantly exhibited the lowest percentage with 17.0% on inhibition, respectively.

Elicitation is known as one of the important techniques to enhance the production of secondary metabolites. Based on the results in Table 1, the basal medium treated with methyl jasmonate significantly recorded the highest increment in the production of total phenolic content, total flavonoid content, total tannin content, DPPH free radical scavenging activity, and superoxide anion radical scavenging activity in *in vitro* propagated leaves of *C. caesia*. Besides methyl jasmonate, supplementation of salicylic acid, silver nitrate, chitosan, and yeast also significantly affected the phenolics content and antioxidant activities. The findings in this study were consistent with previous studies reported on *Dysphania ambrosioides*, *Baccharoides anthelmintica*, *Aloe barbadensis*, *Salvia virgata* and *Rauvolfia serpentina* (Dey *et al.* 2020; de Carvalho *et al.* 2020; Rajan *et al.* 2022; Dowom *et al.* 2022; Shafighi *et al.* 2022).

Elicitors were supplemented exogenously in the basal medium to induce stress in plant, which directly influenced the production of plant secondary metabolites. According to Kim *et al*. (2020), application of methyl jasmonate, chitosan, and salicylic acid in the basal medium of *Pimpinella brachycarpa* significantly increased the expression levels of phenylpropanoid-related genes and enhanced the phenolic compounds accumulation. In the plant, phenylpropanoids play important biological functions such as antioxidants, phytoalexins, and signaling molecules against biotic and abiotic stresses (Eray *et al.* 2020). The increment of key enzymes activity such as chalcone isomerase and ammonium lyase in the phenylalanine pathway leads to an increment of secondary metabolites production (Dixon *et al.* 2002; Rachidi *et al.* 2021).

Table 1. Effect of Different Concentrations of Elicitors on Phenolics Content and Antioxidant Properties of *in vitro* Propagated Leaves of *C. caesia*

Type of Elicitor	Concentration	Total Phenolic Content (mg GAE/g DW)	Total Flavonoid Content (mg RE/g DW)	Total Tannin Content (mg TAE/g DW)	DPPH (mg TE/g DW)	Ferrous Ion Chelating (%)	Superoxide Anion (%)
Control	0	18.39 l	15.04 f	1.89 j	18.29 g	84.46 d	22.15 hi
Salicylic acid	100 µM	20.31 g	15.40 f	2.00 h	17.97 h	79.47 l	26.23 e
	150 µM	20.70 f	15.67 ef	2.05 g	19.77 c	80.08 k	22.81 gh
	200 µM	21.33 e	14.50 f	2.09 f	17.36 i	81.19 i	17.04 k
Methyl jasmonate	100 µM	20.40 g	17.12 d	2.19 e	20.44 a	80.42 j	32.34 c
	150 µM	23.66 c	17.93 cd	2.32 c	19.06 e	83.62 g	39.04 b
	200 µM	25.51 a	23.60 a	2.48 a	18.90 f	84.84 c	41.21 a
Silver nitrate	100 µM	19.52 j	18.20 cd	2.05 g	19.05 e	84.04 f	19.50 j
	150 µM	24.36 b	21.35 b	2.36 b	19.26 d	84.56 d	24.10 f
	200 µM	19.89 i	15.22 f	1.91 ij	20.00 b	85.01 bc	27.48 d
Chitosan	100 mg/L	20.67 f	16.85 de	1.92 i	20.11 b	84.58 d	19.09 j
	150 mg/L	17.65 m	17.39 d	1.52 l	16.18 j	86.18 a	31.97 c
	200 mg/L	22.37 d	17.21 d	2.23 d	18.29 g	83.14 h	19.45 j
Yeast	100 mg/L	18.72 k	14.50 f	1.81 k	14.83 k	85.12 b	21.45 i
	150 mg/L	20.14 h	19.19 c	2.00 h	17.30 i	84.60 d	23.07 g
	200 mg/L	22.34 d	20.81 b	2.35 bc	20.01 b	84.25 e	26.38 e

Means followed by the same letter within the columns were not significantly different at P<0.05 using Duncan's Multiple Range Test.

Phenolics Content and Antioxidant Properties in *In Vitro* Propagated Rhizomes of *C. caesia* as Affected by Supplementation of Elicitors

The effect of elicitors on total phenolic, flavonoid, and tannin also were quantified in *in vitro* propagated rhizomes of *C. caesia* (Table 2). The total phenolic content recorded ranged between 17.6 and 24.7 mg GAE/g DW. Based on the results in Table 2, basal medium supplemented with 200 µM methyl jasmonate significantly produced the highest total phenolic content with 22.5 mg GAE/g DW. The basal medium supplemented with 150 µM salicylic acid recorded the second highest total phenolic content with 23.8 mg GAE/g DW. The lowest total phenolic content was recorded from the basal medium supplemented with 100 µM silver nitrate with 17.6 mg GAE/g DW. The results in Table 2 show that the accumulation of total phenolic content in the basal medium supplemented with silver nitrate increased as the concentration of silver nitrate increased from 100 to 150 µM with 17.6 and 23.8 mg GAE/g DW and decrement of total phenolic content observed when the concentration of silver nitrate was increased to 200 µM with 22.5 mg GAE/g DW. Meanwhile, addition of methyl jasmonate and chitosan in the basal medium successfully enhanced the total phenolic content production as the value recorded was increased from 22.3 to 24.7 mg GAE/g DW for methyl jasmonate and 18.8 to 22.1 mg GAE/g DW for chitosan, respectively.

The total flavonoid content in *in vitro* propagated rhizomes of *C. caesia* ranged from 11.7 to 27.3 mg RE/g DW (Table 2). The supplementation of 200 µM methyl jasmonate significantly produced the highest total flavonoid content with 27.5 mg RE/g DW. In addition, methyl jasmonate at 150 and 100 µM also significantly produced the second and third highest in accumulation of total flavonoid content with 22.5 and 20.2 mg RE/g DW, respectively. The supplementation of 100 µM silver nitrate resulted the lowest accumulation of total flavonoid content with 11.7 mg RE/g DW. The total flavonoid content accumulated from the salicylic acid, methyl jasmonate and chitosan showed the same trend in which increment of concentration lead to the increment of total flavonoid content. In salicylic acid, methyl jasmonate and chitosan treatment, the total flavonoid content was increased from 11.7 to 18.2, 20.2 to 27.3, and 12.5 to 15.4 mg RE/g DW, respectively. In the silver nitrate and yeast treatment, the highest accumulation of total flavonoid content achieved was 18.2 and 16.4 mg RE/g DW from the concentration of 200 µM and 200 mg/L, respectively.

Based on the results in Table 2, the total tannin content recorded was between 1.94 to 2.92 mg TAE/g DW. The highest total tannin content was significantly produced from the basal medium fortified with 200 µM methyl jasmonate with 2.92 mg TAE/g DW, followed by the treatment of 200 µM silver nitrate with 2.83 mg TAE/g DW. Meanwhile, the lowest accumulation of total tannin content was obtained from the treatment of 100 and 150 µM silver nitrate and 100 mg/L yeast. The values were 1.94, 1.97 and 1.96 mg TAE/g DW, respectively, which were not significantly different from each other. Based on the results in Table 2, the application of salicylic acid, methyl jasmonate, silver nitrate, chitosan, and yeast showed a rising trend of total tannin content production as the elicitors concentrations were increased. The increment of total tannin content recorded was from 2.07 to 2.83 mg TAE/g DW for salicylic acid, 2.36 to 2.92 mg TAE/g DW for methyl jasmonate, 1.94 to 2.42 mg TAE/g DW for silver nitrate, 2.14 to 2.36 mg TAE/g DW for chitosan, and 1.96 to 2.23 mg TAE/g DW for yeast treatment, respectively. Besides, the results also showed that application of 100 and 150 µM silver nitrate and 100 mg/L yeast were produced lowered accumulation of total tannin content as compared to the control treatment.

The application of elicitors significantly affected the antioxidant properties, including DPPH free radical scavenging activity, ferrous ion chelating activity, and superoxide anion radical scavenging activity of *in vitro* propagated rhizomes of *C. caesia*. The results in Table 2 show that application of 200 µM silver nitrate significantly exhibited the highest DPPH free radical scavenging activity with 23.63 mg TE/g DW. This was closely followed by the treatment of 150 µM salicylic acid and 200 µM methyl jasmonate with 22.90 and 22.79 mg TE/g DW, respectively. Application of 100 µM salicylic acid significantly produced the lowest DPPH free radical scavenging activity with 13.36 mg TE/g DW. In this analysis, most of the treatments applied including 100 and 200 µM silver nitrate, 100 and 150 µM methyl jasmonate and all concentrations of chitosan and yeast treatments exhibited lower DPPH free radical scavenging activity as compared to the control treatment.

The ferrous ion chelating activity from the *in vitro* propagated rhizomes of *C. caesia* ranged between 47.7 and 77.7% of chelating activity (Table 2). The highest ferrous ion chelating activity was recorded from the basal medium supplemented with 100 mg/L chitosan with 77.8% of chelating activity, followed by 74.8 and 73.5% of chelating activity from the treatment of 100 µM salicylic acid and 200 mg/L yeast treatment, respectively.

Meanwhile, the lowest ferrous ion chelating activity was significantly produced from the control and 100 µM methyl jasmonate treatment with 47.7 and 48.1% of chelating activity. Based on the observation in Table 2, all the treatments except the control and 100 µM methyl jasmonate exhibited above 50% of chelating activity. In addition, 70% of chelating activity was recorded from the treatment of 100 and 200 µM salicylic acid, 100, 150, and 200 mg/L chitosan and 150 and 200 mg/L yeast.

As for superoxide anion radical scavenging activity, basal medium treated with 100 µM salicylic acid exhibited the highest scavenging activity with 48.1% (Table 2). The application of elicitors significantly reduced the superoxide anion radical scavenging activity, as the values recorded were lower than the control treatment except for the treatment of 100 µM salicylic acid. The treatment of chitosan at all concentrations produced the lowest superoxide anion radical scavenging activity with the value ranging from 27.7 to 29.7%. In contrast, the previous findings on *Origanum vulgare* and *Salvia bulleyana* reported that application of elicitors were able to enhance the superoxide anion radical scavenging activity (Li *et al.* 2021b; Krzemińska *et al.* 2022). The variation in the results obtained might be due to the effective concentration of elicitors may vary for different plant species, culture methods, and classes of bioactive compounds present in the plant (Wang *et al.* 2016; Du *et al.* 2020).

Table 2. Effect of Different Concentrations of Elicitors on Phenolics Content and Antioxidant Properties of *in vitro* Propagated Rhizomes of *C. caesia*

Type of Elicitor	Concentration	Total Phenolic Content (mg GAE/g DW)	Total Flavonoid Content (mg RE/g DW)	Total Tannin Content (mg TAE/g DW)	DPPH (mg TE/g DW)	Ferrous Ion Chelating (%)	Superoxide Anion (%)
Control	0	21.06 e	13.81 g	2.04 h	22.22 d	47.66 j	46.03 b
Salicylic acid	100 µM	17.63 k	11.71 j	2.07 h	13.36 l	74.84 b	48.08 a
	150 µM	23.84 b	15.61 f	2.54 c	22.90 b	62.90 ef	31.05 h
	200 µM	22.49 c	18.21 d	2.83 b	19.28 gh	70.33 d	28.14 j
Methyl jasmonate	100 µM	22.32 cd	20.21 c	2.36 e	18.92 hi	48.06 j	42.31 d
	150 µM	22.41 cd	22.51 b	2.56 c	19.59 g	64.31 e	40.29 e
	200 µM	24.68 a	27.31 a	2.92 a	22.79 bc	52.17 i	44.70 c
Silver nitrate	100 µM	19.30 h	15.71 f	1.94 i	22.25 d	61.96 fg	43.97 c
	150 µM	18.08j	12.91 hi	1.97 i	22.51 cd	52.37 i	37.31 f
	200 µM	21.12 e	18.21 d	2.42 d	23.63 a	60.31 g	33.37 g
Chitosan	100 mg/L	18.79 i	12.51 i	2.14 g	20.10 f	77.67 a	29.69 i
	150 mg/L	19.86 g	13.41 gh	2.21 f	20.38 f	72.35 c	28.33 j
	200 mg/L	22.13 d	15.41 f	2.36 e	21.69 e	72.02 cd	27.74 j
Yeast	100 mg/L	18.70 i	15.21 f	1.96 i	18.57 ji	56.07 h	37.57 f
	150 mg/L	18.10 j	12.91 hi	2.08 h	18.46 j	72.48 c	30.13 i
	200 mg/L	20.41 f	16.41 e	2.23 f	15.30 k	73.59 bc	33.00 g

Means followed by the same letter within the columns were not significantly different at $P<0.05$ using Duncan's Multiple Range Test.

In this study, different concentrations of salicylic acid, methyl jasmonate, silver nitrate, chitosan, and yeast were applied in the basal medium to regulate the production of phenolics content and antioxidant activities. It was found that application of 200 µM methyl jasmonate significantly accumulated the highest total phenolic, total flavonoid, and total tannin content in *in vitro* propagated rhizomes of *C. caesia*. The efficiency of methyl jasmonate was also reported on secondary metabolites accumulation in *Mentha spicata* and *Aster scaber* (Ghimire *et al.* 2019; Yousefian *et al.* 2020). According to Ghimire *et al*. (2019), rises in total phenolic and total flavonoid content were observed after application of methyl jasmonate and yeast into the hairy root culture of *Aster scaber*. A recent study conducted on *Salvia virgata* revealed that the production amounts of total phenolic, total flavonoid, rosmarinic acid, and salvianolic acid A were significantly affected after application of yeast, silver nitrate, and methyl jasmonate (Dowom *et al.* 2022). It is proposed that methyl jasmonate can function as a signaling molecule to stimulate the biosynthesis of phenolics production *via* the activation of methyl jasmonate responsive transcription factors (Sun *et al.* 2019; Deng *et al.* 2020). Meanwhile, the stimulatory effect of yeast on secondary metabolites production might be related to its organic and compounds such as polysaccharide and peptide (Baenas *et al.* 2014).

Theoretically, signal transduction pathways are induced by the application of elicitors, resulting in the plant secondary metabolites production. The specific receptors in the plasma membrane are perceived by elicitors and result in depolarization of plasma membrane. This can lead to the activation of plasma membrane channels, such as the K^+/H^+ antiport channel. The exchange of K^+/H^+ caused the transient cytoplasmic acidification. The Cl^- efflux further acts as a signal for the secondary metabolites production (Zhao *et al.* 2005; Narayani and Srivastava 2017). In addition, perception of elicitor by the receptors may also activate the G-protein couples to them or the mitogen activated protein kinase (MAPK) cascade. The MAPK cascade contains a MAPK kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK, all of which are activated by phosphorylation. The Ca^{2+}/ion fluxes are induced by the elicitor, resulting in elevated levels of cytoplasmic free calcium ions. As a result, many intracellular processes are triggered, including plant defense responses (Narayani and Srivastava 2017). Although the observed increases in phenolics content and antioxidant activities may appear modest in absolute terms, they are nonetheless biologically and commercially meaningful. *In vitro* elicitation offers a reproducible and scalable platform for enhancing the accumulation of targeted bioactive compounds, especially in species such as *C. caesia*, for which conventional propagation is slow and metabolites yields are highly variable under field conditions. Moreover, even a relatively small increase of bioactive compounds can significantly impact the overall antioxidant potential and therapeutic efficacy of the extracts, particularly in pharmaceutical and nutraceutical applications.

Total Curcumin Content in *In vitro* Propagated Leaves and Rhizomes of *C. caesia* as Affected by Supplementation of Elicitors

In this present study, the total curcumin content in the *in vitro* propagated leaves and rhizome of *C. caesia* were evaluated using the UV-vis spectrophotometry method. Based on the results in Table 3, the highest total curcumin content recorded in leaves was from the application of 200 µM salicylic acid and 100 µM methyl jasmonate with 3.83 and 3.78 mg CE/g DW, respectively. The same treatments also recorded the highest percentage of curcumin content in leaves with 5.25 and 5.14%, respectively. It was found

that the lowest total curcumin content and percentage of curcumin content were from the control treatment with 3.23 mg CE/g DW and 4.11%, respectively. The results in Table 3 revealed that application of elicitors significantly enhanced the total curcumin content and percentage of curcumin content in leaves. However, among all the elicitor treatments, only the treatment of 200 µM of salicylic acid and 100 µM methyl jasmonate successfully enhanced the percentage of curcumin content above 5%.

Table 3. Effect of Different Concentrations of Elicitors on Total Curcumin Content and Curcumin Percentage of *in vitro* Propagated Leaves of *C. caesia*

Type of Elicitor	Concentration	Curcumin Content (mg CE/g DW)	Curcumin Percentage (%)
Control	0	3.23 g	4.11 h
Salicylic acid	100 µM	3.30 f	4.25 g
	150 µM	3.38 e	4.40 ef
	200 µM	3.83 a	5.25 a
Methyl jasmonate	100 µM	3.78 a	5.14 a
	150 µM	3.50 cd	4.63 cd
	200 µM	3.55 bc	4.71 bc
Silver nitrate	100 µM	3.51 cd	4.65 c
	150 µM	3.56 bc	4.73 bc
	200 µM	3.43 de	4.50 de
Chitosan	100 mg/L	3.50 cd	4.62 cd
	150 mg/L	3.61 b	4.83 b
	200 mg/L	3.50 cd	4.62 cd
Yeast	100 mg/L	3.58 bc	4.77 bc
	150 mg/L	3.53 c	4.69 bc
	200 mg/L	3.31 f	4.27 fg

Means followed by the same letter within the columns were not significantly different at $P<0.05$ using Duncan's Multiple Range Test.

The analysis of total curcumin content and percentage of curcumin content in *in vitro* rhizomes of *C. caesia* is tabulated in Table 4. The findings in Table 4 show that the treatment of 200 µM methyl jasmonate significantly accumulated the highest total curcumin content and percentage of curcumin content in the rhizomes with 5.79 mg CE/g DW and 8.94%, respectively. In addition, application of 100 and 150 µM salicylic acid, 200 µM silver nitrate and 100 mg/L chitosan were able to accumulate more than 4 mg CE/g DW of total curcumin content. The lowest total curcumin content recorded was not significantly different between the treatment of 200 µM salicylic acid, 100 µM methyl jasmonate, 200 mg/L chitosan, and 200 mg/L yeast with 3.11, 3.20, 3.19, and 3.44 mg CE/g DW, respectively.

Table 4. Effect of Different Concentrations of Elicitors on Total Curcumin Content and Curcumin Percentage of *in vitro* Propagated Rhizomes of *C. caesia*

Type of Elicitor	Concentration	Curcumin Content (mg CE/g DW)	Curcumin Percentage (%)
Control	0	3.65 def	4.92 defg
Salicylic acid	100 µM	4.31 b	6.16 b
	150 µM	4.25 b	6.04 b
	200 µM	3.11 g	3.90 h
Methyl jasmonate	100 µM	3.20 fg	4.06 fgh
	150 µM	3.69 cdef	4.98 defg
	200 µM	5.79 a	8.94 a
Silver nitrate	100 µM	3.84 bcde	5.27 bcde
	150 µM	3.85 bcde	5.29 bcde
	200 µM	4.19 bc	5.93 bc
Chitosan	100 mg/L	4.01 bcd	5.58 bcd
	150 mg/L	3.88 bcde	5.33 bcde
	200 mg/L	3.19 fg	4.04 gh
Yeast	100 mg/L	3.66 def	4.92 defg
	150 mg/L	3.70 cdef	5.00 cdef
	200 mg/L	3.44 efg	4.52 efgh

Means followed by the same letter within the columns were not significantly different at $P<0.05$ using Duncan's Multiple Range Test.

Curcumin is a specific bioactive compound that is specifically present in *Curcumin* species. The quantification of curcumin is commonly conducted using chromatography in order to determine the specific compounds and amounts present in the turmeric sample (Ricciutelli *et al.* 2020; Setyaningsih *et al.* 2021; Insuan *et al.* 2022; Shen *et al.* 2023). However, quantification of total curcumin content can be conducted using UV-vis spectrophotometer at 425 nm (Pawar *et al.* 2018; Maithaa *et al.* 2019). The recent study conducted by Khatun *et al*. (2021) showed that the percentage of total curcumin present in *Curcuma longa* sample was 3.53%. Furthermore, the quantification of curcumin content in *Curcuma longa* collected from different districts in India found that the percentage of curcumin present in Bhandara district was significantly higher (4.32%) as compared to turmeric collected in Satara, Dhule, Nashik, and Mumbai (Pawar *et al.* 2014). It was found that the curcumin content in turmeric was unstable and largely influenced by various factors including climate and plant maintenance. Hence, propagation using plant tissue culture could be a suitable propagation technique for a stable curcumin production.

CONCLUSIONS

1. Elicitor application was found to significantly influence phenolics content, antioxidant activities, and curcumin accumulation in *in vitro* leaves and rhizomes of *Curcuma caesia.*
2. Methyl jasmonate at a concentration of 200 µM was found to be the most effective elicitor, yielding the highest total phenolic, total flavonoid, and total tannin content in both leaves and rhizomes.
3. Elicitor treatment enhanced antioxidant activities in *C. caesia* leaves and rhizomes. Salicylic acid, methyl jasmonate, and silver nitrate significantly increased DPPH and superoxide anion radical scavenging activity. Meanwhile, chitosan and yield extract exhibited the best ferrous ion chelating activity.
4. The highest total curcumin content and curcumin percentage recorded in leaves were from the treatment of 200 µM silver nitrate and 100 µM methyl jasmonate. In rhizomes, 200 µM methyl jasmonate yielded the highest total curcumin content and curcumin percentage.
5. Although the increment in metabolite production may appear moderate, the use of elicitor under *in vitro* conditions offers a controlled and reproducible system to enhance bioactive compound accumulation, particularly valuable for medicinal and high-value crops such as *C. caesia.* These improvements may justify the elicitation process in contexts where standardization, quality and compound yield are critical particularly in pharmaceutical and nutraceutical industries.
6. The possibility that certain elicitors or their metabolites may interact with colorimetric reagents or influence spectrophotometric measurements cannot be entirely excluded. Further investigations utilizing more selective and sensitive analytical techniques such as high-performance liquid chromatography or liquid chromatography-mass spectrometry are recommended to further validate compound specificity and quantification accuracy.

ACKNOWLEDGMENTS

The authors are grateful for the support from the Univesiti Putra Malaysia for the facilities and research funding.

REFERENCES CITED

Amalraj, A., Pius, A., Gopi, S., and Gopi, S. (2017). "Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives – A review," *Journal of Traditional and Complementary Medicine 7*(2), 205-233. DOI: 10.1016/j.jtcme.2016.05.005

Baenas, N., García-Viguera, C., and Moreno, D. A. (2014). "Elicitation: A tool for enriching the bioactive composition of foods," *Molecules* 19(9), 13541-13563. DOI: 10.3390/molecules190913541

Bayraktar, M., Naziri, E., Akgun, I. H., Karabey, F., Ilhan, E., Akyol, B., Bedir, E., and

Gurel, A. (2016). "Elicitor induced stevioside production, *in vitro* shoot growth, and biomass accumulation in micropropagated *Stevia rebaudiana*," *Plant Cell, Tissue and Organ Culture* 127, 289-300. DOI: 10.1007/s11240-016-1049-7

Bhaskar, R., Xavier, L. S. E., Udayakumaran, G., Kumar, D. S., Venkatesh, R., and Nagella, P. (2021). "Biotic elicitors: A boon for the *in-vitro* production of plant secondary metabolites," *Plant Cell, Tissue and Organ Culture* 149*,* 7-24. DOI: 10.1007/s11240-021-02131-1

Borah, A., Paw, M., Gogoi, R., Loying, R., Sarma, N., Munda, S., Pandey, S. K., and Lal, M. (2019). "Chemical composition, antioxidant, anti-inflammatory, anti-microbial and *in-vitro* cytotoxic efficacy of essential oil of *Curcuma caesia* Roxb. leaves: An endangered medicinal plant of North East India," *Industrial Crops and Products* 129, 448-454. DOI: 10.1016/j.indcrop.2018.12.035

Chandana, B. C., Nagaveni, H. C., Lakshmana, D., Kolakar, S. S., and Heena, M. S. (2018). "Role of plant tissue culture in micropropagation, secondary metabolites production and conservation of some endangered medicinal crops," *Journal of Pharmacognosy and Phytochemistry* 7(3S), 246-251.

Das, S., Mondal, P., and Zaman, M. K. (2013). "*Curcuma caesia* Roxb. and it's medicinal uses: A review," *International Journal of Research in Pharmacy and Chemistry* 3*(*2), 370-375.

de Carvalho, A. A., Bertolucci, S. K. V., Honorato, A. D. C., Rocha, T. T., Silva, S. T., and Pinto, J. E. B. P. (2020). "Influence of light spectra and elicitors on growth and ascaridole content using *in vitro* cultures of *Dysphania ambrosioides* L," *Plant Cell, Tissue and Organ Culture* 143(2), 277-290. DOI: 10.1007/s11240-020-01892-5

Deng, C., Wang, Y., Huang, F., Lu, S., Zhao, L., Ma, X., and Kai, G. (2020). "SmMYB2 promotes salvianolic acid biosynthesis in the medicinal herb *Salvia miltiorrhiza*," *Journal of Integrative Plant Biology* 62(11), 1688-1702. DOI: 10.1111/jipb.12943

Dey, A., Nandy, S., Nongdam, P., Tikendra, L., Mukherjee, A., Mukherjee, S., and Pandey, D. K. (2020). "Methyl jasmonate and salicylic acid elicit indole alkaloid production and modulate antioxidant defence and biocidal properties in *Rauvolfia serpentina* Benth. ex Kurz. *in vitro* cultures," *South African Journal of Botany* 135, 1-17. DOI: 10.1016/j.sajb.2020.07.020

Dixon, R. A., Achnine, L., Kota, P., Liu, C. J., Reddy, M. S., and Wang, L. (2002). "The phenylpropanoid pathway and plant defense—A genomics perspective," *Molecular Plant Pathology* 3(5), 371-390. DOI: 10.1046/j.1364-3703.2002.00131.x

Dosoky, N. S., and Setzer, W. N. (2018). "Chemical composition and biological activities of essential oils of *Curcuma* species," *Nutrients* 10(9), 1196-1238. DOI: 10.3390/nu10091196

Dowom, S. A., Abrishamchi, P., Radjabian, T., and Salami, S. A. (2022). "Elicitor-induced phenolic acids accumulation in *Salvia virgata* Jacq. hairy root cultures," *Plant Cell, Tissue and Organ Culture* 148(1), 107-117. DOI: 10.1007/s11240-021-02170-8

Du, L., Li, D., Zhang, J., Du, J., Luo, Q., and Xiong, J. (2020). "Elicitation of *Lonicera japonica* Thunb suspension cell for enhancement of secondary metabolites and antioxidant activity," *Industrial Crops and Products* 156, article 112877. DOI: 10.1016/j.indcrop.2020.112877

Eray, N., Dalar, A., and Turker, M. (2020). "The effects of abiotic stressors and signal molecules on phenolic composition and antioxidant activities of *in vitro* regenerated *Hypericum perforatum* (St. John's Wort)," *South African Journal of Botany* 133, 253-

263. DOI: 10.1016/j.sajb.2020.07.037

Fozia, I., Fouzia, H., Baqir Shyum, N., Iqbal, A., Sabahat, J., and Syed, M. F. H. (2012). "Studies on antibacterial activity of some traditional medicinal plants used in folk medicine," *Pakistan Journal of Pharmaceutical Sciences* 25, 669-674.

Ghimire, B. K., Thiruvengadam, M., and Chung, I. M. (2019). "Identification of elicitors enhances the polyphenolic compounds and pharmacological potential in hairy root cultures of *Aster scaber*," *South African Journal of Botany* 125, 92-101. DOI: 10.1016/j.sajb.2019.07.006

Gupta, R., and Jain, D. (2022). "Transforming plant secondary metabolite production through the application of PTC and elicitor technology," *Journal of Advanced Scientific Research* 13(01), 75-86.

Haida, Z., Sinniah, U. R., Nakasha, J. J., and Hakiman, M. (2022). "Shoot induction, multiplication, rooting and acclimatization of black turmeric (*Curcuma caesia* Roxb.): An important and endangered curcuma species," *Horticulturae* 8(8), 740. DOI: 10.3390/horticulturae8080740

Haida, Z., Nakasha, J. J., and Hakiman, M. (2020). "*In vitro* responses of plant growth factors on growth, yield, phenolics content and antioxidant activities of *Clinacanthus nutans* (Sabah Snake Grass)," *Plants* 9(8), 1030. DOI: 10.3390/plants9081030

Insuan, W., Hansupalak, N., and Chahomchuen, T. (2022). "Extraction of curcumin from turmeric by ultrasonic-assisted extraction, identification, and evaluation of the biological activity," *Journal of Herbmed Pharmacology* 11(2), 188-196. DOI: 10.34172/jhp.2022.23

Jain, A., Jain, P., and Parihar, D. K. (2019). "Comparative study of *in-vitro* antidiabetic and antibacterial activity of non-conventional curcuma species," *Journal of Biologically Active Products from Nature* 9(6), 457-464.

Jana, L. Š., Tran, H. D., Nguyen, Q. B., Kristyna, H., Nguyen, Q. D., Nguyen, T. T., and Mark, N. (2019). "The identity of *Amomum trilobum* and *Amomum unifolium* (Zingiberaceae: Alpinioideae), and description of four new related species from Vietnam," *Phytotaxa* 401(3), 149-165.

Kataria, V., Masih, A., Chauhan, S., Sharma, S. K., Kant, A., and Arya, I. D. (2022). "Clonal propagation, a tested technique for increasing productivity: A review of bamboos, *Eucalyptus* and *Chirpine*," *Agricultural Biotechnology: Latest Research and Trends* 2021, 37-51.

Kaur, R., Kaur, B., Suttee, A., and Kalsi, V. (2018). "Comparative assessment of *in vitro* antimicrobial activity of *curcuma caesia* Roxb. and *Curcuma amada* Roxb," *Asian Journal of Pharmaceutical and Clinical Research* 11(14), 94-97. DOI: 10.22159/ajpcr.2018.v11s2.28591

Khatun, M., Nur, M. A., Biswas, S., Khan, M., and Amin, M. Z. (2021). "Assessment of the anti-oxidant, anti-inflammatory and anti-bacterial activities of different types of turmeric (*Curcuma longa*) powder in Bangladesh," *Journal of Agriculture and Food Research* 6, article 100201. DOI: 10.1016/j.jafr.2021.100201

Kim, N. S., Jung, D. H., Jung, C. R., Jeon, K. S., Park, H. W., and Park, S. U. (2020). "Improvement of phenylpropanoid production with elicitor treatments in *Pimpinella brachycarpa* Nakai," *Horticulturae* 6(4), 108-124. DOI: 10.3390/horticulturae6040108

Maithaa, M. J., Iman, H. A., Raya, S. A., Marya, A. A., Sharifa, Y. A., Tanveer, A., Maksood, A., and Shah, A. K. (2019). "Comparison of curcuminoids content, *in vitro* anti-oxidant and anti-diabetic activity of *Curcuma longa* collected from four different

countries," *Asian Journal of Biological Sciences* 12, 577-582. DOI: 10.3923/ajbs.2019.577.582

Narayani, M., and Srivastava, S. (2017). "Elicitation: A stimulation of stress in *in vitro* plant cell/tissue cultures for enhancement of secondary metabolite production," *Phytochemistry Reviews* 16, 1227-1252. DOI: 10.1007/s11101-017-9534-0

Paeizi, M., Karimi, F., and Razavi, K. (2018). "Changes in medicinal alkaloids production and expression of related regulatory and biosynthetic genes in response to silver nitrate combined with methyl jasmonate in *Catharanthus roseus in vitro* propagated shoots," *Plant Physiology and Biochemistry* 132*,* 623-632. DOI: 10.1016/j.plaphy.2018.10.015

Pawar, H. A., Gavasane, A. J., and Choudhary, P. D. (2018). "A novel and simple approach for extraction and isolation of curcuminoids from turmeric rhizomes," *Natural Products Chemistry and Research* 6(1), 1-4. DOI: 10.4172/2475-7675.1000300

Pawar, H., Karde, M., Mundle, N., Jadhav, P., and Mehra, K. (2014). "Phytochemical evaluation and curcumin content determination of turmeric rhizomes collected from Bhandara District of Maharashtra (India)," *Medicinal Chemistry* 4(8), 588-591. DOI: 10.4172/2161-0444.1000198

Pham, N. K., Nguyen, H. T., and Nguyen, Q. B. (2021). "A review on the ethnomedicinal uses, phytochemistry and pharmacology of plant species belonging to *Kaempferia* L. genus (Zingiberaceae)," *Science Asia* 48, 1-24. DOI: 10.29090/psa.2021.01.19.070

Rachidi, F., Benhima, R., Kasmi, Y., Sbabou, L., and Arroussi, H. E. (2021). "Evaluation of microalgae polysaccharides as biostimulants of tomato plant defense using metabolomics and biochemical approaches," *Scientific Reports* 11(1), 1-16. DOI: 10.1038/s41598-020-78820-2

Rai, N., Shukla, T. P., Loksh, K. R., and Karole, S. (2020). "Synthesized silver nanoparticle loaded gel of *Curcuma caesia* for effective treatment of acne," *Journal of Drug Delivery and Therapeutics* 10(6-s), 75-82. DOI: 10.22270/jddt.v10i6-s.4453

Rajan, M., Chandran, V., and Mathew, L. (2022). "Influence of methyl jasmonate and salicylic acid on rhamnetin production in adventitious root cultures of *Baccharoides anthelmintica* (L.) Moench," *Natural Product Research* 36(16), 4182-4186. DOI: 10.1080/14786419.2021.1961251

Rasouli, D., Werbrouck, S., Maleki, B., Jafary, H., and Schurdi-Levraud, V. (2021). "Elicitor-induced *in vitro* shoot multiplication and steviol glycosides production in *Stevia rebaudiana*," *South African Journal of Botany* 137, 265-271. DOI: 10.1016/j.sajb.2020.10.023

Ricciutelli, M., Nzekoue, F. K., Caprioli, G., Sagratini, G., Alesi, A., Vici, G., and Polzonetti, V. (2020). "Study of the effect of marination treatment on garlic bioactive compounds through an innovative HPLC-DAD-MS method for alliin and curcuminoids analysis," *LWT-Food Science and Technology* 131, article 109788. DOI: 10.1016/j.lwt.2020.109788

Setyaningsih, D., Santoso, Y. A., Hartini, Y. S., Murti, Y. B., Hinrichs, W. L., and Patramurti, C. (2021). "Isocratic high-performance liquid chromatography (HPLC) for simultaneous quantification of curcumin and piperine in a microparticle formulation containing *Curcuma longa* and *Piper nigrum*," *Heliyon* 7(3), article e06541. DOI: 10.1016/j.heliyon.2021.e06541

Shafighi, S., Moieni, A., and Monfared, S. R. (2022). "Effects of methyl jasmonate, salicylic acid and phenylalanine on aloe emodin and aloin in diploid and tetraploid

Aloe barbadensis," *International Journal of Horticultural Science* 28, 86-96. DOI: 10.31421/ijhs/28/2022/9304

Shen, R., Yang, X., and Lin, D. (2023). "pH sensitive double-layered emulsions stabilized by bacterial cellulose nanofibers/soy protein isolate/chitosan complex enhanced the bioaccessibility of curcumin: *In vitro* study," *Food Chemistry* 402, article 134262. DOI: 10.1016/j.foodchem.2022.134262

Sultana, S., Munir, N., Mahmood, Z., Riaz, M., Akram, M., Rebezov, M., Kuderinova, N., Moldabayeva, Z., Shariati, M. A., Rauf, A., and Rengasamy, K. R. (2021). "Molecular targets for the management of cancer using *Curcuma longa* Linn. phytoconstituents: A Review," *Biomedicine and Pharmacotherapy* 135, article 111078. DOI: 10.1016/j.biopha.2020.111078

Sun, M., Shi, M., Wang, Y., Huang, Q., Yuan, T., Wang, Q., Wang, C., Zhou, W., and Kai, G. (2019). "The biosynthesis of phenolic acids is positively regulated by the JA-responsive transcription factor ERF115 in *Salvia miltiorrhiza*," *Journal of Experimental Botany* 70(1), 243-254.

Thakur, M., Bhattacharya, S., Khosla, P. K., and Puri, S. (2019). "Improving production of plant secondary metabolites through biotic and abiotic elicitation," *Journal of Applied Research on Medicinal and Aromatic Plants* 12, 1-12. DOI: 10.1016/j.jarmap.2018.11.004

Van, H. T., Thang, T. D., Luu, T. N., and Doan, V. D. (2021). "An overview of the chemical composition and biological activities of essential oils from *Alpinia* genus (Zingiberaceae)," *RSC Advances* 11(60), 37767-37783. DOI: 10.1039/d1ra07370b

Vanda, G. F., Shabani, L., and Razavizadeh, R. (2019). "Chitosan enhances rosmarinic acid production in shoot cultures of *Melissa officinalis* L. through the induction of methyl jasmonate," *Botanical Studies* 60, 1-10. DOI: 10.1186/s40529-019-0274-x

Wang, H. Q., Jin, M. Y., Paek, K. Y., Piao, X. C., and Lian, M. L. (2016). "An efficient strategy for enhancement of bioactive compounds by protocorm-like body culture of *Dendrobium candidum*," *Industrial Crops and Products* 84, 121-130. DOI: 10.1016/j.indcrop.2016.02.001

Yadav, R. R., Negi, P. S., and Singh, J. (2021). "Climate change and plant biodiversity in Himalaya, India," *Proceedings of the Indian National Science Academy* 87(2), 234-259. DOI: 10.1007/s43538-021-00034-5

Yousefian, S., Lohrasebi, T., Farhadpour, M., and Haghbeen, K. (2020). "Effect of methyl jasmonate on phenolic acids accumulation and the expression profile of their biosynthesis-related genes in *Mentha spicata* hairy root cultures," *Plant Cell, Tissue and Organ Culture* 142(2), 285-297. DOI: 10.1007/s11240-020-01856-9

Zhao, J., Davis, L. C., and Verpoorte, R. (2005). "Elicitor signal transduction leading to production of plant secondary metabolites," *Biotechnology Advances* 23(4), 283-333. DOI: 10.1016/j.biotechadv.2005.01.003

Article submitted: April 1, 2025; Peer review completed: May 10, 2025; Revised version received: May 19, 2025; Further revised version received July 16, 2025; Accepted: July 17, 2025; Published: July 25, 2025.
DOI: 10.15376/biores.20.3.7574-7591