Arch Biol Sci. 2025;77(4):313-322 https://doi.org/10.2298/ABS250626025N

Methyl jasmonate elicitation improves antioxidant and antibacterial activities in *Portulaca oleracea*

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Received: June 26, 2025; **Revised**: September 2, 2025; **Accepted**: September 4, 2025; **Published online**: September 8, 2025

Abstract: The application of elicitors has been shown to enhance the biological activities of crops and medicinal plants by modulating their phytochemical content. This study evaluated the effect of methyl jasmonate elicitation on the biological activities of *Portulaca oleracea*. Antioxidant activity of methanol and aqueous extracts from methyl jasmonate-elicited and non-elicited plants was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Antibacterial activity was evaluated against *Bacillus cereus*, *Serratia marcescens*, *Escherichia coli*, and *Staphylococcus aureus* using the well-diffusion method. Toxicity was tested using the brine shrimp lethality assay. The methyl jasmonate-elicited methanol extract showed the highest antioxidant activity with half maximal inhibitory concentration (IC_{50}) values of 1556.52±30.81 μg/mL (DPPH), 3353.33±29.83 μg/mL (ABTS), and ferric reducing power of 273.24±3.49 mg ferrous (II)/g dry extract. Only methyl jasmonate-elicited methanol and aqueous extract inhibited the growth of *Serratia marcescens*, with inhibition zones of 15.1±0.06 mm and 11±0.06 mm, respectively. No inhibition was observed against the other tested bacteria. Toxicity results confirmed the non-toxic nature of all extracts, with median lethal concentration (LC_{50}) values >1000 μg/mL. Overall, methyl jasmonate elicitation enhanced the antioxidant and selective antibacterial activity of *P. oleracea*.

Keywords: *Portulaca oleracea,* elicitation, methyl jasmonate, antioxidant activity, antibacterial activity

INTRODUCTION

Purslane (*Portulaca oleracea* L.) is an underutilized leafy vegetable from the Portulacaceae family. It is widely distributed in the tropical and subtropical areas of the world. In many countries, *P. oleracea* is well known for its use in folk medicine [1]**.** *P. oleracea* is recognized for its nutritional value, primarily due to its high content of omega-3 fatty acids and antioxidants. [2]. In the Mediterranean and tropical Asian countries, the edible parts (leaves and purplish stems) of *P. oleracea* are added to soups and salads [3]. According to the World Health Organization, this plant is considered a "global panacea", signifying a cure for all diseases [4]. It is used in herbal medicine as an aperient, cardiac tonic, muscle relaxant, anti-inflammatory, and diuretic [5]. Traditionally, it has been used to treat fever, abscesses, urinary disorders, headaches, piles, scurvy, wounds, and sores [6]. Coumarin, flavonoids, and saponins have been reported as phytochemicals in *P. oleracea* [7]. It has also been documented to contain various bioactive compounds with biological activities including antioxidant, anti-osteoporotic, anticancer, anti-inflammatory, antiasthmatic, neuroprotective, hepatoprotective, antimicrobial, gastroprotective, muscle relaxant, and renoprotective effects [8].

Elicitation is the activation of natural plant defense mechanisms in response to biotic or abiotic elicitors [9]. The application of elicitors has been reported to enhance health-promoting properties in plants by inducing and modulating beneficial secondary metabolites [10], as well as improving growth and yield [11] and visual characteristics [12]. Methyl jasmonate (MeJA), a widely used elicitor, is an endogenous and lipid-derived plant hormone. The use of phytohormones

such as MeJa as elicitors has gained importance in recent years as an eco-friendly practice, since it is non-toxic and classified as a generally recognized as safe (GRAS) compound [13]. Previous studies have reported that the application of MeJa significantly enhanced the active constituents in *Salvia verticillata* [14], *Curcuma wenyujin* [15], *Solanum lycopersicum* [16], and *Lycoris longituba* [17]. Moreover, MeJA-induced increases in phytochemical levels have been reported to enhance free radical scavenging in broccoli [18], anticancer activity in hairy root cultures of *Senna obtusifolia* [19], and antidiabetic and antiseptic effects of essential oil from *Ocimum basilicum* [20]. Previously, we had reported that the application of MeJa improved the growth performance and production of antioxidant metabolites in *P. oleracea,* thereby modulating reactive oxygen species (ROS) generation [21].

P. oleracea exhibits strong antioxidant activity comparable to synthetic antioxidants, with high levels of phenolics and beneficial fatty acids [22]. Both water and ethanol extracts of *P. oleracea* demonstrate significant antioxidant and antimicrobial properties against various pathogens, including *Helicobacter pylori* and *Staphylococcus aureus* [23]. Methanolic and ethanolic extracts of *P. oleracea* leaves show particularly potent antibacterial activity against multiple drug-resistant (MDR) clinical isolates, with the highest inhibition observed against *Escherichia coli* and *Staphylococcus aureus[24]*. Phytochemical analysis reveals the presence of various bioactive compounds in *P. oleracea*, including saponins, alkaloids, flavonoids, and phenolics, which likely contribute to its medicinal properties [24].

Previous studies have suggested that the application of various elicitors, such as salicylic acid [25], sodium chloride [26], and heat [27], as well as MeJa [21, 28], positively influences the levels of phytochemicals in *P. oleracea*. However, few studies have investigated the influence of elicitation on the biological activity of *P. oleracea*. Our previous study [21] observed differences in the metabolite composition of MeJA-elicited versus non-elicited *P. oleracea* extracts, suggesting that MeJA elicitation may influence bioactivity. Therefore, the present study aimed to evaluate whether MeJA elicitation enhances antioxidant and antibacterial activities in *P. oleracea* and to assess the toxicity of MeJA-treated extracts.

MATERIALS AND METHODS

Ethics statements

This study did not involve humans or vertebrate animals. The brine shrimp lethality assay was conducted using *Artemia salina* nauplii, an invertebrate model not requiring ethical approval.

Plant growth and elicitor treatment

The plant growth conditions and foliar treatment of MeJa (Sigma, USA) in *P. oleracea* were outlined in our previous manuscript [21]. *P. oleracea* seeds were sown in trays, and 14-day-old seedlings were transplanted into 300-mL pots filled with commercial potting mix (one plant per pot). Plants were watered regularly. On the 35th day after sowing (DAS), seedlings were treated with foliar sprays of MeJa prepared in 0.1% ethanol containing 0.05% Tween 20 at concentrations of 0.1-1.0 mM. Plants were harvested on 60 DAS and stored at -80°C. In this study, non-elicited *P. oleracea* (control, 0.0 mM) plants and *P. oleracea* plants elicited with 0.4 mM MeJa (the optimal concentration) were used.

Aqueous extraction

Aerial tissues from 50 plants per treatment were pooled and cut into pieces to homogenize the composite. Due to the limited dry extract yield per plant, pooling was necessary to ensure sufficient sample quantity and reduce variability between plants. From this composite, 20 g of fresh foliage from 0.0 mM and 0.4 mM MeJa-elicited plants were taken and heated at 80°C in 500 mL of distilled water for 1 h. The decoctions were then filtered, and the filtrates were subjected to freeze-drying. The freeze-dried powder of the *P. oleracea* aqueous extracts, designated as 0.0Aqs (from 0.0 mM MeJa/control plants) and 0.4Aqs (from 0.4 mM MeJa treated plants), was then stored at -80°C [29]. All biochemical assays were performed in three technical replicates for each pooled extract.

Methanol extraction

Aerial tissues from 50 plants per treatment were pooled to obtain a composite sample. Due to the limited dry extract yield per plant, pooling was necessary to ensure

sufficient sample quantity and reduce variability between plants. The pooled material was freeze-dried for 4 days at -50°C and was then ground finely into a powder form. The powder from non-elicited (0.0 mM MeJa) and elicited (0.4 mM MeJa) plants was immersed in methanol at 1:30 (w/v) for 48 h at 4°C. The sample mixture was then filtered, and the filtrates were subjected to rotary evaporation at 40°C to yield the dry *P. oleracea* methanol extract. The resulting dry extracts (DE), designated as 0.0Meth (from 0.0 mM MeJa/control plants) and 0.4Meth (from 0.4 mM MeJa treated plants), were stored at -80°C [21]. All biochemical assays were performed in three technical replicates for each pooled extract.

DPPH free radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of *P. oleracea* extracts was analyzed according to the standard procedure [30]. The methanolic extract was dissolved in absolute methanol, and the aqueous extract in distilled water, to prepare working solutions ranging from 1.0 to 6.0 mg/mL. Into each well of a 96-well plate, 150 µL of DPPH reagent (0.3 mM) and 50 µL of the extract solutions were mixed and incubated at room temperature for 30 min. The absorbance was read at 515 nm. A graph of the percentage of inhibition against concentration was constructed to obtain IC_{50} from the fitted linear curve. Ascorbic acid served as a comparative standard.

ABTS radical scavenging activity

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay was carried out as described previously [31], with slight modifications. The methanolic extract was dissolved in absolute methanol, and the aqueous extract in distilled water to prepare working solutions ranging from 0.2-1.0 mg/mL. Into each well of a 96-well plate, 200 µL ABTS solution and 20 µL of the extract solutions were mixed and incubated for 30 min at room temperature. The absorbance was read at 734 nm. A graph of the percentage of inhibition against the concentration was plotted to obtain IC_{50} from the fitted linear curve. Ascorbic acid served as a comparative standard.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out following previous procedures [32], with slight modifications. The methanolic extract was dissolved in absolute methanol, and the aqueous extract in distilled water, to prepare working solutions at 0.6 mg/mL. Into each well of a 96-well plate, 180 µL of FRAP reagent and 20 µL of the extract solutions were mixed and incubated for 30 min at room temperature. The absorbance was read at 595 nm, and FRAP activity was expressed as ferrous equivalent (mg Fe (II)/g DE). Ascorbic acid served as a comparative standard.

Screening of extracts for antibacterial property

The bacteria examined in this investigation included *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus,* and *Serratia marcescens*. The bacterial strains were sourced from the Microbiology Department, Institute of Biological Sciences, Universiti Malaya, Kuala Lumpur, Malaysia. The antibacterial properties of *P. oleracea* extract were evaluated using the well-diffusion method. The methanolic extract was dissolved in absolute methanol, and the aqueous extract in sterile distilled water, to prepare working solutions at 60, 100, and 500 mg/mL. Precultures of *S. aureus*, *B. cereus*, *E. coli*, and *S. marcescens* were spread onto Mueller-Hinton agar (MHA) plates using a sterile cotton swab. Then, five wells with a 6-mm diameter were created using a sterile cork borer. Into each well, 100 µL of the extracts was added. Ciprofloxacin (5 µg/mL) was used as the positive control. After 24 h of incubation, the diameter of the inhibition zone was recorded.

***Artemia salina* (brine shrimp) toxicity assay**

The toxicity of *P. oleracea* extract was evaluated using brine shrimp larvae as a bioassay model. The brine shrimp eggs were hatched by incubating the eggs in simulated seawater for 48 h at room temperature with constant aeration and irradiation of light. The simulated seawater was prepared by dissolving 19 g of NaCl salt in 500 mL of distilled water. After 48 h, active brine shrimp were transferred to 6-well plates using a microscope and paintbrush, with 10 shrimp placed in each well containing artificial seawater. Methanolic extracts were dissolved in 1% dimethyl sulfoxide (DMSO) to

prepare concentrations of 10, 100, 250, 500, 750, and 1000 µg/mL; 1% DMSO is widely used as a safe solvent in brine shrimp lethality assays [33]. Aqueous extracts were directly dissolved in seawater at the same concentration range. The numbers of live and dead shrimp were recorded at 24 and 48 h post-incubation. Shrimp observation and counting were performed using a light microscope, with potassium dichromate as the positive control. The mortality percentage of brine shrimp was estimated using the following equation:

$$\%\ mortality = \frac{Nc - Nl}{Nc} \times 100$$

where *Nc*=total number of shrimps used in each well, and *Nl*=number of living shrimps in each well. The lethality concentration of 50% (LC_{50}) was determined following the probit analysis using SPSS.

Statistical analysis

Data from all assays, performed in triplicate per treatment, were analyzed using IBM SPSS Statistics Version 26 (IBM Corporation, Armonk, NY, USA). Mean differences were evaluated by one-way ANOVA followed by Duncan's multiple range test (DMRT). Principal component analysis (PCA) was conducted in R to assess relationships among measured parameters, incorporating previously published enzymatic and non-enzymatic antioxidant data [21] alongside current results.

RESULTS

Antioxidant potential of *P. oleracea* extracts (ABTS, DPPH, and FRAP assays)

The free radical scavenging properties and reducing potential of *P. oleracea* extracts were evaluated. As shown in Table 1, the 0.4Meth extract demonstrated the highest DPPH free radical scavenging activity (lowest IC_{50} = 1556.52±30.81 µg/mL), ABTS scavenging activity (lowest IC_{50} = 3353.33±29.83 µg/mL), and ferric reducing power (273.24±3.49 mg Fe^{2+}/g DE) compared with the 0.0Meth extract. However, no significant difference was observed in the ABTS and DPPH free radical scavenging activity between 0.4Meth extracts and 0.0Meth extracts. A significant increase in the ferric-reducing power of 0.4Meth extracts was observed compared to 0.0Meth extracts. Similarly, 0.4Aqs extracts also exhibited the highest DPPH free radical scavenging activity (IC_{50}=7516.60±216.25 µg/mL), ABTS free radical scavenging activity (IC_{50}=3715.87±131.71 µg/mL), and ferric reducing power (130.18±10.32 Fe (II) mg/g DE) compared to the 0.0Aqs extracts. A significant increase in ABTS and DPPH scavenging activity was observed in 0.4Aqs extracts compared with 0.0Aqs extracts, whereas ferric-reducing power showed no significant difference. Methanol extracts of *P. oleracea* exhibited significantly higher antioxidant activity than aqueous extracts. All extracts displayed significantly lower antioxidant potential than the positive control (ascorbic acid).

Table 1. Antioxidant potential of MeJa-treated *P. oleracea* extracts

Extract	ABTS (IC_{50} µg/mL)	DPPH (IC_{50} µg/mL)	FRAP (Fe (II) mg/ g DE)
0.0Meth	1662.83±7.49[b]	3551.50±58.79[b]	212.93±1.32[c]
0.4Meth	1556.52±30.81[b]	3353.33±29.83[b]	273.24±3.49[b]
0.0Aqs	4694.67±212.10[d]	10961.02±389.89[d]	88.80±7.15[d]
0.4Aqs	3715.87±131.71[c]	7516.60±216.25[c]	130.18±10.32[d]
Ascorbic acid	48.92±2.01[a]	37.36±0.25[a]	6337.99±37.60[a]

[a] – Data are reported as mean values±standard error (n=3). [b] – Means bearing identical letters within each group are not significantly different (P<0.05); [c] – 0.0Meth-non-elicited *P. oleracea* (methanol extracts); 0.4Meth – 0.4 mM of MeJa-elicited *P. oleracea* (methanol extracts); 0.0Aqs-non-elicited *P. oleracea* (aqueous extracts); 0.4Aqs-0.4 mM of MeJa-elicited *P. oleracea* (aqueous extracts).

Antibacterial activity of *P. oleracea* extracts

The antibacterial properties of *P. oleracea* extracts were tested against four bacterial species. Extract efficacy was quantified by measuring the diameter of the inhibition zones. The results of the antibacterial activity are summarized in Table 2 and Fig. 1. None of the *P. oleracea* extracts at 60, 100, or 500 mg/mL inhibited the tested bacteria, except for 0.4Meth and 0.4Aqs extracts at 500 mg/mL, which inhibited *S. marcescens* with zones of 15.10±0.06 mm and 11.00±0.06 mm, respectively. Clear inhibition zones were observed in wells containing the standard antibiotic ciprofloxacin (5 µg/mL) against all tested bacteria.

Table 2. Antibacterial activity of MeJa-treated *P. oleracea* extracts

Bacteria	Extracts	Diameter of the inhibition zone			Positive control CIP (5 µg/mL)	Negative control (Meth/ sdH_2O)
		Concentration of extracts (mg/ mL)				
		60	100	500		
Escherichia coli	0.0Meth	ND	ND	ND	38.00±0.06	ND
	0.4Meth	ND	ND	ND		
	0.0Aqs	ND	ND	ND	39.00±0.06	ND
	0.4Aqs	ND	ND	ND		
Serratia marcescens	0.0Meth	ND	ND	ND	25.93±0.07	ND
	0.4Meth	ND	ND	15.10±0.06		
	0.0Aqs	ND	ND	ND	28.07±0.07	ND
	0.4Aqs	ND	ND	11.00±0.06		
Bacillus cereus	0.0Meth	ND	ND	ND	33.10±0.06	ND
	0.4Meth	ND	ND	ND		
	0.0Aqs	ND	ND	ND	33.17±0.09	ND
	0.4Aqs	ND	ND	ND		
Staphylococcus aureus	0.0Meth	ND	ND	ND	24.97±0.03	ND
	0.4Meth	ND	ND	ND		
	0.0Aqs	ND	ND	ND	26.07±0.07	ND
	0.4Aqs	ND	ND	ND		

Data reported as mean values±standard error (n=3). ND – Not detected; CIP – ciprofloxacin (positive control), sdH_2O – sterile distilled water (negative control), Meth – methanol (negative control); 0.0Meth – non-elicited *P. oleracea* (methanol extracts), 0.4Meth – 0.4 mM of MeJa-elicited *P. oleracea* (methanol extracts), 0.0Aqs – non-elicited *P. oleracea* (aqueous extracts), and 0.4Aqs –0.4 mM of MeJa-elicited *P. oleracea* (aqueous extracts).

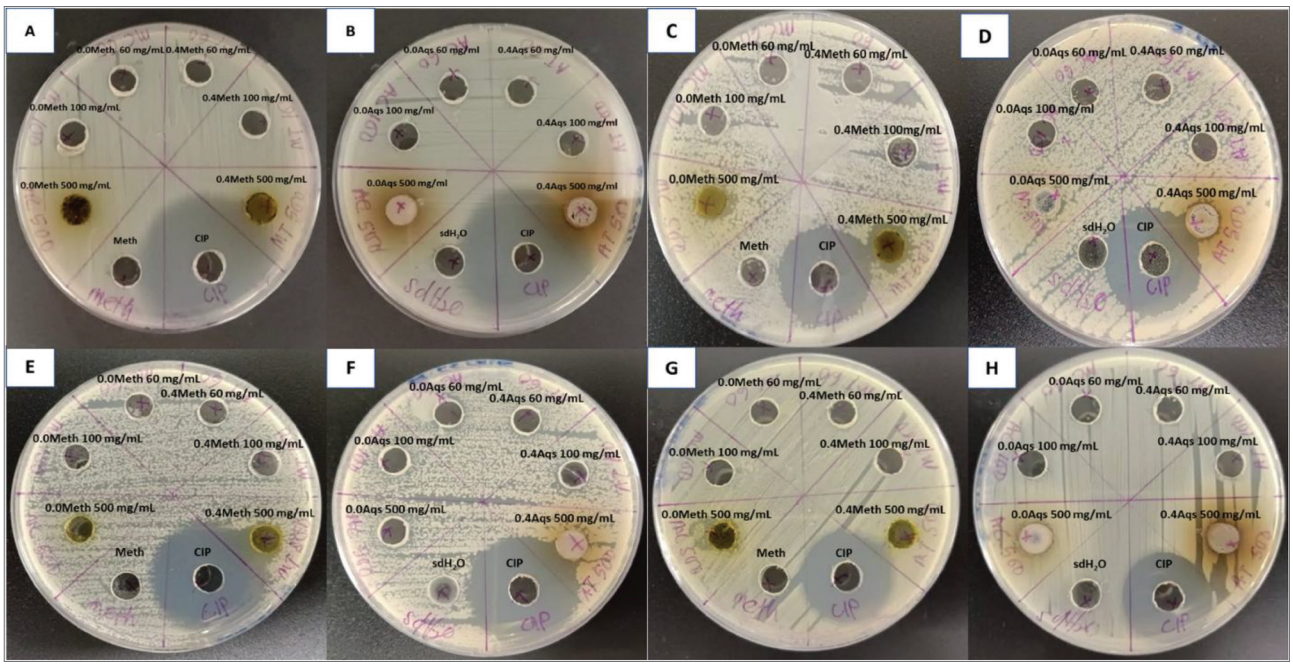

Fig. 1. Antibacterial activity of *P. oleracea* at various concentrations (60, 100, and 500 mg/mL). (**A, B** – *E. coli*), (**C, D** – S. *marcescens*), (**E, F** – *B. cereus*), and (**G, H** – S. *aureus*).

Table 3. Mortality percentage and LC_{50} of methanol and aqueous *P. oleracea* extract

Extract	Concentration (µg/mL)	After 24 h		After 48 h	
		% mortality	LC_{50} (µg/mL)	% mortality	LC_{50} (µg/mL)
0.0Meth	1000	0	>1000	10±5.77	>1000
	750	0		0	
	500	0		0	
	250	0		0	
	100	0		0	
	10	0		0	
	0	0		0	
0.4Meth	1000	0	>1000	13.33±3.33	>1000
	750	0		0	
	500	0		0	
	250	0		0	
	100	0		0	
	10	0		0	
	0	0		0	
0.0Aqs	1000	0	>1000	10±0.0	>1000
	750	0		0	
	500	0		0	
	250	0		0	
	100	0		0	
	10	0		0	
	0	0		0	
0.4Aqs	1000	0	>1000	10±0.0	>1000
	750	0		0	
	500	0		0	
	250	0		0	
	100	0		0	
	10	0		0	
	0	0		0	
Potassium dichromate	100	60±0.0	63.36±2.69	100±0.0	26.86±0.56
	75	50±0.0		100±0.0	
	50	46.67±3.33		100±3.33	
	25	36.67±3.33		36.67±3.33	
	10	0±0.0		6.67±0.0	
	1	0±0.0		0±0.0	
	0	0±0.0		0±0.0	

Data reported as mean values±standard error (n=3). 0.0Meth – non-elicited *P. oleracea* (methanol extracts); 0.4Meth –0.4 mM of MeJa-elicited *P. oleracea* (methanol extracts); 0.0Aqs – non-elicited *P. oleracea* (aqueous extracts); 0.4Aqs – 0.4 mM of MeJa-elicited *P. oleracea* (aqueous extracts).

Toxicity of *P. oleracea* extracts

The toxicity of the *P. oleracea* extracts was tested against brine shrimp larvae. After 24 h of incubation, all shrimp remained alive in wells containing any of the six concentrations of 0.0Meth, 0.4Meth, 0.0Aqs, and 0.4Aqs extracts (Table 3), indicating LC_{50} values >1000 µg/mL for all extracts. After 48 h, the highest concentration (1000 µg/mL) of all extracts caused only slight mortality (10–13%), while all other concentrations showed 0% mortality. As mortality was below 50%, the LC_{50} of all extracts remained >1000 µg/mL. Brine shrimp mortality increased with rising concentrations of potassium dichromate after 24 and 48 h of incubation. The LC_{50} was 63.36±2.69 µg/mL at 24 h and 26.86±0.56 µg/mL at 48 h.

Relationships between MeJa elicitation and antioxidant status in *P. oleracea*

A multivariate analysis (PCA) was conducted to analyze the relationships between MeJa elicitation with antioxidant metabolites and enzymes, and the antioxidant activity in *P. oleracea* (Fig. 2). Previously reported antioxidant metabolite and enzyme data [21] were included in this analysis. The scree plot (Fig. 2A) illustrates the proportion of variance explained by each principal component. The first principal component (PC1) accounted for 73.6% of the total variance and PC2 for 19.8%, together explaining 93.4% of the dataset variability. As discerned in the individual PCA plots (Fig. 2B), 0.0Meth and 0.0Aqs were separated from those elicited with MeJa (0.4Meth and 0.4Aqs). Along dimension 1, 0.0Aqs and 0.0Meth exhibited negative scores, while 0.4Aqs and 0.4Meth exhibited positive scores. Among dimension 1, variables such as SOD (superoxide dismutase activity), CAT (catalase activity), TPC (total phenolic content), TFC (total flavonoid content), TAC (total anthocyanin content), TChl (total chlorophyll), CAR (total carotenoid), and FRAP were positively loaded (Fig. 2C), while AsA (ascorbic acid), proline, DPPH, and ABTS were negatively loaded The PCA biplot (Fig. 2C) shows that MeJA elicitation increased antioxidant metabolites and enzyme levels (TPC, TFC, TAC, TChl, CAR, SOD, CAT) and enhanced antioxidant activity

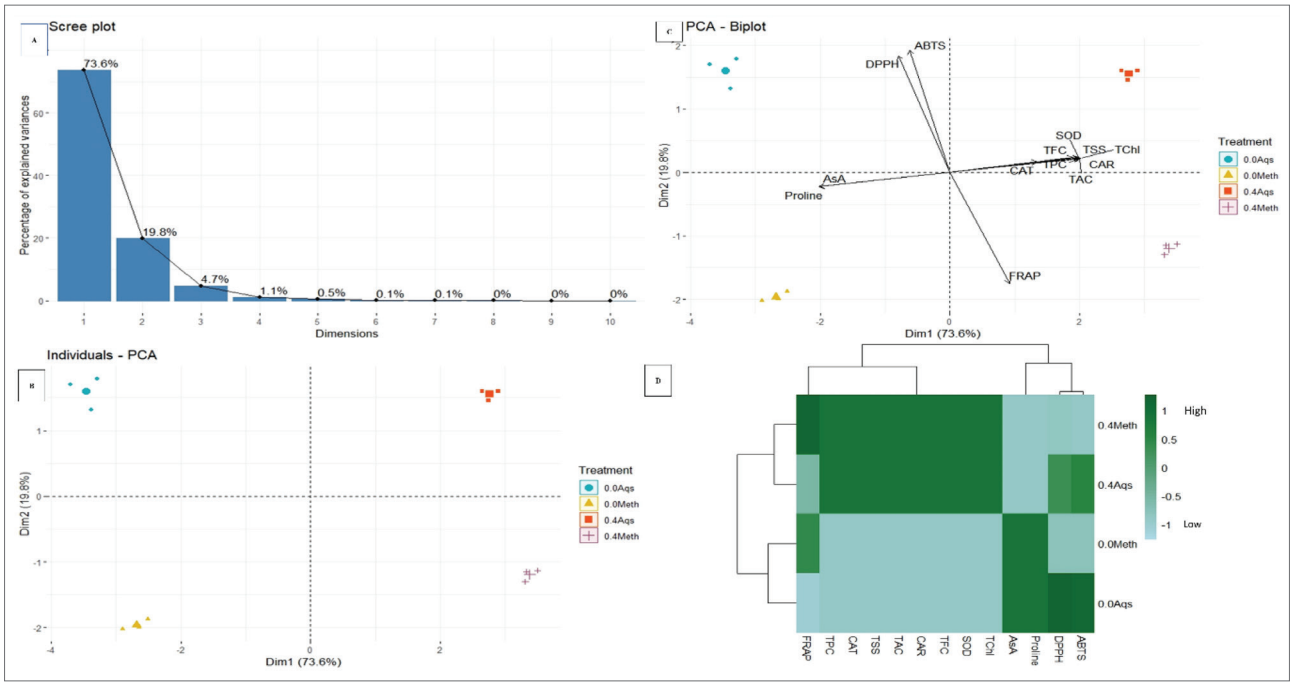

Fig. 2. PCA analysis. **A** – Scree plot, **B** – individual PCAs, **C** – PCA-Biplot, **D** – heat map showing relationship between the enzymatic and non-enzymatic antioxidants, and antioxidant activities of MeJa-elicited *P. oleracea* extracts. 0.0Meth – non-elicited *P. oleracea* (methanol extracts); 0.4Meth – 0.4 mM of MeJa-elicited *P. oleracea* (methanol extracts); 0.0Aqs – non-elicited *P. oleracea* (aqueous extracts); 0.4Aqs – 0.4 mM of MeJa-elicited *P. oleracea* (aqueous extracts).

(FRAP, DPPH, ABTS) in *P. oleracea* extracts. These results align with the heat map analysis (Fig. 2D) and are consistent with the ANOVA findings (Table 1).

DISCUSSION

The results of this study support the antioxidant activity of *P. oleracea*, consistent with previous scientific reports [34,35]. Furthermore, MeJa-elicitation enhances the antioxidant activity of *P. oleracea* extract, as observed in the decrease in IC_{50} values and increase in ferric reducing power of 0.4Meth and 0.4Aqs in comparison to 0.0Meth and 0.0Aqs. The increased antioxidant activity in response to elicitor supplementation is mainly due to the improved production of enzymatic and non-enzymatic antioxidants [36,37]. Similar enhancements of antioxidant activity in response to MeJA have been reported in several plant species, including pak choi with increased chlorophyll and glucosinolate contents [38], Chinese chives with elevated phenolic levels [39], callus cultures of *Hibiscus sabdariffa* with higher phenolic, flavonoid, and anthocyanin contents [40], and callus cultures of *Bergenia ciliata* with increased phenolic and flavonoid contents and higher activities of enzymatic antioxidants such as superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase [41]. Given that elicitors can enhance and induce various secondary metabolites in plants, the antibacterial activity of non-elicited and MeJA-elicited *P. oleracea* extracts was compared. In this study, 0.0Meth and 0.0Aqs extracts showed no antibacterial activity against any tested bacteria, contradicting previous reports of *P. oleracea* antibacterial effects [8]. However, consistent with the current findings, Truc and Bui [42] reported that 50 µL of *P. oleracea* methanol extract failed to inhibit *E. coli* and *B. cereus*, and El-Sayed and Awad [43] found that 150 mg/mL of ethanol, hot water, and cold water leaf extracts (100-300 µL) showed no antibacterial activity against *S. aureus* and *E. coli*.

The primary factor affecting differences in *P. oleracea* antibacterial activity is the variation in its biochemical composition [44]. The biochemical composition of *P. oleracea* extracts can be affected by factors including the extraction solvent [45], extraction method [46], extract purity (crude versus isolated compounds) [47], plant part tested [48], and growth conditions [49]. In this study, MeJA elicitation increased antibacterial activity only against S. marcescens, with no effect on the

other tested bacterial strains. This study supports the role of MeJa in enhancing the antibacterial activity of *P. oleracea* against *S. marcescens.* Previously, we identified active compounds such as bergenin, fumaric acid, apigenin, and aconitic acid in MeJa-elicited *P. oleracea* extract [21], which may contribute to its antibacterial properties against *S. marcescens.*

Similarly, jasmonic acid-treated hairy root cultures of *Momordica charantia* significantly inhibited *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli* compared with non-elicited cultures [50]. Złotek and Rybczyńska-Tkaczyk [20] reported that essential oil from jasmonic acid-elicited *Ocimum basilicum* exhibited the highest antibacterial activity against *S. aureus* and *E. coli*. Comparable results were observed in jasmonic acid-elicited extracts of *Acalypha indica*, which strongly inhibited *P. vulgaris*, *E. coli*, *S. typhi*, and *P. aeruginosa* relative to untreated extracts [51].

The brine shrimp lethality assay has been widely used as a preliminary screening approach to study the toxicity [52]. The toxicity screening of various traditional medicinal plant species has been reported following the brine shrimp lethality assay [53,54]. According to Meyer's toxicity scale, crude extracts with LC_{50}<1000 µg/mL and LC_{50}>1000 µg/mL are considered toxic and non-toxic, respectively [55]. Since the LC_{50} of all *P. oleracea* extracts were >1000 µg/mL following 24 and 48 h of incubation, the *P. oleracea* extract, regardless of whether they were MeJa-elicited or non-elicited, was non-toxic.

CONCLUSION

MeJA elicitation of *P. oleracea* enhanced antioxidant activity and antibacterial activity against *S. marcescens*, likely through upregulation of bioactive secondary metabolites. These results indicate that elicitor treatment can effectively improve plant extract bioactivity, supporting the potential use of *P. oleracea* extracts in pharmaceutical, nutraceutical, and cosmetic applications as functional, health-promoting products.

Funding: This research was funded by Universiti Malaya (SATU Joint Research ST009-2021).

Acknowledgments: The authors thank Universiti Malaya, Malaysia for the experimental facilities and financial support (SATU Joint Research ST009-2021) provided.

Author contributions: JSY, SR, and SST conceived and designed the experiments; LN performed the experiments and analyzed the data; JSY, SR, and SST contributed reagents/materials/ analysis tools; LN wrote the paper; JSY and SR revised and proofread the paper.

Conflict of interest disclosure: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability: The dataset supporting the findings of this study has been deposited in https://doi.org/10.5281/zenodo.15720695

REFERENCES

1. Amirul Alam M, Juraimi AS, Rafii M, Hamid AA, Kamal Uddin M, Alam MZ, Latif MA. Genetic improvement of Purslane (Portulaca oleracea L.) and its future prospects. Mol Biol Rep. 2014;41:7395-411. https://doi.org/10.1007/s11033-014-3628-1
2. Oliveira I, Valentão P, Lopes R, Andrade PB, Bento A, Pereira JA. Phytochemical characterization and radical scavenging activity of Portulaca oleraceae L. leaves and stems. Microchem J. 2009;92(2):129-34. https://doi.org/10.1016/j.microc.2009.02.006
3. Palaniswamy UR, McAvoy RJ, Bible BB. Stage of Harvest and Polyunsaturated Essential Fatty Acid Concentrations in Purslane (Portulaca oleraceae) Leaves. J Agric Food Chem 2001;49(7):3490-3. https://doi.org/10.1021/jf0102113
4. Lim YY, Quah EPL. Antioxidant properties of different cultivars of Portulaca oleracea. Food Chem. 2007;103(3):734-40. https://doi.org/10.1016/j.foodchem.2006.09.025
5. Uddin MK, Juraimi AS, Hossain MS, Nahar MAU, Ali ME, Rahman M. Purslane weed (Portulaca oleracea): A prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. Sci World J. 2014;2014(1):951019. https://doi.org/10.1155/2014/951019
6. Sangeetha S, Kiran RS, Abbulu K, Battu S. A review on traditional herb Portulaca oleracea. World J Pharm Res. 2020;9(3):578-601.
7. Adrien KM, Calixte B, Honoré T, Lucien BG, Vincent E, Joseph DA, David NJ. Antifungal Activity of Roots Barks Extract of Securinega virosa (Roxb. ex Willd.) Baill and Anogeissus leiocarpa (DC.) Guill. & Perr, Two Plants Used in the Traditional Treatment of Candidiasis in Northern Côte d'Ivoire. Int J Biochem Res Rev. 2015;8(1):1-11. https://doi.org/10.9734/IJBCRR/2015/17481
8. Li K, Xia T, Jiang Y, Wang N, Lai L, Xu S, Yue X, Xin H. A review on ethnopharmacology, phytochemistry, pharmacology and potential uses of Portulaca oleracea L. J Ethnopharmacol. 2024;319:117211. https://doi.org/10.1016/j.jep.2023.117211
9. Wei X, Vrieling K, Kim HK, Mulder PPJ, Klinkhamer PGL. Application of methyl jasmonate and salicylic acid lead to contrasting effects on the plant's metabolome and herbivory. Plant Sci. 2021;303:110784. https://doi.org/10.1016/j.plantsci.2020.110784

10. Baek MW, Choi HR, Lee HC, Lee JH, Lee O-H, Hong JS, Jeong CS, Tilahun S. Preharvest methyl jasmonate and salicylic acid treatments improve the nutritional qualities and postharvest storability of tomato. Sci Hortic. 2023;321:112332. https://doi.org/10.1016/j.scienta.2023.112332
11. El-Ziat RA, Soliman DM, El-Sayed IM. Exogenous utilization of jasmonic acid and methyl jasmonate stimulates growth and biochemical composition of lavender (Lavandula angustifolia) plant. Egypt Pharm J. 2023;22(3):372-9. https://doi.org/10.4103/epj.epj_23_23
12. Ahmed ZFR, Al Shaibani FYY, Kaur N, Maqsood S, Schmeda-Hirschmann G. Improving Fruit Quality, Bioactive Compounds, and Storage Life of Date Palm (Phoenix dactylifera L., cv. Barhi) Using Natural Elicitors. Horticulturae. 2021;7(9):293. https://doi.org/10.3390/horticulturae7090293
13. Modesti M, Petriccione M, Forniti R, Zampella L, Scortichini M, Mencarelli F. Methyl jasmonate and ozone affect the antioxidant system and the quality of wine grape during postharvest partial dehydration. Food Res Int. 2018;112:369-77. https://doi.org/10.1016/j.foodres.2018.06.061
14. Rahmani N, Radjabian T. Integrative effects of phytohormones in the phenolic acids production in Salvia verticillata L. under multi-walled carbon nanotubes and methyl jasmonate elicitation. BMC Plant Biol. 2024;24(1):56. https://doi.org/10.1186/s12870-023-04719-5
15. Jiang CX, Yu JX, Zhu NN, Zhou D, Yuan ST, Lu MQ, Pan XJ, Dai LS, Qi Y, Li XK, Wu ZG. Integrative omic and transgenic analyses reveal methyl jasmonate- and abscisic acid-responsive regulator CwbHLH2 that positively regulates terpenoid biosynthesis in Curcuma wenyujin. Ind Crops Prod. 2024;209:118039. https://doi.org/10.1016/j.indcrop.2024.118039
16. Meza SLR, de Castro Tobaruela E, Pascoal GB, Magalhães HCR, Massaretto IL, Purgatto E. Induction of Metabolic Changes in Amino Acid, Fatty Acid, Tocopherol, and Phytosterol Profiles by Exogenous Methyl Jasmonate Application in Tomato Fruits. Plants. 2022;11(3):366. https://doi.org/10.3390/plants11030366
17. Li Q, Xu J, Zheng Y, Zhang Y, Cai Y. Transcriptomic and metabolomic analyses reveal that exogenous methyl jasmonate regulates galanthamine biosynthesis in lycoris longituba seedlings. Front Plant Sci. 2021;12:713795. https://doi.org/10.3389/fpls.2021.713795
18. Wang J, Mao S, Liang M, Zhang W, Chen F, Huang K, Wu Q. Preharvest Methyl Jasmonate Treatment Increased Glucosinolate Biosynthesis, Sulforaphane Accumulation, and Antioxidant Activity of Broccoli. Antioxidants. 2022;11(7):1298. https://doi.org/10.3390/antiox11071298
19. Kowalczyk T, Sitarek P, Merecz-Sadowska A, Szyposzyńska M, Spławska A, Gorniak L, Bijak M, Śliwiński T. Methyl Jasmonate Effect on Betulinic Acid Content and Biological Properties of Extract from Senna obtusifolia Transgenic Hairy Roots. Molecules. 2021;26(20):6208. https://doi.org/10.3390/molecules26206208
20. Złotek U, Rybczyńska-Tkaczyk K, Michalak-Majewska M, Sikora M, Jakubczyk A. Potential Acetylcholinesterase, Lipase, α-Glucosidase, and α-Amylase Inhibitory Activity, as well as Antimicrobial Activities, of Essential Oil from Lettuce Leaf Basil (Ocimum basilicum L.) Elicited with Jasmonic Acid. Appl Sci. 2020;10(12):4315. https://doi.org/10.3390/app10124315
21. Nyanasaigran L, Ramasamy S, Gautam A, Guleria P, Kumar V, Yaacob JS. Methyl jasmonate elicitation improves the growth performance and biosynthesis of antioxidant metabolites in Portulaca oleracea through ROS modulation. Ind Crops Prod. 2024;216:118709. https://doi.org/10.1016/j.indcrop.2024.118709
22. Ercıslı S, Coruh I, Gormez A, Sengul M. Antioxidant and antibacterial activities of Portulaca oleracea L. grown wild in Turkey. Ital J Food Sci. 2008;20(4).
23. Cho YJ, Ju IS, Kwon OJ, Chun SS, An BJ, Kim JH. Biological and antimicrobial activity of Portulaca oleracea. J Korean Soc Applied Biol Chem. 2008;51(1). https://doi.org/10.3839/jksabc.2008.048
24. Wasnik DD, Tumane P. Preliminary phytochemical screening and evaluation of antibacterial activity of Portulaca oleracea L. against multiple drug resistant (MDR) pathogens isolated from clinical specimen. World J Pharmac Res. 2014;3(10):920-31.
25. Saheri F, Barzin G, Pishkar L, Boojar MMA, Babaeekhou L. Foliar spray of salicylic acid induces physiological and biochemical changes in purslane (Portulaca oleracea L.) under drought stress. Biologia. 2020;75(12):2189-200. https://doi.org/10.2478/s11756-020-00571-2
26. He J, You X, Qin L. High Salinity Reduces Plant Growth and Photosynthetic Performance but Enhances Certain Nutritional Quality of C4 Halophyte Portulaca oleracea L. Grown Hydroponically Under LED Lighting. Front Plant Sci. 2021;12(457). https://doi.org/10.3389/fpls.2021.651341
27. Jin R, Wang Y, Liu R, Gou J, Chan Z. Physiological and Metabolic Changes of Purslane (Portulaca oleracea L.) in Response to Drought, Heat, and Combined Stresses. Front Plant Sci. 2016;6(1123). https://doi.org/10.3389/fpls.2015.01123
28. Mohammadi Azni M, Moradi H, Ghasemi K, Biparva P. Elicitation of dopamine biosynthesis in common purslane as affected by methyl jasmonate and silicon. J Plant Nutr. 2021;44(20):3083-98. https://doi.org/10.1080/01904167.2021.1936027
29. Mahmud N, Ramasamy S, Manickam S, Wan-Mohtar WAAQI. Synergistic-antagonistic interaction of vegetable extracts, Acalypha indica, Centella asiatica, and Sesbania grandiflora: Wound healing, antioxidant, protectivity, and antimicrobial properties. Malays J Microbiol. 2020;16(6). https://doi.org/10.21161/mjm.200792
30. Sulaiman SF, Ooi KL. Polyphenolic and vitamin C contents and antioxidant activities of aqueous extracts from mature-green and ripe fruit fleshes of Mangifera sp. J Agric Food Chem 2012;60(47):11832-8. https://doi.org/10.1021/jf303736h
31. Mohd Yusof FF, Yaacob JS, Osman N, Ibrahim MH, Wan-Mohtar WAAQI, Berahim Z, Mohd Zain NA. Shading effects on leaf gas exchange, leaf pigments and secondary metabolites of Polygonum minus Huds., an aromatic medicinal herb. Plants. 2021;10(3):608. https://doi.org/10.3390/plants10030608

32. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-6. https://doi.org/10.1006/abio.1996.0292
33. Sahgal G, Jayanthi P, Siah C, Ong MT. Interference from ordinarily used solvents in the outcomes of Artemia salina lethality test. J Adv Pharm Technol Res. 2013;4:179-82. https://doi.org/10.4103/2231-4040.121411
34. Erkan N. Antioxidant activity and phenolic compounds of fractions from Portulaca oleracea L. Food Chem. 2012;133(3):775-81. https://doi.org/10.1016/j.foodchem.2012.01.091
35. Sicari V, Loizzo MR, Tundis R, Mincione A, Pellicano TM. Portulaca oleracea L.(Purslane) extracts display antioxidant and hypoglycaemic effects. J Appl Bot Food Qual. 2018;91(1):39-46.
36. Singh S. Salicylic acid elicitation improves antioxidant activity of spinach leaves by increasing phenolic content and enzyme levels. Food Chem Adv. 2023;2:100156. https://doi.org/10.1016/j.focha.2022.100156
37. Gorni PH, Pacheco AC, Lima Moro A, Silva JFA, Moreli RR, de Miranda GR, Pelegrini JM, Zaniboni CB, Spera KD, Bronzel Junior JL, da Silva RM. Elicitation improves the leaf area, enzymatic activities, antioxidant activity and content of secondary metabolites in Achillea millefolium L. grown in the field. J Plant Growth Regul. 2021;40:1652-66. https://doi.org/10.1007/s00344-020-10217-x
38. Baek MW, Choi HR, Solomon T, Jeong CS, Lee OH, Tilahun S. Preharvest methyl jasmonate treatment increased the antioxidant activity and glucosinolate contents of hydroponically grown pak choi. Antioxidants. 2021;10(1):131. https://doi.org/10.3390/antiox10010131
39. Wang C, Zhang J, Lv J, Li J, Gao Y, Patience BE, Niu T, Yu J, Xie J. Effect of Methyl Jasmonate Treatment on Primary and Secondary Metabolites and Antioxidant Capacity of the Substrate and Hydroponically Grown Chinese Chives. Front Nutr. 2022;9. https://doi.org/10.3389/fnut.2022.859035
40. Jirakiattikul Y, Rithichai P, Kwanthong P, Itharat A. Effect of jasmonic acid elicitation period on enhancement of bioactive compounds and antioxidant activity in callus cultures of Hibicus sabdariffa Linn. Hortic Environ Biotechnol. 2021;62(4):629-36. https://doi.org/10.1007/s13580-021-00332-3
41. Sardar T, Ishtiaq M, Mazhar MW, Maqbool M, Moussa IM, Zaman W, Mahmoud EA. Methyl jasmonate and iron oxide nanoparticles act as elicitors to stimulate production of bioactive antioxidants and metabolites in the in vitro callus cultures of Bergenia ciliata (haw.) Sternb. S Afr J Bot. 2023;162:201-10. https://doi.org/10.1016/j.sajb.2023.09.016
42. Truc TT, Bui PH, Phan NTT, My DH, Minh TNK. Bioactive Compounds from Portulaca oleracea L. Extract. Chem Eng Trans 2023;106:637-42.
43. El-Sayed M, Awad S, Ibrahim A. Impact of purslane (Portulaca oleracea L.) extract as antioxidant and antimicrobial agent on overall quality and shelf life of Greek-style yoghurt. Egypt J Agric Res 2019;47(1):51-64.
44. Moumni S, Elaissi A, Trabelsi A, Merghni A, Chraief I, Jelassi B, Chemli R, Ferchichi S. Correlation between chemical composition and antibacterial activity of some Lamiaceae species essential oils from Tunisia. BMC Complement Med Ther. 2020;20:1-15. https://doi.org/10.1186/s12906-020-02888-6
45. Thouri A, Chahdoura H, El Arem A, Omri Hichri A, Ben Hassin R, Achour L. Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). BMC Complement Altern Med. 2017;17(1):248. https://doi.org/10.1186/s12906-017-1751-y
46. Traversier M, Gaslonde T, Lecso M, Michel S, Delannay E. Comparison of extraction methods for chemical composition, antibacterial, depigmenting and antioxidant activities of Eryngium maritimum. Int J Cosmet Sci. 2020;42(2):127-35. https://doi.org/10.1111/ics.12595
47. Kengne IC, Feugap LDT, Njouendou AJ, Ngnokam CDJ, Djamalladine MD, Ngnokam D, Voutquenne-Nazabadioko L, Tamokou JD. Antibacterial, antifungal and antioxidant activities of whole plant chemical constituents of Rumex abyssinicus. BMC Complement Med Ther 2021;21(1):164. https://doi.org/10.1186/s12906-021-03325-y
48. Chlif N, Diouri M, El Messaoudi N, Ed-Dra A, Filali F, Bentayeb A. Phytochemical composition, antioxidant and antibacterial activities of extracts from different parts of Brocchia cinerea (Vis.). Biointerface Res Appl Chem. 2022;12:4432-47. https://doi.org/10.33263/BRIAC124.44324447
49. Walia S, Mukhia S, Bhatt V, Kumar R, Kumar R. Variability in chemical composition and antimicrobial activity of Tagetes minuta L. essential oil collected from different locations of Himalaya. Ind Crops Prod. 2020;150:112449. https://doi.org/10.1016/j.indcrop.2020.112449
50. Chung I-M, Thiruvengadam M, Rekha K, Rajakumar G. Elicitation enhanced the production of phenolic compounds and biological activities in hairy root cultures of bitter melon (Momordica charantia L.). Braz Arch Biol Technol. 2016;59:e160393. https://doi.org/10.1590/1678-4324-2016160393
51. Shireesha S, Khan A. Phytochemical analysis and antimicrobial activity of salicylic acid (SA) and jasmonic acid (JA) treated plants of Acalypha indica Linn. Int J Res Biosci Agric Technol. 2021;17:645-56.
52. Konan AML, Golly KJ, Kra AKM, Adima AA, Lohoues EEC. Phytochemical screening and toxicity assessment of Imperata cylindrica (L.) P. Beauv.(Poaceae) raw extracts with brine shrimp (Artemia salina) lethality assay. J Biosci Med. 2022;10(8):153-71. https://doi.org/10.4236/jbm.2022.108014
53. Clemen-Pascual LM, Macahig RAS, Rojas NRL. Comparative toxicity, phytochemistry, and use of 53 Philippine medicinal plants. Toxicol Rep. 2022;9:22-35. https://doi.org/10.1016/j.toxrep.2021.12.002
54. Otang WM, Grierson DS, Ndip RN. Assessment of potential toxicity of three South African medicinal plants using the brine shrimp (Artemia salina) assay. Afr J Pharm Pharmacol. 2013;7(20):1272-9. https://doi.org/10.5897/AJPP12.264
55. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982;45(5):31-4. https://doi.org/10.1055/s-2007-971236