ACADEMIA JOURNAL OF BIOLOGY 2024, 46(4): 91–102
DOI: 10.15625/2615-9023/20779

INFLUENCE OF THE ELICITORS ON THE ACCUMULATION OF SECONDARY METABOLITES AND ANTIOXIDANT ACTIVITIES IN THE *Polyscias fruticosa* (L.) Harms *in vitro* ROOTS

Le Thi Van Anh, Tran Ngoc Trung Anh, Duong Phuong Anh, Ha Tran Bao Chau, Dinh Thi Hien Diu, Mai Thi Phuong Nga*

University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Ha Noi, Vietnam

Received 13 May 2024; accepted 6 December 2024

ABSTRACT

Polyscias fruticosa (L.) Harms, belonging to the Ginseng family (Araliaceae), is a valuable medicinal plant in Vietnam. Recognized in the Vietnamese Pharmacopoeia, it is esteemed for its antioxidant, anti-inflammatory, and antipyretic properties, making it a significant contributor to traditional medicine. This research contributes to the understanding of elicitor-induced changes in antioxidant activity, and secondary metabolite content of *in vitro* root extract of *P. fruticosa.* The results showed that the antioxidant activity of the root extract tested by DPPH radical scavenging assay with an IC_{50} value of 1.28 mg/mL in the baseline and the lowest IC_{50} value of 0.89 mg/mL in the medium treated with yeast extract (YE). The highest total phenolic and flavonoid content were displayed in jasmonic acid-treated samples at 24.31 ± 2.86 mg gallic acid/g extract and 29.25 ± 0.26 mg quercetin/g extract, respectively. The highest saponin was also produced in roots elicited by jasmonic acid, where it reached about 167.19 ± 3.29 mg aescin/g extract. Furthermore, investigation with antioxidant enzyme activities showed another superiority when using elicitors YE (64.4 ± 3,14 U/mg protein) and mannitol (Man) (65.39 ± 3.85 U/mg protein) in peroxidase (POD) activity and using jasmonic acid (8.87 ± 0.74 U/mg) in catalase (CAT) activity. Lastly, TPC and TFC exhibited a positive correlation of r = 0.53 while the TPC and DPPH scavenging activity and TFC and POD showed a remarkably negative correlation of r around -0.8. In conclusion, this study highlights the advancements in eliciting root cultures to enhance specific phytochemicals and antioxidant activity. The information provided holds significant value and will be of interest to scientists engaged in plant biotechnology, particularly in the elicitation of medicinal plant roots.

Keywords: Antioxidant, elicitors, *in vitro* tissue culture, *Polyscias fruticosa* (L.) Harms, secondary metabolites.

Citation: Le Thi Van Anh, Tran Ngoc Trung Anh, Duong Phuong Anh, Ha Tran Bao Chau, Dinh Thi Hien Diu, Mai Thi Phuong Nga, 2024. Influence of the elicitors on the accumulation of secondary metabolites and antioxidant activities in the *Polyscias fruticosa* (L.) Harms *in vitro* roots. *Academia Journal of Biology*, 46(4): 91–102. https://doi.org/10.15625/2615-9023/20779

**Corresponding author email*: mai-thi-phuong.nga@usth.edu.vn

INTRODUCTION

Traditional medical practices have been researched and practiced for numerous ailments for millennia. Ancient people, benefiting from favorable natural conditions with abundant medicinal herbs, have made significant discoveries in the field of remedies that exhibit both efficacy and safety in their usage. Ming aralia, scientifically known as *Polyscias fruticosa* (L.) Harms belongs to the Araliaceae family. These plants thrive in temperatures below 25 ^{o}C (Ho, 2017). It has been reported that *P. fruticosa* is composed of amino acids, saponins, alkaloids, glycosides, polyphenols, flavonoids, tannins, and vitamins (C, B1, B2, and B6); thus, this herb possesses potent antioxidant properties and antibacterial activity against Gram-positive, Gram-negative bacteria and molds (Mawea et al., 2021; Ly et al., 2022). *In vivo* testing in old male rat demonstrated that the root extract of *P. fruticosa* could stimulated sexual activity, and increased fertility (Boye et al., 2018). Additionally, this plant is rich in saponins, a class of bitter-tasting steroids (Ly et al., 2022). Saponins showed a wide range of biological effects, such as reducing inflammation and blood sugar levels and inhibiting the formation of tumors (Khoang et al., 2022). Moreover, saponins also exhibit diverse biological activities, including hemolytic, anti-inflammatory, antifungal, antibacterial, molluscicidal, cytotoxic, and antitumor properties, potentially playing crucial roles in plant defense (Hussain et al., 2019).

Elicitors are stressors that trigger the production of biologically active secondary metabolites (phytoalexins) in plants, including cultivated plant cells. These substances function as signals and evoke defense responses when recognized by elicitor-specific receptors on the plant cell membrane (Halder et al., 2019). Consequently, secondary metabolites are synthesized and accumulated at an increased rate. Different elicitors have been used in plant cultures; however, the effectiveness of elicitors strongly depends on the concentration, time exposure, elicitor type, and cell culture type (Dhiman et al., 2018). Recently, only one study has used yeast extract, salicylic acid, and silver nitrate as elicitors to study their effect on the growth of suspension cells of *P. fruticosa* (Kim et al., 2018) but not yet on the antioxidant metabolites and enzyme activities. However, all elicitors used in this study inhibited cell growth.

In our study, different elicitors, including mannitol, yeast extract, and jasmonic acid, were used to study their effectiveness in stimulating secondary metabolite production and antioxidant enzyme activities in *P. fruticosa*. The results from our study can open new insights into the application of elicitors to enhance the production of valuable metabolites for therapeutic application.

MATERIALS AND METHODS

Materials

Biological materials

The *in vitro* culture plantlets of *P. fruticosa* sub-cultured in our laboratory were used as the culture specimen.

Chemicals

Murashige and Skoog medium (MS), 1-Naphthaleneacetic acid (NAA), 6-Benzylaminopurine (BAP), and mannitol were purchased from Duchefa (Duchefa Biochemie, Haarlem, Netherlands). Yeast extract and jasmonic acid were purchased from Merk (Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA) respectively. Guanicol and H_2O_2 were purchased from Silong, China. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), $NaNO_2$, $AlCl_3$, Na_2CO_3, and Follin-Ciocalteu were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

Preparation for *Polyscias fruticosa* (L.) Harms extractions

The root samples obtained from the *in vitro* culture and wild-type roots were subjected to a series of steps for extracting bioactive compounds, following the protocol of Zhou et al. (2020) with some modifications. Firstly, the samples were cleaned and dried at 60 ^{o}C until they reached a constant weight. Once dried, the roots were ground into a fine powder. Next, all samples

mixed with methanol were sonicated at 38 ^{o}C for 30 minutes for a better extraction. After sonication, the vials were centrifuged for 6 minutes to separate the solid particles from the solution. The supernatant, containing the crude extract, was then evaporated in an oven set at 60 ^{o}C to remove the methanol and concentrate the bioactive compounds. Finally, methanol was added to each extract, resulting in a 40 mg/mL solution.

Total phenolic content assay (TPC)

The TPC in the methanol extracts of *P. fruticosa in vitro* roots was estimated by the Folin Ciocalteu (FC) colorimetric method (Singleton et al., 1999) with some modifications. The formation of blue compounds from the TPC assay, which is the reduction of the blue-colored FC reagent with gallic acid as the standard, was measured at 765 nm to determine the content of TPC.

Preparation of standard curve of gallic acid and TPC quantification

The gallic acid solution was prepared for the standard calibration curve. One mg of gallic acid (GAE) powder was dissolved in 1 mL of methanol 80 % and Milli-Q water at a ratio of 1:9 to prepare various concentrations of GAE solution (0.04, 0.02, 0.01, 0.005, and 0.0025 mg/mL). In each concentration, 40 µL of the gallic solution was mixed with 480 µL of FC reagent (diluted with Milli-Q water 1:10), then the mixture was incubated at 40 ^{o}C for 1 minute. Then, 480 µL of Na_2CO_3 (6 %) was added to the mixture and continuously incubated at 40 ^{o}C for 15 minutes. The absorbance of the blue-colored mixtures was measured at 765 nm by a UV-Vis spectrophotometer (SpectraMax® iD5) against the blank solution. A linear equation was used to calculate the amount of phenolic compounds in the extraction samples.

Subsequently, TPC in root extracts followed the protocol of standard GAE, except for gallic acid, which was replaced by a root extract sample. The concentration of TPC in samples was calculated based on the standard curve built with GAE.

$$\mathrm{TPC} = \frac{\text{Concentration of gallic acid}\,(\mathrm{mg/mL})}{\text{Concentration of extracts}\,(\mathrm{g/mL})}$$

The TPC was calculated as GAE equivalent (mg of GAE/g of dry weight of extract).

Total flavonoid content assay (TFC)

The TFC in the methanolic extracts of *P. fruticosa* was estimated by the aluminium chloride colorimetric method (Phuyal et al., 2020).

Preparation of Standard curve of Quercetin and TFC quantification

To prepare the standard curve, 1 mg of quercetin powder was dissolved in 1 mL Milli-Q water and diluted to different concentrations (0.18, 0.09,0.045,0.0225 and 0.01125 mg/mL). Next, 240 µL of each concentration's solution was transferred into an Eppendorf tube, mixed with 40 µL of 5% $NaNO_2$ solution, then incubated at 27 ^{o}C for 6 minutes. After incubation, 40 µL of 10% $AlCl_3$ solution was added and continuously incubated for 6 minutes. Thus, 400 µL of NaOH 1M and 280 µL of ethanol 30% are added simultaneously and left for 30 minutes. Finally, the absorbance of mixtures was measured at 510 nm by UV-Vis spectrophotometer (SpectraMax® iD5) against the blank solution.

To quantify the TFC of root extracts, a similar procedure as described for quercetin solution was followed, except a root extract sample replaced quercetin. The TFC values of the extracts were calculated using the formula:

$$\mathrm{TFC} = \frac{\text{Concentration of quercetin}\,(\mathrm{mg/mL})}{\text{Concentration of extracts}\,(\mathrm{g/mL})}$$

The TFC was calculated as quercetin equivalent (mg of quercetin/g of dry weight of extract).

Total saponin content assay

The total saponin content (TSC) in the methanolic extracts of *P. fruticosa* was estimated following the total saponin content assay (Khoang et al., 2022) with some modifications.

Preparation of Aescin standard curve and TSC quantification

One mg of aescin powder was dissolved in 1 mL of methanol and then diluted to different concentrations (0.08, 0.04, 0.02, 0.01, and 0.005 mg/mL). In each concentration, 150 µL of the solution was taken to the Eppendorf tube and mixed with 600 µL of the reagent mixture (mixture of acetic acid and sulfuric acid in proportion 1:1). Then, the mixture was vortexed and incubated at 60 ^{o}C for 30 minutes. The absorbance of mixtures was measured at 527 nm by UV-Vis spectrophotometer (SpectraMax® iD5) against the blank solution, subsequently plotting the calibration curve.

The TSC content in root extracts of *P. fruticosa* followed the aescin standard curve except that aescin was replaced by a root extract solution. The absorbance was measured, and the TSC was calculated using the formula:

$$\text{TSC} = \frac{\text{Concentration of aescin}\left(\text{mg/mL}\right)}{\text{Concentration of extracts}\left(\text{g/mL}\right)}$$

The TSC was calculated as quercetin equivalent (mg of aescin/g of dry weight of extract).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

In vitro antioxidant activities of the root extracts were determined using the DPPH free radical scavenging assay, described by Phuyal et al. (2020).

Preparation of ascorbic acid standard curve and DPPH activity in root sample

Two mg of ascorbic acid was dissolved in 1 mL of Milli-Q water and was diluted in various concentrations (0.00625, 0.003125, and 0.0015625 mg/mL). In each concentration, 40 µL of solution was taken to add with 760 µL of DPPH 0.25 mM, then incubated the mixture at room temperature for 20 minutes in the dark. Thus, the absorbance of the mixture was measured at 517 nm by UV-Vis spectrophotometer (SpectraMax® iD5) against the blank solution.

Various concentrations of extracts of *P. fruticosa* (2, 1.5, 1, 0.75, and 0.5 mg/mL) were prepared by diluting the stock solution of extracts with methanol. The procedure described for ascorbic acid solution was followed for each concentration. The absorbance was measured and plot to the calibration curve of the percentage of inhibition (I%) to determine the IC_{50} based on the optical density of the control (OD_C) and the sample (OD_S):

$$\text{I\%} = \frac{\text{OD}_\text{C} - \text{OD}_\text{S}}{\text{OD}_\text{C}} \times 100\%$$

Antioxidant enzyme activity

The antioxidant enzyme activity of POD and CAT was determined following the protocol of Chen & Zhang (2016).

Preparation of extracts

One-fifth of the fresh young roots of *P. fruticosa* were collected and ground with a mortar and pestle in liquid nitrogen. Three mL of 100 mM PBS buffer pH 7.8 was added to homogenize the sample, then transferred the homogenate into a 15 mL centrifuge tube and centrifuged at 4,000 rpm for 20 minutes at 4 ^{o}C. After centrifugation, the supernatant was transferred to the new tube and kept on ice. The concentration of crude protein (mg/mL) was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

POD activity assay

The reaction buffer for 15 reactions was prepared by adding 8.4 µL of guaiacol 0.2 % in 15 mL of PBS 100 mM pH 7.0, then, adding 5.7 µL of H_2O_2 30%. For measurement, 200 µL of PBS 100 mM pH 7.8 and 1 mL of reaction solution were added into a cuvette and considered blank. To measure the enzyme activity of root extract, 200 µL of each crude extract and 1 mL reaction solution were into the cuvette. Subsequently, the absorbance was measured at 470 nm against the blank every 15 seconds for 1 minute.

CAT activity assay

The reaction buffer for 15 reactions was prepared by adding 23.25 µL of H_2O_2 30% in

15 mL of PBS 100 mM pH 7.0. For measurement, 200 µL of PBS 100 mM pH 7.8 and 1 mL of reaction solution were added into a cuvette and considered blank. The enzyme activity of root extract was measured by adding 200 µL of each crude extract into 1 mL reaction solution in the cuvette. Subsequently, the absorbance was measured at 240 nm against the blank every 15 seconds for 1 minutes.

Statistical analysis

All the experiments were repeated three times. The data was presented by means of three replications ± standard deviations. The difference between parameters was analyzed using ANOVA one way and Turkey posthoc test to assess the statistical significance of the means, with $p < 0.05$ considered significant.

RESULTS

***in vitro* root culture**

Our previous study confirmed that MS supplemented with 2 mg/L NAA strongly stimulated root induction from leaves of *P. fruticose.* Therefore, this medium was chosen for the following experiments.

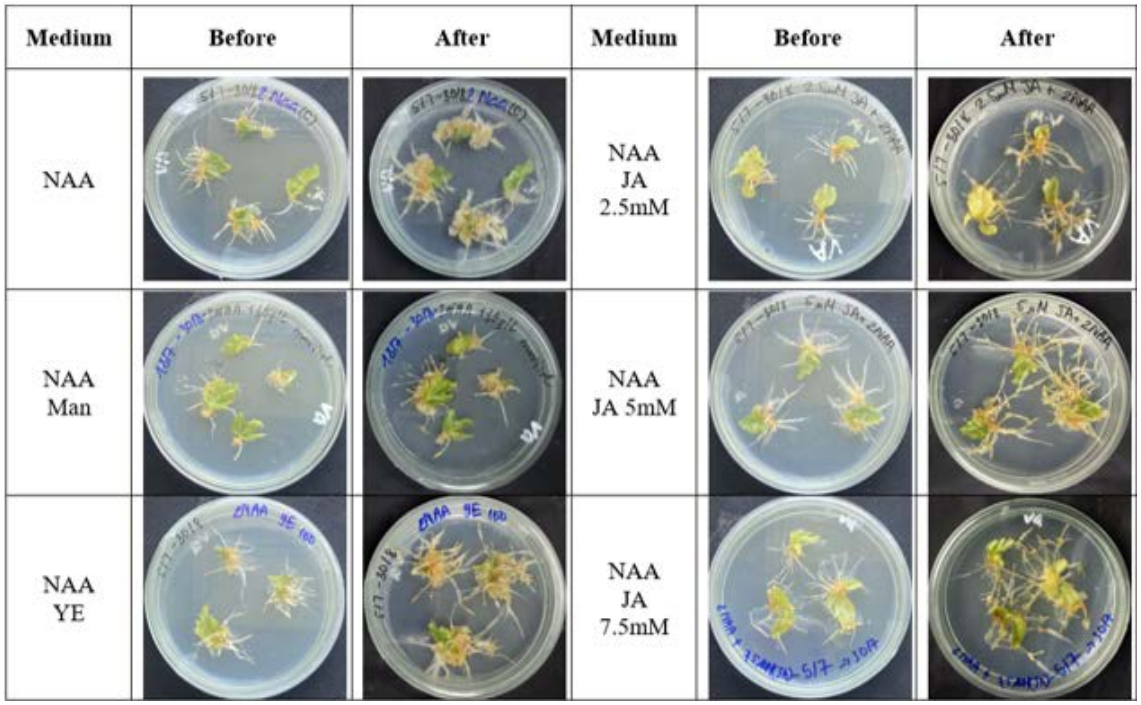

Figure 1. Morphological changes in roots following a month of elicitor exposure

After four weeks of culture in MS medium supplemented with 2 mg/L NAA, roots started to be produced from the leaf pieces of *P. fruticose*. Two weeks later, root-induced leaves were transferred to another MS medium containing both NAA hormone and an elicitor. After a month of growing in media with elicitors, roots were harvested for investigation.

We investigated the effects of elicitors at different concentrations on *in vitro* root formation: Mannitol (20, 30, 40 50 g/L), YE (100, 200, 300, 400, 500 mg/L), and JA (2.5, 5, 7.5 mM). Root development was measured following a month. The root biomass produced by the explants was used to determine suitable concentrations of elicitors for stimulating root growth: Man 30 g/L, YE 100 mg/L, and three concentrations of JA at 2.5, 5, and 7.5 mM. Consequently, increasing the number of roots collected, the quantity of secondary compounds

the plant contains, and other activities were the goals.

According to Figure 1, the root biomass had developed after a month. Leaf fragments in a medium containing NAA only produced more calli than roots, although new root formation was still observed. However, the biomass proliferated with the formation of large numbers of long roots in the medium containing YE and JA at a concentration of 5 mM. Roots in a medium treated with Man were slowly regenerated and in small quantities.

Total phenolic content

The TPC was expressed in milligrams of Gallic acid equivalent per gram root extract (mgGAE/g extract) (Table 1). The baseline extract treated with NAA exhibited a TPC value of 10.35 ± 0.84 mgGAE/g. Notably, introducing Man led to a substantial increase in TPC, reaching 17.36 ± 1.85 mgGAE/g ($p < 0.05$). Similarly, adding YE resulted in a significantly higher TPC value of 18.95 ± 2.55 mgGAE/g ($p < 0.05$). Among concentrations of JA, NAA + JA 2.5 mM exhibited the highest TPC value at 24.31 ± 2.86 mgGAE/g ($p < 0.01$). In contrast, the wild root extract showed a comparatively lower TPC value of 6.35 ± 1.13 mgGAE/g than the roots grown in NAA 2 mg/L.

Table 1. TPC values of *Polyscias fruticosa* root extracts elicited by elicitors

Extracts	TPC values (mgGAE/g extract)
NAA	10.35 ± 0.84
NAA + Man	17.36 ± 1.85*
NAA + YE	18.95 ± 2.55*
NAA + JA 2.5	24.31 ± 2.86**
NAA + JA 5	22.56 ± 2.43*
NAA + JA 7.5	21.49 ± 3.19*
R_W	6.35 ± 1.13*

Note: (*, **) indicate the significant difference between treatments and NAA only with $p < 0.05$ and 0.01, respectively. R_W: wildtype roots.

Total flavonoid content

The TFC in *P. fruticosa* roots was quantified in milligrams of quercetin equivalent per gram (mgQE/g extract). Table 2 elucidates the intricate influence of diverse elicitation strategies on the flavonoid composition. The baseline extract of NAA-treated samples exhibited a TFC value of 22.44 ± 0.27 mgQE/g. The addition of Man significantly reduced to 13.59 ± 1.30 mgQE/g ($p < 0.05$). Conversely, the combination of NAA with YE yielded a TFC value of 18.99 ± 2.64 mgQE/g, a non-significant difference from the TFC produced by roots grown in NAA only ($p > 0.05$). Notably, NAA + JA 2.5 mM and NAA + JA 7.5 mM demonstrated statistically significant increases in TFC (27.65 ± 0.15 mgQE/g and 29.25 ± 0.26 mgQE/g, respectively) ($p < 0.01$), highlighting a positive modulation of flavonoid synthesis. Furthermore, the wild root extract (R_W) exhibited a TFC value of 16.46 ± 0.89 mgQE/g, which was lower than the baseline.

Total saponin content

The TSC in *P. fruticosa* roots was expressed in milligrams of aescin equivalent per gram (mgAE/g extract) (Table 3). The extract of NAA-treated samples established a foundation with a TSC value of 54.08 ± 2.40 mgAE/g. The treatment with Man showed a significant increase in TSC to 73.01 ± 1.40 mgAE/g ($p < 0.05$). Meanwhile, the combination with YE demonstrated a substantial elevation to 123.67 ± 8.56 mgAE/g, suggesting a significant positive modulation of saponin synthesis. Notably, NAA + JA 2.5 mM, NAA + JA 5 mM, and NAA + JA 7.5 mM exhibited statistically significant

increases in TSC (167.19 ± 3.29 mgAE/g, 140.69 ± 1.94 mgAE/g, and 136.90 ± 0.81 mgAE/g, respectively) ($p < 0.05$), highlighting a dose-dependent positive modulation of saponin synthesis. Furthermore, the R_W extract displayed a TSC value of 137.37 ± 2.84 mgAE/g, a significant increase compared to the baseline.

Table 2. TFC values of *Polyscias fruticos* root extracts elicited by elicitors

Extracts	TPC values (mgQE/g extract)
NAA	22.44 ± 0.27
NAA + Man	$13.59 \pm 1.3^{*}$
NAA + YE	18.99 ± 2.64^{ns}
NAA + JA 2.5	$27.65 \pm 0.15^{**}$
NAA + JA 5	21.58 ± 0.35^{ns}
NAA + JA 7.5	$29.25 \pm 0.26^{**}$
R_W	$16.46 \pm 0.89^{*}$

Note: (*, **, and ns) indicate the significant difference between treatments and NAA only with $p < 0.05$, 0.01, and non-significant difference, respectively. R_W: wildtype roots.

Table 3. TSC values of *Polyscias fruticosa* root extracts elicited by elicitors.

Extracts	TSC values (mgAE/g extract)
NAA	54.08 ± 2.4
NAA + Man	$73.02 \pm 1.4^{*}$
NAA + YE	$123.67 \pm 8.56^{**}$
NAA + JA 2.5	$167.19 \pm 3.29^{**}$
NAA + JA 5	$140.69 \pm 1.94^{**}$
NAA + JA 7.5	$130.9 \pm 0.81^{**}$
R_W	$137.37 \pm 2.84^{**}$

Note: (*, **) indicate the significant difference between treatments and NAA only with $p<0.05$ and 0.01, respectively. R_W: wildtype roots.

Antioxidant enzyme activities

The activity of the enzyme in the *P. fruticosa* root extracts was measured and shown in Figure 2. The results showed that the measured POD activity in the control sample was approximately 17.85 ± 5.05 U/mg protein. Treatment with Man and YE showed a remarkable increase in POD activity to 65.385 ± 3.846 and 64.397 ± 3.140 U/mg ($p < 0.01$), respectively. Meanwhile, there was a slight increase in the POD activity in the roots treated with JA. As the concentration of JA increased, the measured POD activities were 24 ± 6.788, 24.826 ± 1.848, and 23.468 ± 3.688 U/mg, which did not show the dose-dependent between studied JA concentration and POD activity. The sample of R_W displayed a significant rise with 85.53 ± 6.847 U/mg.

As a reference, the baseline's measured CAT activity was roughly 2.261 ± 0.246 U/mg in roots grown in an MS medium containing only NAA. There was a significant rise to 7.772 ± 0.314 U/mg ($p < 0.01$) when YE was introduced. Adding Man did not significantly decrease CAT activity, which was 1.923 ± 0.544 U/mg. The enzyme activity was not affected by the concentration of JA, and it was highly boosted to 8.532 ± 0.389 (JA 2.5 mM), 8.737 ± 1.002 (JA 5 mM), and 8.866 ± 0.738 U/mg (JA 7.5 mM) in roots elicited by JAs. The CAT activity in R_W, which was 20.656 ± 0.913 U/mg, was significantly higher than the roots grown in MS medium containing only NAA ($p < 0.05$).

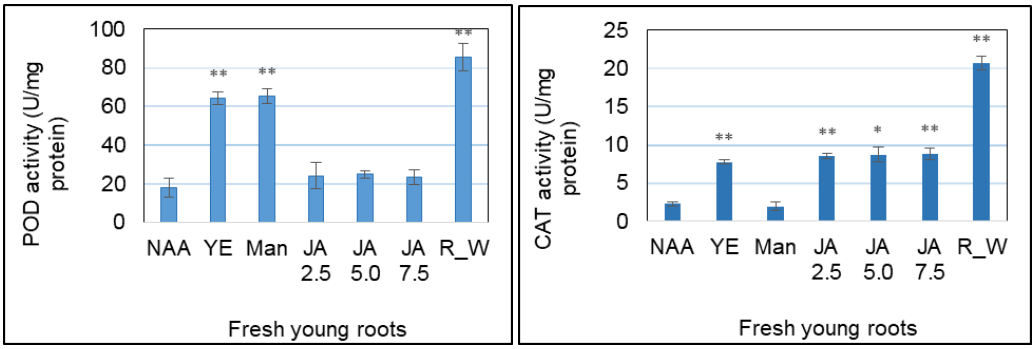

Figure 2. Peroxidase (left) and catalase (right) activities of *Polyscias fruticosa* root elicited by elicitors. (*, **) indicate the significant difference between treatments and NAA only with $p < 0.05$ and 0.01, respectively

DPPH scavenge activity

The assessment of DPPH scavenges activity, measured through IC_{50} values in milligrams per milliliter (mg/mL), shown in Figure 3, sheds light on the efficacy of diverse elicitation strategies in *P. fruticosa* extracts. As a positive control, ascorbic acid demonstrated robust antioxidant potential with an impressively low IC_{50} value of 0.005 mg/mL (Fig. 3). In comparison, the extract of NAA-treated samples exhibited a moderate IC_{50} value of 1.28 mg/mL, as a reference. The addition of Man increased the IC_{50} to 1.39 mg/mL. Conversely, the YE treatment yielded a noteworthy decrease in the IC_{50} to 0.89 mg/mL. Concentrations of JA (2.5, 5, 7.5 mM) in conjunction with NAA demonstrated the IC_{50} values of 1.065, 1.004, and 1.08 mg/mL, respectively, which did not indicate the correlation between studied JA concentration and DPPH activity. In contrast, the wild root extract (R_W) displayed a higher IC_{50} value of 2.66 mg/mL, indicative of comparatively lower DPPH antioxidant activity.

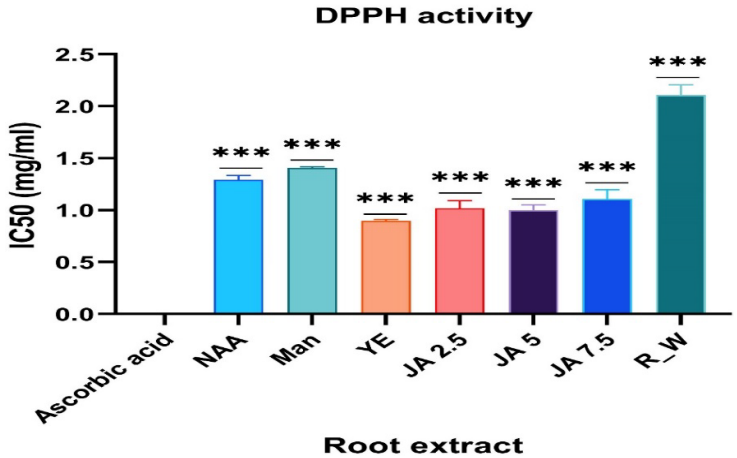

Figure 3. DPPH scavenge activity of root extracts elicited by elicitors. *** indicates the significant difference between treatments and NAA only with $p < 0.001$

Correlation between values obtained

The correlation study presented in Table 4 provides valuable insights into the relationships among different assays conducted on *P. fruticosa* root extract. Notably, TPC and TFC exhibited a positive correlation of $r = 0.53$, indicating a

simultaneous increase in phenolic and flavonoid compounds. However, the TPC and IC_{50} value showed a remarkably negative correlation of r = -0.825. Surprisingly, despite this negative correlation, the lower the IC_{50} value, the higher the antioxidant activity, suggesting a positive correlation between TPC and DPPH scavenging activity.

On the other hand, TFC and POD activity displayed a substantial negative correlation of r = -0.794. This implied that as the flavonoid content increased, the POD activity decreased. The correlations between IC_{50} and both enzymes revealed exciting dynamics. Although IC_{50} showed a positive correlation with r values of approximately 0.6, indicating a potential increase in antioxidant activity, the actual radical scavenging and enzyme activities displayed an inverse relationship.

Furthermore, TSC exhibited a high positive correlation with CAT activity, suggesting a potential link between TSC and catalase activity. The results of other assays exhibited moderate correlations with lower r values, highlighting the complex interactions within the chemical composition and antioxidant properties of the sample.

Table 4. The correlation between values obtained

	TPC	TFC	TSC	IC_{50}	POD	CAT
TPC	1					
TFC	0.530701	1				
TSC	0.484951	0.462225	1			
IC_{50}	-0.82468	-0.50201	-0.12361	1		
POD	-0.51885	-0.79433	-0.02639	0.629969	1	
CAT	-0.35704	-0.04861	0.625613	0.613259	0.492034	1

DISCUSSION

Overall, the present results demonstrated that YE and JA exhibited the best results among all elicitors. The results of *P. fruticosa* treated with JA show greater comprehensiveness. JA shows a comprehensive development in the overall indexes, while YE shows an increase in antioxidant enzyme POD activity, more notably the DPPH value. With the lowest IC_{50} value compared to other samples, the YE treatment exhibits strong antioxidant activity. Elicitors trigger defensive responses of plants, enhancing the secondary metabolite accumulation (Halder et al., 2019).

Although *P. fruticosa* treated with YE presents the highest antioxidant enzyme POD activity among all, its TPC and TFC are lower than that of JA treatment. One theory is that YE promotes the synthesis of other chemicals that enhance plants' antioxidant activity rather than phenolic and flavonoid molecules; for example, it increases the POD and CAT antioxidant activity (Rarison et al., 2023). In this research, both enzymes exhibit substantial activity with YE treatment, particularly POD, with a result that is three times greater than the control sample, leading to a higher antioxidant activity. The studies conducted by Woch et al. (2023) showed that the application of JA, and salicylic acid, to *Givotia moluccana* (L.), resulted in increased levels of POD and CAT enzymes compared to the control group. In addition, the age of plants can also influence the concentration of enzymes, and the relationship is complex and dependent on the particular enzyme and plant species. The investigation conducted on the leaves of Ragi has demonstrated that the levels of specific enzymes, including catalase, may escalate as the plant ages. Moreover, our investigation demonstrated that the correlation between POD and CAT was negative, which was consistent with the research of K.B. Kumar (Kumar & Khan, 1983).

TPC and TFC function as antioxidants by reacting with free radicals via hydrogen atom transfer, electron transfer, etc. (Zeb, 2020). In

our research, generally, roots treated with elicitors increased TPC and TFC to neutralize any toxic effect caused by elicitors. This phenomenon has been observed in diverse plants under stress conditions (Kumar et al., 2023). The same results were also obtained in the research of (Nguyen et al., 2020), on *P. fruticosa*, regarding the direct correlation between the antioxidant activity of the plant and its phenolic and flavonoid content. In the experiments of Marjia et al. (1999), they proved that plants that a TPC value of more than 20 µgGAE/mg, were generally considered to have strong antioxidant activity. Interestingly, in our study, TPC produced by roots treated with JA was higher than 20 µgGAE/mg.

The total saponin content obtained by treating JA at a concentration of 2.5 mM is the most notable value. Moreover, compared to wildtype *P. fruticosa* grown for an extended period, roots treated with JA for three weeks had a higher concentration of saponin. Therefore, using JA as an elicitor is a strategy not only to increase saponin content but also to reduce the culturing time. In addition, JA treatment was found to increase the secondary metabolites, including phenolic and flavonoid, better than those other elicitors. Our results demonstrate a resemblance in TSC to the findings of Le et al. (2022). While in the study of Le et al. (2022), the TSC of *P. fruticosa* was around 41.24 mg/g, our research demonstrates a somewhat higher result of 54.08 mg/g for the control sample. Except for Man administration, which only slightly enhanced the TSC in the plants, the remaining samples exhibit a significantly greater TSC concentration. The average amount of TSC produced from plants treated with YE and JA is nearly threefold more than that produced by control plants. Therefore, our results strongly demonstrated a very promising method to increase highly valuable TSC by using elicitors, which could be applied in therapeutic applications.

Regarding DPPH scavenge activity, the study of Mai et al. (2017) affirmed the reliability of our research since their DPPH results and our results show some similarity. The IC_{50} of *P. fruticosa* was found to be 2,110.08 µg/mL in Mai's study, whereas it was 2,089.94 µg/mL for the wild type of *P. fruticosa* in our investigation. Given that Jumina et al. (2019) indicated that antioxidant activity was weak at IC_{50} greater than 150 µg/mL, it is possible to draw the conclusion from these data that this plant exhibits poor antioxidant activity. However, the study by Nguyen et al. (2020) on *P. fruticosa* demonstrated the opposite effect. In this experiment, the IC_{50} value fell around 96,14 µg/mL, ten times smaller than our IC_{50} results. The reasons contributing to this phenomenon could be the difference between culture conditions, source of plant harvest, extraction solvent (ethanol instead of methanol), extraction time, etc.

CONCLUSION

In this research, the metabolites produced by *P. fruticosa* showed minimal reaction to Man, in contrast to the elicitors JA and YE, which exhibited some considerable responses. Overall, Man does stimulate secondary metabolites and enzymes in *P. fruticosa*. However, JA and YE proved to be more effective. The application of YE considerably increased the antioxidant activity of the plant, while JA treatment significantly elevated the production of almost all the secondary compounds, remarkably saponin content. Our results also confirmed the positive correlation between the TPC and TFC with antioxidant activity. In summary, the experiment affirmed that elicitation is a potential tool for influencing plants' antioxidant activity, secondary metabolites, and antioxidant enzymatic activity. This investigation unfolds perspectives for further exploration into the specific compounds synthesized under different elicitor treatments, exploring the diverse chemical responses induced by various elicitors. Further studies could also delve into the molecular mechanisms triggered by elicitors to elucidate the signalling pathways involved in secondary metabolite enhancement.

Acknowledgements: We would like to thank the University of Science and Technology of Hanoi for supporting the facilities for us to perform this study.

REFERENCES

Boye A., OseiOwusu A., Koffuor G., Barku V., Asiamah E., Asante E., 2018. Assessment of *Polyscias fruticosa* (L.) Harm (Araliaceae) leaf extract on male fertility in male Wistar Rats. *J. Intercult. Ethnopharmacol.* 7: 45–56. doi: 10.5455/jice.20170701035248

Chen T., Zhang B., 2016. Measurements of proline and malondialdehyde content and antioxidant enzyme activities in leaves of drought stressed cotton. *Bio-protocol* 6. doi: 10.21769/BIOPROTOC.1913

Dhiman N., Patial V., Bhattacharya A., 2018. The current status and future applications of hairy root cultures. *Biotechnological Approaches for Medicinal and Aromatic Plants. Springer* 87–155.

Halder M., Sarkar S., Jha S., 2019. Elicitation: A biotechnological tool for enhanced production of secondary metabolites in hairy root cultures. *Eng. Life Sci.*, 19: 880–895. doi: 10.1002/ELSC.201900058

Ho P. H., 2017. Vietnamese plants. Young publisher.

Hussain M., Debnath B., Qasim M., Steve Bamisile B., Islam W., Hameed M. S., 2019. Role of saponins in plant defense against specialist herbivores. *Molecules*, 24(11): 2067. doi: 10.3390/ MOLECULES24112067

Jumina Siswanta D., Zulkarnain A. K., Triono S., Priatmoko Yuanita, E., 2019. Development of C-Arylcalix[4]resorcinarenes and C-Arylcalix[4]pyrogallolarenes as antioxidant and UV-B protector. *Indones. J. Chem.*, 19: 273–284. doi: 10.22146/IJC.26868

Khoang L., Huyen H., Chung H., Duy L., Toan T., Bich, H., 2022. Optimization of total saponin extraction from *Polyscias fruticosa* roots using the ultrasonic-assisted method and response surface methodology. *Processes*, 10(10): 2034.

Kim P. T. A., Ngan N. T. H., Thu L. T. A., Huan L. V. T., 2018. Effects of carbon sources and elicitors on growth of *Polyscias fruticosa* (L.) harms suspension cells. *Hueuni-jns*, 127(1): 85–94.

Kumar K. B., Khan P. A., 1983. Age-related changes in catalase and peroxidase activities in the excised leaves of *Eleusine coracana Gaertn*. cv PR 202 during senescence. *Exp. Gerontol.*, 18: 409–417. doi:10.1016/0531-5565(83)90019-0

Kumar K., Debnath P., Singh S., Kumar N., 2023. An overview of plant phenolics and their involvement in abiotic stress tolerance. *Stress*, 3: 570–585. doi: 10.3390/STRESSES3030040

Ly H. T., Nguyen T. T. H., Le V. M., Lam B. T., Mai T. T. T., Dang T. P. T., 2022. Therapeutic potential of *Polyscias fruticosa* (L.) harms leaf extract for parkinson's disease treatment by drosophila melanogaster model. *Oxid. Med. Cell. Longev*, 2022. doi: 10.1155/2022/5262677

Mai D. V., Ngoc Trung Dung H., Kim Ngoc T., 2017. Research and screening of medicinal plants with antioxidant activity in Can Tho city. *J. Sci. Res. and Eco. Dev., Tay Do Uni.*, 1: 143–152.

Mawea F., Indrayati A., Rahmawati I., 2021. Review: Antibacterial Activity of Polyscias fruticosa and Molecular Mechanism of Active Compounds. *Int. J. Health Med. Curr. Res.*, 6: 2062–2087. doi: 10.22301/IJHMCR.2528-3189.2062

Nguyen N. Q., Nguyen M. T., Nguyen V. T., Le V. M., Trieu L. H., Le X. T., 2020. The effects of different extraction conditions on the polyphenol, flavonoids components and antioxidant activity of *Polyscias fruticosa* roots. *IOP Conf. Ser. Mater. Sci. Eng.*, 736: 022067. doi:10.1088/1757-899X/736/2/022067

Phuyal N., Jha P. K., Raturi P. P., Rajbhandary S., 2020. Total phenolic,

flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* dc. *Scientific World Journal.*, 2020: 8780704. doi: 10.1155/2020/8780704

Rarison R. H. G., Truong V. L., Yoon B. H., Park J. W., Jeong W. S., 2023. Antioxidant and anti-inflammatory mechanisms of lipophilic fractions from *Polyscias fruticosa* leaves based on network pharmacology, in silico, and in vitro approaches. *Foods*, 12: 3643. doi: 10.3390/FOODS12193643/S1

Singleton V. L., Orthofer R., Lamuela-Raventós R. M., 1999. Methods in enzymology - oxidants and antioxidants Part A. *Methods Enzymol.*, 299: 152–178. doi:10.1016/S0076-6879(99)99017-1

Woch N., Laha S., Gudipalli P., 2023. Salicylic acid and jasmonic acid induced enhanced production of total phenolics, flavonoids, and antioxidant metabolism in callus cultures of *Givotia moluccana* (L.) Sreem. *Vitr. Cell. Dev. Biol. Plant*, 59: 227–248. doi: 10.1007/S11627-023-10335-7/METRICS

Zeb A., 2020. Concept, mechanism, and applications of phenolic antioxidants in foods. *J. Food Biochem.* 44: e13394. doi: 10.1111/JFBC.13394.

Zhou Y., Feng J., Li Q., Huang D., Chen X., Du Z., Lv Z., Xiao Y., Han Y., Chen J., Chen W., 2020. SmMYC2b enhances tanshinone accumulation in salvia miltiorrhiza by activating pathway genes and promoting lateral root development. *Front Plant Sci.*, 11: 559438. doi: 10.3389/fpls.2020.559438