Original Article

Enhancement of PAL enzyme activity and production of phenylpropanoids and antioxidant contents of *Cynara scolymus* L. callus under the influence of precursor and elicitor

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ABSTRACT

Secondary metabolites (SMs) are valuable compounds with different applications in various industries. Nowadays application of suitable precursor and elicitor is an effective method for increasing Secondary Metabolites under *in vitro* conditions. The effect of phenylalanine as precursor (0, 5, 10 and 20 mg/l) and salicylic acid (SA) as elicitor (0 and 200 µmol) on the phenylalanine ammonia lyase (PAL) and antioxidant activity were investigated in callus culture of artichoke. The changes in total phenols and flavonoids under the effect of treatments were also studied. Based on the obtained results, phenylalanine had a significant effect on the measured parameters. Under the combination effect of salicylic acid (SA) and phenylalanine, all measured parameters except antioxidant activity were significantly influenced. For instance, by using the combination of 200 µmol of SA and 20 mg/l phenylalanine, the highest PAL activity (4.398 mmol/g FW), phenol content (0.638 mg/g FW), flavonoid content (0.577 mg/g FW) and antioxidant activity (89.405%) were recorded. According to the results of this experiment, for increasing the medicinal compounds of artichoke, it is recommended to use the stimulant compounds such as phenylalanine and salicylic acid.

1. Introduction

Medicinal plants produce a wealth of secondary metabolites (SMs) also known as natural products, which are small molecular weight compounds with enormous structural diversity and show various biological activities. These plants are very important in medicinal, food, cosmetics and health industries [1]. Plant cell culture technology has shown great priority as an alternative to the whole plant system for producing commercially important bioactive products. There are various methods to increase secondary metabolites production under *in vitro* conditions, such as medium

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optimization, cell line selection, cell immobilization, precursor feeding, culture of capillary roots and metabolite engineering and elicitation [2].

Elicitors are compounds with biotic or abiotic origin that induce the biosynthesis and accumulation of active compounds via stimulating defensive responses increase the activity of key enzymes involved in the synthesis of low-molecular antioxidant, e.g., activities of tyrosine/phenylalanine ammonia-lyase, chalcon synthase in the phenylpropanoid pathways (phenolics synthesis) [3]. Elicitations are considered to be an important strategy towards improved *in vitro* production of SMs. Signaling compounds such as salicylic acid (SA) act as abiotic elicitors and play a role in several processes such as plant growth regulation, plant development. They also act as a signal molecule in responses to biotic and abiotic stresses [4]. The SA reportedly enhanced the activity of PAL enzyme and the production of antioxidant SMs such as phenol and flavonoid compounds in *Carthamus tinctorius* [5], *Cynara scolymus* [6] and *mentha piperita* [7].

Phenylalanine (PHE) is an aromatic amino acid, the precursor of the phenylpropanoid pathway, converted to those phenolic acids, flavonoids and other phenolic compounds via phenylalanine ammonia-lyase (PAL) activity. Phenylalanine has been successfully used to increase the metabolite production of many plants via in *vitro* cultures [8]. Phenylalanine supplementation has been reported to enhance secondary metabolite production in plant cell cultures of *Psoralea corylifolia* L [9]. In an experiment, phenylalanine increased the alkaloid content of *Hyoscyamus muticus*. The highest value of hyoscyamine alkaloid content (3.01 mg/g dry weight) was recorded with phenylalanine at 200 mg/l ([0]. It has been reported that the antioxidant activity and phenolic content of *Daucus carota* callus cultures were increased as affected by 1000 mg/l PHE under light and dark conditions [11]. Buckwheat sprouts had maximum phenolics and flavonoids content by using of 0.1 mmol PHE. Also, PAL enzyme and anti-radical activity of plant were increased at this concentration ([2].

Artichoke (*Cynara scolymus* L., *Asteraceae*) is an ancient crop and medicinal plant, a perennial plant, native to the Mediterranean region (13). It is considered a healthy food, due to its nutritional and phytochemical composition. It contains proteins, minerals, low amount of lipids, dietary fiber and a high proportion of phenolics (14). The phenolicsinclude cynarin, luteolin, cynaroside scolmoside; phenolic acids such as caffeic, coumaric, hydroxycinnamic, ferulic, caffeoylquinic acid derivatives; mono- and dicaffeoylquinic acids, including chlorogenic acid; acid alcohols; flavonoid glucosides, among others (14). Artichoke is commonly eaten as a vegetable; its leaves are used in folk medicine for treating blood cholesterol, hepatitis, hyperlipidemia, obesity, dyspeptic disorders and liver diseases (15). Due to the interesting reaction of plant cells to stimulators compounds, in this study we investigated the enzymatic behavior and the metabolite production of the artichoke callus in the presence of biosynthetic precursor (PHE) and elicitor (SA).

2. Materials and Methods

2.1. Explants and treatments

The artichoke seeds were sterilized using hypochlorite 5% for 15 min and inoculated in $\frac{1}{2}$ MS medium. The explants were taken from the petiole of the *in vitro* seedlings and were cultured in MS medium containing 5 mg l^{-1} NAA + 2 mg l^{-1} BA and placed at 28±2 °C in darkness for callogenesis. The formed callus was transferred to the similar medium containing different concentrations of phenylalanine (0, 5, 10 and 20 mg/l) and salicylic acid (0 and 200 µmol). The samples were maintained under controlled conditions similar to callogenesis phase (at 28±2 °C in darkness) for four weeks. Then, after four weeks, the PAL enzyme and antioxidant activity as well as the phenylpropanoid content of treated callus were estimated.

2.2. Phenylalanine ammonia lyase (PAL) enzyme activity

For measuring the PAL activity, initially, 250 µL enzyme extract (0.1 g fresh tissue in 1 mL phosphate buffer with pH = 7), 250 µL sodium borate buffer 10 mmol (pH = 8.8), 250 µL distilled water and 250 µL phenylalanine substrate (50 mmol) as a reaction mixture were mixed and kept at 40 °C for 30 minutes. Then, the color spectrum of samples was spectrophotometerically recognized at 290 nm. The PAL activity of each extract was determined by Beer-Lambert law with extinction coefficient of 9630 $\mu^{-1}cm^{-1}$ and expressed in nmol g^{-1} FW min^{-1} (16).

2.3. Total phenols

Total phenolic compounds of callus were measured according to the FolinCiocalteu method. First, 20 µL of plant extract (in the ratio of 1 g plant sample:10 mL methanol 80%) was mixed with 100 µL FolinCiocalteu and 1.16 mL distilled water and kept in room temperature for 5-8 minutes. Then, 300 µL of sodium carbonate 1 M was added to the mixture. The reaction mixture was placed in 40°C water bath for 30 min in darkness and immediately read at 765 nm; total phenols were expressed as mg gallic acidper gram dray matter [17].

2.4. Total flavonoids

Flavonoid content was estimated by aluminum chloride method [18]. Relatively 0.5 mL of plant extract, 1.5 mL of methanol, 0.1 mL of aluminum chloride 10% in ethanol (10 g aluminum chloride in 100 mL ethanol and distilled water), 0.1 mL of potassium acetate 1 M and 2.8 mL of distilled water were mixed and kept in darkness for 30 min. The color spectrum of prepared sample was immediately read at 415 nm. The obtained value was calculated based on the used callus weight and the results were expressed as mg quercetin per gram dray matter.

2.5. Free radicals scavenging activity

Free radicals scavenging activity (antioxidant activity) of callus extract was calculated using free radical DPPH method. So, 0.1 mmol of DPPH and 2 mL of plant extract were placed in darkness for 15 minutes. Then, the color spectrum of sample was read at 517 nm. Control sample contained 2 mL DPPH and 2 mL methanol [19].

Free radical scavenging percentage= ((Ac–As)/Ac) × 100

Where As stands for Sample absorption, and Ac signifies Control absorption.

2.6. Statistical analysis

This experiment was performed as factorial based on a completely randomized design under control conditions with four concentrations of phenylalanine, two concentrations of SA and five replications. Mean data was compared by LSD test at $p < 0.05$ using SAS statistical package (SAS Institute, Cary, NC, USA) and the graphs were drawn by MS-Excel software (Microsoft Corp., Redmond, Washington, USA).

3. Results

According to the results, phenylalanine significantly affected the activity of PAL at the 5% and total phenols, total flavonoids and antioxidant activity at 1%. SA and interaction between phenylalanine and SA had a significant effect on the measured parameters except antioxidant activity (Table 1).

Table1. Analysis of variance of effect of phenylalanine and salicylic acid on biochemical compounds of Artichoke callus

		Mean Square			
Sources	**DF**	**PAL enzyme activity**	**Phenol**	**Flavonoid**	**Antioxidant activity**
PHE	3	1.326 *	0.155 **	0.153 **	44.183 **
SA	1	10.628 **	0.066 **	0.007 **	1.44 n.s
PHE*SA	3	1.157 *	0.021 **	0.007 **	2.043 n.s
Error	24	0.37	0.001	0.001	4.088
CV	-	15.666	10.671	7.729	2.284

*, **: Significant differences in levels 5 and %1 and n.s: no significant differences

3.1. Effect of phenylalanine (PHE) and Salicylic acid (SA) on PAL enzyme activity

Based on the obtained results (Figure 1), the maximum PAL enzyme activity (4.752 nmol/g FW) was observed between treated callus with 200 µmol of SA+ 5 mg/l phenylalanine, but no significant difference was observed with 200 µmol SA+ 10 and 20 mg/l PHE and 0 µmol of SA+ 20 mg/l PHE. The minimum of PAL activity (2.688 nmol/g FW) was observed in control.

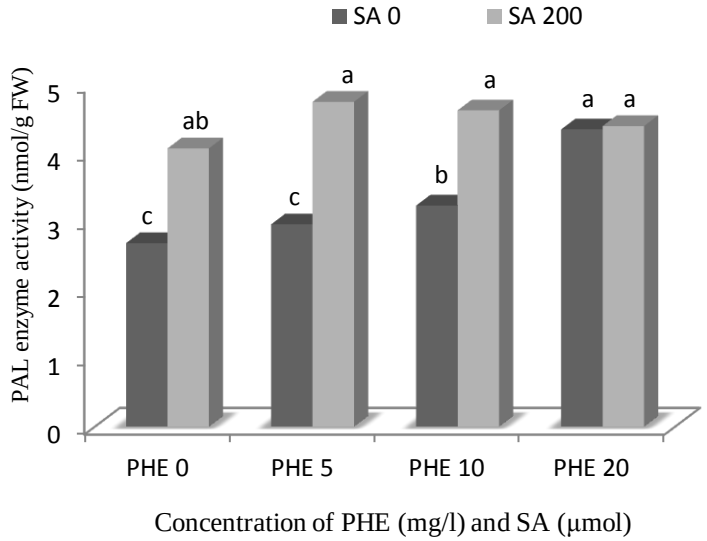

Figure 1. Effect of Phenylalanine and SA on PAL enzyme activity

3.2. Effect of PHE and SA on total phenol accumulation

The highest amount of total phenol with significant difference by control was observed in callus fed with 200 µmol of SA and 20 mg/l PHE (0.637 mg/g FW). In this treatment the phenol accumulation was nearly 2 folds higher than the control case (0.317 mg/g FW). Samples cultured in media fed with PHE alone had acceptable amounts of phenol (0.420 mg/g FW). In contrast to media supplemented with 5mg/l PHE, the lowest amount of total phenol (0.162 mg/g FW) was observed (Figure 2).

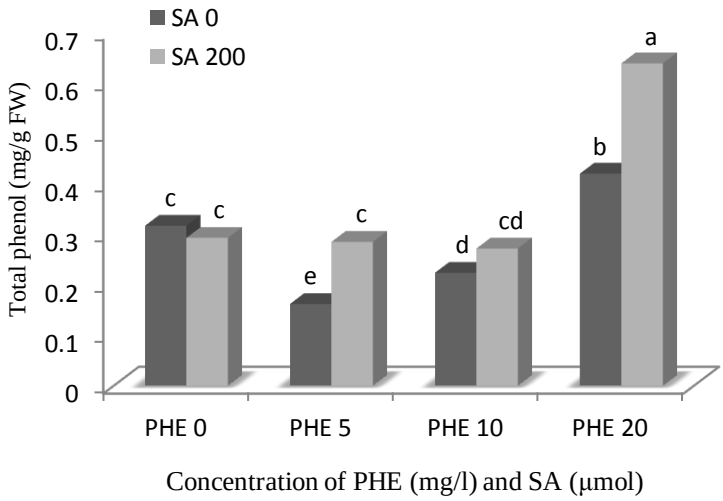

Figure 2. Effect of Phenylalanine and SA on total phenols

3.3. Effect of PHE and SA on total flavonoids accumulation

As shown in Figure 3, the maximum number of flavonoids obtained from samples cultured in 0 µmol of SA+ 20 mg/l PHE (0.632 mg/g FW) with significant increase was compared with the control (1.38 fold). Also, 200 µmol of SA+ 20 mg/l PHE had high number of flavonoids. In contrast and similar to the phenolic compounds, the lowest flavonoid content was observed in media containing 0 µmol of SA+ 5mg/l (0.235 mg/g FW).

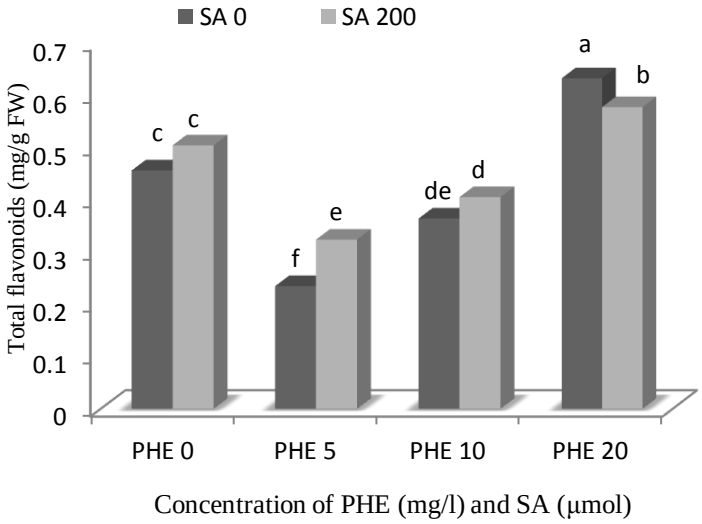

Figure 3. Effect of Phenylalanine and SA on total flavonoids

3.4. Effect of PHE and SA on the antioxidant activity

SA had no significant effect on antioxidant activity. PHE was effective treatment in the antioxidant activity of artichoke callus and with increasing concentration, antioxidant activity increased. The maximum antioxidant activity (90%) was observed in samples fed by 20 mg/l PHE (Figure 4) which were significantly higher than that of control samples.

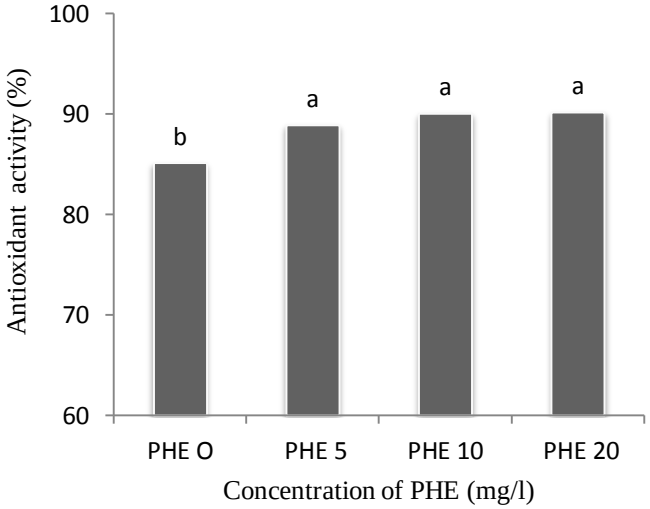

Figure 4. Effect of PHE on antioxidant activity

3.5. Effect of PHE and SA on the correlation between PAL activity and phenylpropanoid compounds

The activity of PAL enzyme of artichoke callus fed by Phenylalanine and SA had positive correlation with total phenol, flavonoid and antioxidant activity (Table 2).

Table 2. Correlation of PAL enzyme, total phenol, flavonoid and antioxidantactivityunder the treatment of Phenylalanine and SA

	PAL Enzyme activity	Total Phenols	Total Flavonoids	Antioxidant activity
PAL Enzyme activity	1			
Total Phenols	0.325 $^{n.s}$	1		
Total Flavonoids	0.304 $^{n.s}$	**0.672	1	
Antioxidant activity	0.302$^{n.s}$	0.112 $^{n.s}$	- 0.073$^{n.s}$	1

*, **: Significant differences in levels 5 and %1 and $^{n.s}$: no significant differences

4. Discussion

As mentioned in the results section, the PAL enzyme activity increased under the influence of precursor and elicitor. PAL is an antioxidant enzyme with defensive role in plants. PAL activity is changed by external factors including hormone composition, nutrients, light, stress, and biotic and abiotic elicitors [20]. SA is known as a key messenger component in activation of protective responses of plants. SA as an elicitor activates the signaling pathway and increases the transcription of special mRNA of PAL enzyme that it leads to plants defense responses and accumulation of defensive compounds such as phenolics [20]. SA significantly affects the PAL enzyme activity of *Cynara scolymus* callus. When the callus was treated with 100 µmol of SA, the maximum activity of enzyme was obtained [6]. In another report in *Cynara scolymus*, it has been shown that PAL enzyme activity of callus was significantly increased compared with the control case [21]. Based on the finding of a research, PAL enzyme activity of quinoa sprouts was increased by addition of phenylalanine [22]. Also, in another research, it has been reported that 0.1 mmol of phenylalanine amino acid had significant effect on PAL enzyme activity of buckwheat sprouts and this increasing was about 1.4 folds of non- treated samples [12]. The highest PAL activity (101.18 mmol CA/g FW) of *Sauropus androgynus* was also attained in light-induced callus cultures fed with 20 mg/l of phenylalanine at week 3 [23]. Phenylalanine is the substrate of PAL that stimulated this enzyme activity by catalyzing the reductive de-amination of L-phenylalanine into trans-cinnamic acid as the first step of the biosynthesis of plant phenolic compounds via phenylpropanoid pathway [24].

Phenolics are SMs with diverse chemical nature and potential and have essential functions in the reproduction and the growth of the plants acting as defense mechanisms against biotic and abiotic elicitors. In the plants, phenolic compounds are synthesized via the phenylpropanoid pathway that begins with the conversion of phenylalanine to cinnamic acid by phenylalanine ammonialyase (PAL) [25]. It has been reported that the exogenous application of SA may induce the expression of many defense genes which encode particular enzymes of secondary metabolic pathway to form bioactive compounds such as phenolics [26]. The positive effects of PHE and SA on the amount of total phenol in the present study were consistent with the findings of many studies. It has been shown that the total phenolic compound contents of *Foeniculum vulgare* treated with 0.25 mM SA increased by 56.95% as compared with the control plants [27]. In *Cynara scolymus*, the highest amount of total phenolic compounds was obtained in callus which were treated with 100 µmol of SA and it was 1.32 folds rather than control [6]. Phenolics are ubiquitous plant components that are primarily derived from phenylalanine via the phenylpropanoid metabolism. It has been previously shown that some treatments may be enhanced by precursors feeding, for example phenylalanine for phenolics synthesis [3]. Phenylalanine increases the metabolic flux

through phenylpropanoid biosynthetic pathway and elevates the level of targeted compound [28]. Phenylalanine supplementation has been reported to enhance secondary metabolite production in plant cell cultures [9]. It has been reported that 100 µM phenylalanine after 72 h was found as optimal feeding conditions for production of silymarin (1.84 fold higher than that of the control) in *Silybum marianum* hairy root cultures [29]. Another research reported that phenolic content of *Daucus carota* callus cultures was increased as affected by 1000 mg/l PHE under light and dark conditions [11]. Phenylalanine addition in the amount of 150 mg/l as precursors led to increase the production of Thymol and Coumarin in Callus Cultures of *Verbascum thapsus* L. [30]. After 3 weeks of phenylalanine treatment at 20 mg/l, the highest levels of total phenolics (246.62 µg/10 g FW) were detected in light-induced callus cultures of *Sauropus androgynous* [23].

Flavonoids are polyphenolic compounds, among the most bioactive secondary metabolites in plants. Flavonoids are originated from phenylalanine, an upstream metabolic precursor through phenylpropanoid pathway (10). All flavonoids are basically derivatives of 1,3-diphenylpropan-1-one (C6–C3–C6), which is derived from the condensation of three malonyl- CoA molecules with one p-coumaroyl-CoA to form a Chalcon intermediate [31]. The SA induces the expression of genes encoding enzymes related to the phenylpropanoid pathway production (among them the flavonoids) and increases the amount or the activity of these enzymes. Chalcon synthase (the first enzyme to branch off from phenylpropanoid metabolism to flavonoid metabolism) activity was increased in plants treated with SA [32]. A significant increase in the synthesis of flavonoids in response to application of SA was observed in various medicinal plant species. In safflower, the highest content of total flavonols (4.2 mg RE g^{-1} FW) was observed under elicitation by 50 mg L^{-1} of SA (33). The highest number of flavonoids *Cynara scolymus* was obtained when the media fed with 100 µmol SA and this increasing was 1.31 folds more than control [6]. The effect of precursor feeding (phenylalanine) on the production of isoflavones in *Psoralea corylifolia* hairy root culture demonstrated that phenylalanine at 2 mM concentration increased the production of daidzein and genistein by 1.3 folds compared with the control [9]. Maximum number of flavonoids (leutiolin, kaempferol, quercetin and total flavonoid) was estimated in eight weeks old tissue fed with 75 mg/100mL phenylalanine (34). After 3 weeks of phenylalanine treatment at 20 mg/l, total flavonoid (636.26 µg/10g FW), naringenin (12081.05 µg/10 g FW), quercetin (134.36 µg/10 g FW), kaempferol (11325.13 µg/10 g FW) were detected in light-induced callus cultures of *Sauropus androgynus* [23]. These results were consistent with our findings.

Antioxidant potential of medicinal plant and food extracts has been qualified to the presence of phenolic compounds [35]. Antioxidants could inhibit free-radical reaction by inhibiting lipid radical formation, disrupting propagation of chain auto oxidation reactions, and suppressing singlet oxygen; they could act as factors that aid in reducing hydrogen peroxides to stable compounds, as compounds chelating transition metal ions and as inhibitors of pro-oxidative enzymes [36]. This activity, as the fundamental property of food, is important for its health protecting ability, including antimutagenic, anticarcinogenic, antiobesity and antiaging effects [22]. DPPH radical scavenging activity assay is widely used to test the ability of compounds acting as free radical scavengers or hydrogen donors, evaluating antioxidant activity. As already mentioned, antioxidant potential of medicinal plant is because of the presence of phenolic compounds [35], thus the synthesis of these compounds from phenylpropanoid pathway started by phenylalanine and it is important in antioxidant potency of plant. Similar to this experiment, several studies demonstrated the role of this amino acid in antioxidant activity of plant extract. For example, the antioxidant activity of *Daucus carota* callus cultures increased as affected by 1000 mg/l PHE under light and dark conditions [11]. Phenylalanine caused increasing the antioxidant activity of Quinoa sprouts [22]. In 0.1 mmol of phenylalanine, the antioxidant activity of buckwheat sprouts was

increased about 1.11 folds higher than control [12]. The highest levels of antioxidant activities (97.35% for DPPH assay) were detected in light-induced callus culture of sweet shoot (*Sauropus androgynus* after 3 weeks of PHE treatment at 20 mg/l [23].

PAL activity had positive correlation with phenylpropanoids compounds and antioxidant activity of artichoke callus under treatment by Phenylalanine and SA. PAL is the first key enzyme in phenylpropanoid pathway via conversion of L- phenylalanine to the cinnamic acid. This is the first and the most important step through biosynthesis of phenolic, flavonoids and antioxidant compounds. The activity and gene expression of PAL enzyme is changed by factors such as hormone composition, nutrients, light, elicitors, and biotic and abiotic stresses [20]. SA as an elicitor induces the expression of genes related to the production of some classes of secondary metabolites in plants and activates phenylalanine ammonia-lyase (PAL), a key enzyme between the primary and secondary metabolism, involved in the phenylpropanoid compound production route of the secondary metabolism [37,38]. It has been reported that the positive correlation between PAL enzyme activity and phenylpropanoid compounds was influenced by SA in artichoke callus [6,21]. As phenylalanine is the substrate of phenylalanine ammonia-lyase (PAL) for production of phenylpropanoid compounds (8), it is expected that as precursor stimulates the PAL activity and increases related compound accumulation. This theory is in agreement with our results, as shown in Table 2. Many inquiries have shown the positive correlation between PAL enzyme and compounds derived from this enzyme. The greater PAL activity in various concentrations of PHE confirmed PAL affinity for the PHE as the upstream biosynthetic precursor of phenylpropanoid pathway [29]. The application of PHE as the upstream biosynthetic precursor of phenyl-propanoid pathway could enhance SMs production in suspension cultures of *P. corylifolia* [9]. A study showed that the addition of PHE enhanced the enzymatic activities in the phenylpropanoid pathway and increased the concentrations of phenolic and flavonoid compounds (naringenin, quercetin, kaempferol) which in turn contributed to the increase in antioxidant activities in light-induced callus cultures of sweet shoot [23].

5. Conclusion

Secondary metabolites of medicinal plants can be increased by some methods such as using elicitors and precursors and it is very important to produce metabolites for industries. In this experiment, salicylic acid (as an elicitor) and phenylalanine (as a precursor) had a significant effect on the activity of the PAL and phenylpropanoids compounds (phenols, flavonoids and antioxidants). Generally, 200 µmol SA+ 20 mg/l phenylalanine had the high amount of enzyme activity and phenylpropanoids SMs. As mentioned, phenylalanine and salicylic acid increased the production of valuable secondary compounds of artichoke. Therefore, for commercial production of secondary metabolites for the pharmaceutical industry and etc, it is suggested to use these compounds in appropriate concentrations.

Conflict of interest

None of the authors have any conflict of interest to declare.

Consent for publications

All authors approved the final manuscript for publication.

Availability of data and material

Data are available on request from the authors.

Authors' contributions

The idea of the experiment belonged to the A.GH. S.Z performed the experiment, did the statistical analysis and wrote the article. A.GH and M.A participated in the review of the final article.

Ethics approval and consent to participate

No human or animals were used in the present research.

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References

1. Harvey A L, Edrada-Ebel R, Quinn R J. (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews Drug Discovery*, *14*: 111–129. **[crossref], [Google Scholar], [Publisher]**
2. Ismailzadeh Bahabadi S, Sharifi M. (2013). Increasing the production of plant secondary metabolites using biotic elicitors. *Journal of Cell and Tissue, 4*(2): 119-128. **[crossref], [Google Scholar], [Publisher]**
3. Świeca M, Sęczyk Ł, Gawlik-Dziki U. 2014. Elicitation and precursor feeding as tools for the improvement of the phenolic content and antioxidant activity of lentil sprouts. *Food Chemistry, 161*: 288–295. **[crossref], [Google Scholar], [Publisher]**
4. Ajungla L, Patil P P, Barmukh R B, Nikam T D. (2009). Influence of biotic and abiotic elicitors on accumulation of hyoscyamine and scopolamine in root cultures of *Datura metel* L. *Indian Journal of Biotechnology*, *8*: 317-322. **[crossref], [Google Scholar], [Publisher]**
5. Shaki F, Ebrahimzadeh Maboud H, Niknam V. (2018). Growth enhancement and salt tolerance of Safflower (*Carthamus tinctorius* L.), by salicylic acid. *Current plant biology*, *13*: 16-22. **[crossref], [Google Scholar], [Publisher]**
6. Zamani Gerailoo, S, Ghasemnezhad, M. (2011). Effect of salicylic acid on antioxidant enzyme activity and petal senescence in 'Yellow Island' cut rose flowers, Journal of Fruit and Ornamental Plant Research, 19(1): 183-193. **[Google Scholar], [Publisher]**
7. Shabrangi A, Mehrabi L. (2014). Evaluation of antioxidant activity and secondary metabolites of *Mentha piperita* L. under effect of acetylsalicylic acid and methyl jasmonate. *International Research Journal of Applied and Basic Sciences*, *8*(3): 337-340. **[Google Scholar], [Publisher]**
8. Arora R. 2010. Medicinal Plant Biotechnology. CABI, Wallingford, Oxfordshire, UK.
9. Shinde A.N, Malpathak N, Fulzele D.P. (2009). Enhanced production of phytoestrogenic isoflavones from hairy root cultures of *Psoralea corylifolia* L. using elicitation and precursor feeding. *Biotechnology Bioprocess Engineering*, *14*: 288-294. **[crossref], [Google Scholar], [Publisher]**
10. Bosila H, AbdelfattahHamza M, and Abd El-Fattah El-Ateeq A. (2016). Enhancement of Callus Growth and Hyoscyamine Alkaloid Production in *Hyoscyamus muticus* by Nanotechnology, Biotic Elicitor and Precursor. *International Journal of Chemistry Technology Research, 9*(7): 135-142. **[Google Scholar], [Publisher]**
11. Arafa N.M, Ibrahim M.M, Aly U.I. (2015). Evaluation of Total Phenolic Contents and Antioxidant Activity of Carrot Callus Extracts as Affected by Phenylalanine Precursor. *Plant Tissue Culture and Biotechnology, 25*(2): 207-221. **[crossref], [Google Scholar], [Publisher]**
12. Świeca M. 2016 b. Elicitation and treatment with precursor of phenolics synthesis improve low-molecular antioxidants and antioxidant caoacity of buckwheat sprouts. *Acta Scientiarum Polonorum, Technologia Alimentaria,* 15(1): 17–28. **[crossref], [Google Scholar], [Publisher]**
13. Lattanzio V, Kroon P.A, Linsalata V, and Cardinali A. (2009). Globe artichoke: A functional food and source of nutraceutical ingredients. *Journal of Functional Foods*, *1*(2):131-144. **[crossref], [Google Scholar], [Publisher]**
14. Fratianni F, Tucci M, De-Palma M, Pepe R, Nazzaro F. (2007). Polyphenolic composition in different parts of some

cultivars of globe artichoke (*Cynara Cardunculus* L. var. scolymus (L) Fiori). *Food Chemistry, 104*(3): 1282-1286. **[crossref], [Google Scholar], [Publisher]**

15. Ziaie S A, DastPak A, NaghdBadi S, PoorHoseini L, Hemmati Moghadam A, Ghorori Naeini M. (2005). Review on *Cynara Scolymus*. *Journal of Medicinal Plants, 13*: 10-13. **[crossref], [Google Scholar], [Publisher]**
16. Saunders J A, McClure J. (1974). The suitability of a quantitative spetrophotometric assay for phenylalarine ammonia lyase activity in barley, buckwheat and pea seedlings. *Journal of Plant Physiology, 54*: 412-413. **[crossref], [Google Scholar], [Publisher]**
17. Slinkard K, Singleton V L. (1977). Total phenol analysis: Automation and comparison with manual methods. *Americian Journal of Enology and Viticulture*, *28*:49-55. **[crossref], [Google Scholar], [Publisher]**
18. Chang C, Yang M, Wen H, Chern J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food Drug Anal*, *10*(3): 178-182. **[crossref], [Google Scholar], [Publisher]**
19. Boudet AM. (2007). Evolution and current status of research in phenolic compounds. *Phytochemistry 68*:2722-2735. **[crossref], [Google Scholar], [Publisher]**
20. Vogt T. (2010). Phenylpropanoid biosynthesis. *Molecular Plant, 3*(1): 2-20. **[crossref], [Google Scholar], [Publisher]**
21. Samadi S, Ghasemnezhad A, Alizadeh M. (2014). Investigation on phenylalanine ammonia-lyase activity of artichoke (*Cynara scolymus* L.) affected by methyl jasmonate and salicylic acid in in-vitro conditions. *Journal of plant production researches, 21*(4): 135-148. **[crossref], [Google Scholar], [Publisher]**
22. Świeca M. (2016) a. Hydrogen Peroxide Treatment and the Phenylpropanoid Pathway Precursors Feeding Improve Phenolics and Antioxidant Capacity of Quinoa Sprouts via an Induction of L-Tyrosine and L-Phenylalanine Ammonia-Lyases Activities. *Journal of Chemistry,* 1-7. **[crossref], [Google Scholar], [Publisher]**
23. Sin Ling W. (2015). The effect of elicitors and precursor on *in vitro* cultures of Sauropus androgynus for sustainable metabolite production and antioxidant capacity improvement. PhD thesis, The university of Nottingham. **[crossref], [Google Scholar], [Publisher]**
24. Kubota N, Yakushiji H, Nishiyama N, Mimura H, Shimamura K. (2001). Phenolic contents and L-phenylalanine ammonia-lyase activity in peach fruit as affected by rootstocks. *Journal of Japanese Society of Horticultural Science*, *70*(2): 151–156. **[crossref], [Google Scholar], [Publisher]**
25. Ferreres F, Pereira D M, Valentao P, Andrade P B, Seabra R M, Sottomayor M. (2008). New phenolic compounds and antioxidant potential of *Catharanthus roseus*. *Journal of Agriculture and FoodChemistry*, *56*: 9967–9974. **[crossref], [Google Scholar], [Publisher]**
26. Ali MB, Hahn EJ, Paek KY. (2007). Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in *Panax ginseng* bioreactor root suspension cultures. *Molecules*, *12*(3): 607-621. **[crossref], [Google Scholar], [Publisher]**
27. Gorni HP, De Oliveira Brozulato M, Da Silva Louren R, Konrad ECG. (2017). Increased biomass and salicylic acid elicitor activity in fennel (*Foeniculum vulgare* Miller). *Brazilian journal of food technology, 20*: 1-7. **[crossref], [Google Scholar], [Publisher]**
28. Ouyang J, Wang X D, Zhao B, Wang Y C. (2005). Enhanced production of phenylethanoid glycosides by precursor feeding to cell culture of *Cistanche deserticola*. Process. Biochemistry, *40*: 3480-3484. **[crossref], [Google Scholar], [Publisher]**
29. Rahimi SH, Hasanloo T, Najafi F, Khavari-Nejad R A. (2011). Enhancement of silymarin accumulation using precursor feeding in *Silybum marianum* hairy root cultures. *Plant omics journal*, *4*(1): 34-39. **[crossref], [Google Scholar], [Publisher]**
30. Al-Jibouri1 A M J, Abed A S, Ali J A, Majeed D M. (2016). Improvement of Phenols

Production by Amino Acids in Callus Cultures of *Verbascum thapsus* L. *American Journal of Plant Sciences, 7*: 84-91. **[crossref], [Google Scholar], [Publisher]**

31. Reyad-ul-ferdous Rahman M, Mahamud K, Aysha S S, Sohel D. (2015). Pharmacologicals and Phytochemicals Potential of *Abutilon indicum*: A Comprehensive Review. *American Journal of BioScience, 3*: 5-11. **[crossref], [Google Scholar], [Publisher]**
32. Yang L, Yang CH, Li CH, Zhao Q, Liu L, Fang X, Chen X.Y. (2016). Recent advances in biosynthesis of bioactive compounds in traditional Chinese medicinal plants. *Science Bulletin, 61*(1): 3–17. **[crossref], [Google Scholar], [Publisher]**
33. Golkar P, Taghizadeh M, Yousefian Z. (2019). The effects of chitosan and salicylic acid on elicitation of secondary metabolites and antioxidant activity of safflower under in vitro salinity stress. *Plant Cell, Tissue and Organ Culture, 137*:575–585. **[crossref], [Google Scholar], [Publisher]**
34. (a) Mathur S, Goswami A. (2012). Effect of Precursor L-phenylalanine on Production of Flavonoids of *Maytenus emarginata in vitro. International Journal of Science and Research, 3*(7): 333-335. **[Paper ID: 02014969], [Google Scholar], [Publisher]**; (b) Salehi Sardoei A, Rezaei H, Ghasemi H. (2021). In Silico Evaluation of Expansin-Gene Function in Softening and Fruit Ripening. *Journal of Plant Bioinformatics and Biotechnology*, 1(2): 73-83. **[crossref], [Publisher], [Download PDF]**
35. Thiruvengadam M, Praveen N, Maria John K M, Yang Y S, Kim S H, Chung I M. (2014). Establishment of *Momordica charantia* hairy root cultures for the production of phenolic compounds and determination of their biological activities. *Plant Cell Tissue and Organ Culture, 118*: 545-557. **[crossref], [Google Scholar], [Publisher]**
36. Carocho M, Ferreira I C F R. (2013). A review on antioxidants, prooxidants and related controversy. Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chemistry Toxicology, 51*(1): 15–25. **[crossref], [Google Scholar], [Publisher]**
37. Durrant W E, Dong X. (2004). Systemic acquired resistance. Annual Review of Phytopathology. *Palo Alto*, *42*(1): 185-209. **[crossref], [Google Scholar], [Publisher]**
38. Ismail S. (2021). Cholinesterase and Aliesterase as a Natural Enzymatic Defense against Chlorpyrifos in Field Populations of Spodoptera Littoralis (Boisdüval, 1833) (Lepidoptera, Noctüidae). *Journal of Plant Bioinformatics and Biotechnology*, *1*(1): 41-50. **[crossref], [Google Scholar], [Publisher]**