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Enhancement of nutraceutical properties of goldenberry (*Physalis peruviana* L.) leaves through foliar application of salicylic acid during cultivation

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ABSTRACT

Goldenberry leaves are widely consumed traditionally due to the multiple health benefits attributed to them. The objective of this study was to evaluate the effect of foliar application of salicylic acid (SA) at different concentrations during goldenberry cultivation on the physicochemical and nutraceutical properties of the leaves. The results showed that 0.5- and 1-mM SA treatments significantly increased the levels of total phenolic compounds, flavonoids, extractable phenolic compounds and proanthocyanins in goldenberry leaves. It also had positive effects on chlorophyll levels, although higher SA concentrations tended to decrease chlorophyll content. No SA treatment affected plant growth, pH, or acidity, however, a slight decrease in the total soluble solids content was observed. Proximate analysis revealed that SA treatment did not alter fat and ash content but increased protein content and decreased total carbohydrate content. Moreover, treatment with 0.5 and 1.0 mM of SA resulted in increased levels of proline and phytosterols and exhibited higher antioxidant capacity, attributed to their rich content of bioactive compounds, particularly phenolics. Additionally, 1.0 mM SA treatment enhanced the inhibitory activity of α-amylase and pancreatic lipase, indicating its potential in managing hyperglycemic conditions. These findings highlight the nutraceutical properties of goldenberry leaves and their potential utilization as a byproduct of goldenberry cultivation, warranting further research to explore their metabolite profile and maximize their benefits.

Keywords: Physalis peruviana, salicylic acid, antioxidant capacity, abiotic stress, phenolic compounds

Introduction

Physalis peruviana Linnaeus, commonly known as goldenberry, is a plant belonging to the Solanaceae family that produces a juicy berry commonly consumed fresh or processed. The production of this fruit has expanded in recent years to various countries around the world due to its high multiplication potential, as the plant has the ability to adapt to a wide variety of enviromental factors (Kasali et al., 2021). Fruit extracts have demonstrated preventive effects against specific carcinogens, in addition to exhibiting antioxidant and anti-hepatotoxic activities (Akhtar et al., 2019). Furthermore, they have been found to possess anti-inflammatory and anti-diabetic properties. In addition to the fruit, its leaves and calyces are traditionally consumed in the form of decoctions or infusions, due to the multiple health benefits attributed to them (Nocetti et al., 2020). It has been shown that aqueous and ethanolic extracts derived from P. peruviana leaves exhibited protective effects against hepatotoxicity induced by CCl4 (Ramadan, 2020). Furthermore, leaf extracts of goldenberry have also demonstrated antidiabetic, antiproliferative, antifibrotic, and antibacterial properties, attributed to their composition of bioactive compounds, such as phenolic acids, flavonoids, alkaloids, glycowithanolides and withanolides (Nocetti et al., 2020).

Furthermore, it is known that when plants are subjected to certain conditions of moderate stress during cultivation, they trigger a defense response, resulting in changes in their profile of bioactive compounds. Recently, the behavior of plants has been studied using a novel strategy for generating abiotic stress, the use of elicitors, which, when administered in gradual doses, help observe the behavior of secondary metabolites in response to the varying degrees of stress induced by the elicitors on the plant (Baenas et al., 2014; Ramos-Sotelo & Figueroa-Pérez, 2023). The use of SA, when applied exogenously to plants, has gained prominence in recent years, as it can act as an elicitor, endogenously regulating plant growth, as well as the interaction and response to environmental stress or stress caused by other organisms. It has been observed that in different plant species, the external application of SA induces the synthesis and accumulation of antioxidant compounds. SA is associated with the activity of the PAL enzyme, which catalyzes reactions for the formation of secondary metabolites. (Hayat et al., 2010).

There are studies supporting the application of salicylic acid for the enhancement of beneficial health metabolites in solanaceous species (Mandal & Gupta, 2016). However, there are few reports involving the use of *Physalis peruviana* Linnaeus and the effect of such stress on the content of bioactive metabolites in the plant, its nutritional content, or the modification of its health-beneficial properties. Therefore, the primary objective of this study is to investigate the impact of SA on the growth, content of bioactive compounds, antioxidant capacity, and inhibition activity of digestive enzymes in goldenberry plants.

Materials and methods

Plant material, growth conditions, and SA treatment

The study was carried out in Culiacan, Sinaloa, Mexico (24° 51' 44.6" N; 107° 20' 56.1" W), from September 2021 to March 2022. Seeds of goldenberry (*Physalis peruviana* L) were acquired from Saflax® (Münster, Germany). These seeds were germinated in a controlled environment at 25°C with a relative humidity of 75%. After fifty days, the germinated seedlings were transplanted directly into the soil within a greenhouse maintained at a temperature of 25-30°C and a relative humidity of 80-85%. The plants were irrigated every three days, and a nutrient solution consisting of calcium nitrate (1.12 g/L), magnesium sulfate (0.50 g/L), potassium nitrate (0.36 g/L), monobasic potassium phosphate (0.30 g/L), iron chelate (0.07 g/L), and manganese sulfate (0.01 g/L) was applied every five days. The experiment followed a completely randomized block design with three replicates, and each replicate included seven plants. After sixty days of transplantation, different concentrations of SA (0, 0.25, 0.50, 1.0 and 2.0 mM) were applied as a foliar spray, approximately 500 ml per plant, at the dew point, with a frequency of every seven days. Leaf samples were collected for analysis on day 81 after planting. To assess the growth response of the plants to the elicitor treatment, measurements were taken from the base to the apex. Afterward the leaves were dried in a convection oven (Fisher Scientific, 650D, USA) at 45°C for 24 hours and then, ground to a particle size of 0.7-1.0 mm using a herb grinder (Krups GX4100, México).

Physicochemical and proximate analysis

The quantification of total soluble solids (TSS), pH, titratable acidity (TA), chlorophyll, protein, carbohydrate, and fat contents were performed according to the AOAC, 1997.

Extractable and non-extractable phenolic and proanthocyanidin contents

For the extraction of phenolic compounds, 0.5 g of dried leaves was subjected to extraction using 20 mL of methanol:water (50:50) at pH 2.0 in an orbital shaker at 1500 x g for 1 hour. Subsequently, the mixture was centrifuged at 4 °C for 10 minutes at 4000 x g, and the supernatant was collected. The residue was then extracted with 20 mL of acetone:water (70:30) and subjected to centrifugation under the same conditions. The resulting supernatants were combined for the quantification of total phenolic content, following the Folin-Ciocalteu colorimetric method (Singleton et al., 1999). The results were expressed in milligrams of gallic acid equivalents per gram. Total flavonoids were determined using a previously described colorimetric method and expressed in milligrams of rutin equivalents per gram (Kim et al., 2003). Total hydrolyzable polyphenols were measured using the residue obtained after the phenol extraction. This residue was subjected to hydrolysis using a methanol:H_2SO_4 (90:10) solution for 20 hours at 85°C. The total polyphenol content was then determined in

the supernatant using the Folin-Ciocalteu method. The results were expressed as milligrams of gallic acid equivalents per gram of dry leaves. Quantification of total extractable proanthocyanidins was conducted by subjecting the extracts obtained from the phenolic compound determination to butanolysis. The butanolysis process involved incubating the extracts with a solution of n-butanol:HCl (95:5), which contained 0.7 g of FeCl3 per liter, at 100 °C for 1 hour. Subsequently, measurements were taken at 450 nm and 555 nm. For the determination of non-extractable proanthocyanidins, the same butanolysis procedure was employed, utilizing 300 mg of the residual material remaining from the phenolic compound extraction. This residue was combined with 4 mL of the extraction solution. The results were expressed as milligrams of extractable or non-extractable proanthocyanidin equivalents per gram of dry sample.

Chlorophyll, proline and phytosterols determination

The determination of total chlorophyll was conducted following the method described in a previous study (Gogoi & Basumatary, 2018). Free proline levels were assessed using a colorimetric method based on the reaction between ninhydrin and amino acids (Bates et al., 1973), with L-Proline used as the standard. For phytosterols analysis, it was assessed the colorimetric method based on the enzymatic reaction of β-sitosterol, described by Prado et al. (2013) with some modifications. 250 mg of dried leaves were weighed and saponified at 80 °C for 1 hour with 5 mL of a 0.5 M KOH solution in methanol. The unsaponifiable fraction was separated by adding 1 mL of water and 2 mL of hexane, followed by centrifugation of the tubes at 2739 x g for 5 minutes. Aliquots of 0.6 mL of the upper hexane phase were transferred to clean tubes and evaporated using a constant air flow and a temperature of 45 °C. The unsaponifiable material was resuspended in 80 µL of isopropanol, vortexed to mix the resuspended pellet, and 300 µL of the enzymatic cholesterol reagent (Colesterol-LQ from Sigma®) was added. The sample was incubated at 35 °C for 15 minutes. After the incubation period, the color intensity was measured at 500 nm. The results were expressed as mg eq. of β-sitosterol per gram of dried sample.

Antioxidant capacity and inhibitory activity of digestive enzymes assays

The ABTS assay was performed according to the procedure previously described by Arnao et al., 2001, and the results were expressed as the mean values in milligrams of Trolox equivalents per gram of dry leaves. The DPPH assay was conducted following the method outlined by Brand-Williams et al., 1995, and the results were expressed as the mean values in milligrams of Trolox equivalents per gram of dry leaves.

The α-amylase inhibitory activity was determined using a modified version of the method described by (Karakaya et al., 2018). The dried leaf extracts were dissolved in a solution of 0.02 M sodium phosphate buffer containing 0.006 M NaCl at pH 6.9. In test tubes, 100 µL of each sample (at assay concentrations of 1.1–2.3 mg/mL) and 100 µL of α-amylase enzyme

solution (at a concentration of 1 mg/mL) were mixed and incubated at 37°C. A sample blank was prepared without adding the enzyme. After incubation for 10 minutes, 100 µL of a 1% (w/v) starch solution was added to the test tubes as substrate, and the reaction mixture was incubated at 37°C again, for 10 minutes. The reaction was stopped by adding 200 µL of a 3,5-dinitro-salicylic acid (DNS) color reagent, which consisted of 96 mM DNSA, 2 M sodium potassium tartrate tetrahydrate, and 2 M NaOH. The reaction mixture was then incubated in a boiling water bath at 100°C for 5 minutes. After cooling to room temperature, 3 mL of MilliQ water was added. The absorbance was measured at 540 nm. Acarbose, an α-amylase inhibitory drug, was used as the positive control at a concentration of 10 µg/mL. Results were expressed as half-maximal inhibitory concentration (IC_{50}) of the leaves extracts to inhibit the activity of the enzyme.

Regarding pancreatic lipase inhibitory activity was performed in the polyphenolic extract using the technique described by Bustanji et al., 2011 with some modifications. A solution of 10 mg/mL of porcine pancreatic lipase enzyme (Sigma®) was prepared in a Tris buffer solution of 100 mM adjusted to pH 8.2 with HCl. The solution was centrifuged at 1600 g for 5 minutes at room temperature, and the supernatant was collected. The substrate was prepared by dissolving 4-nitrophenyl butyrate (0.08% w/v) in a 5 mM sodium acetate solution adjusted to pH 5.0 with HCl and 1% Triton X-100 mM. The mixture was boiled to clarify the solution. Afterwards, 80 µL of 100 mM Tris solution (pH 8.2), 30 µL of pancreatic lipase solution (10 mg/mL), 90 µL of 4-nitrophenyl butyrate solution, and 10 µL of leaves phenolic extracts were mixed. The reaction was incubated at 37 °C for 2 hours, and the absorbance was read at 400 nm. Orlistat (a pancreatic lipase inhibitor drug) was used as positive control. Results were expressed as half-maximal inhibitory concentration (IC_{50}) of the leaves extracts to inhibit the activity of the enzyme.

Statistical analysis

The data were analyzed using MINITAB ver. 20 software through analysis of variance (ANOVA). Tukey's test was conducted at a significance level of $P<0.05$ with a 95% confidence limit to determine significant differences among the mean parameters for the two treatments and the control.

Results and discussion

Plant height, physicochemical and proximate analysis

It is known that inducing stress in plants or applying certain elicitors can have effects on plant growth, so it is important to consider that this does not impact the yield or production of edible plants when subjected to treatments aimed at enhancing their phytochemical content during cultivation. The impact of exogenous SA on plant growth varies depending on the concentration applied and the specific plant species involved. Different concentrations of SA can either stimulate or inhibit the growth of plants and their organs, exhibiting species-specific effects (Li et al., 2022). Figure 1 shows that the application of SA had no significant effects on the height of goldenberry plants at any of the evaluated concentrations.

Furthermore, Table 1 presents the results of the physicochemical analyses conducted. It can be observed that there were no significant differences in pH levels or titratable acidity of goldenberry leaves treated with SA at any concentration. However, a slight decrease of approximately 40-45% in the total soluble solids content was observed in the plants treated with SA compared to the control plants. This decrease was observed across all tested concentrations. It has been reported that the total soluble solids content significantly increases in tomato fruits after foliar application of 0.50 mM SA (Yıldırım & Dursun, 2009), this could be associated with a slight reduction of this parameter in the leaves of the plant, as the sugar reserves in these tissues are transported towards the fruit, particularly in the adjacent leaves (Luengwilai et al., 2010).

Regarding the proximate analysis, the results showed that the application of SA did not produce changes in the fat and ash content of the leaves (Table 1). However, a slight increase in the protein content was observed, ranging from 17% to 42%, with the highest effect seen in the 2.0 mM SA treatment. As for the total carbohydrate content, slight decreases were observed in the plants treated with SA at all concentrations compared to the control plants, which can be related to the decrease observed in the total soluble solids content. Additionally, the moisture content in the plants treated with the elicitor at a concentration of 0.50 mM was slightly higher than in the control plants. It has been reported that SA exerts a direct effect on protein content in different parts of plants. Recent findings have demonstrated that SA significantly reduces protease activities, promotes the synthesis of protease protein inhibitors, and inhibits the ubiquitin-proteasome degradation of protein repressors involved in the activation of jasmonate-inducible genes by transcription factors (Tarchevsky et al., 2011).

Phytochemical components

Phytochemicals are valuable compounds present in medicinal plants that are not essential for the regular functioning of the human body, however, they exhibit activity and provide significant health benefits, including disease mitigation or improvement (Ghasemzadeh et al., 2015). Table 2 displays the content of hydrolysable and non-hydrolysable phenolic compounds, flavonoids, proanthocyanidins, chlorophylls, proline, and phytosterols present in goldenberry leaves treated with SA during cultivation. The results demonstrate that the application of SA at concentrations of 0.5 and 1.0 mM significantly increased the total phenolic compound (1.3 to 2.3-fold), flavonoid content (1.8 to 3-fold) and extractable phenolic compounds (1.2 to 1.7-fold) in goldenberry leaves compared to the control plants. Exogenous application of SA has been shown to stimulate the synthesis and accumulation of phenolic compounds in various plant species, this effect depends on factors such as the applied concentration, growth stage, among others, which is associated with the ability of this elicitor to regulate PAL enzyme activity, which plays a pivotal role in catalyzing key biosynthetic reactions for the production of secondary metabolites that act as defense mechanisms against environmental stresses (Ramos-Sotelo & Figueroa-Pérez, 2023).

Regarding extractable proanthocyanidins, the application of SA at concentrations of 0.5 and 1.0 mM showed the most favorable effects on this parameter, resulting in increases of up to double compared to untreated plant leaves. Conversely, for non-extractable proanthocyanidins, the opposite effect occurred, as a significant decrease of 35% in these compounds was observed in leaves of plants treated with 1.0 mM SA compared to control plant leaves (Table 2). Based on the previous results, the increase in flavonoid content could be related to the depolymerization of proanthocyanidins stored in the plant's cell wall (Koskimäki et al., 2009) or to a negative regulation in the production pathway of 2,3-trans-flavan 3-ols (precursors of proanthocyanidins), leading to the production of flavonoid precursors such as flavan-3,4-diols, which represent the branching point for the synthesis of polymeric compounds (Abeynayake et al., 2012).

On the other hand, significantly higher levels of chlorophyll were found in the leaves of plants treated with 1.0 mM SA, and tended to decrease at higher concentrations of SA compared to the control plants (Table 2). It has been demonstrated that the application of 0.5 mM SA significantly increases the chlorophyll content in *Amaranthus tricolor* L. plants (Khandaker et al., 2011). Chlorophyll is widely distributed throughout plant leaves and various plant tissues and it serves a vital role in the process of photosynthesis, facilitating the conversion of sunlight into energy. Due to its antioxidant properties and photodynamic capabilities, chlorophyll has been explored as a valuable resource for cancer therapy (Mishra et al., 2012). It was found that the application of low concentrations of SA increased photosynthetic activity in basil and marjoram, resulting in improved plant height, increased number of internodes, branches, and leaves, as well as enhanced leaf area, fresh weight, and dry weight (Liu et al., 2022).

Regarding proline levels, all evaluated concentrations of SA induced an increase in the levels of this compound in goldenberry leaves. The highest amounts were found at concentrations of 0.5 and 1.0 mM, while the content of phytosterols slightly increased (15-30%) in the leaves of plants treated with SA compared to untreated plants. Proline is an indispensable amino acid for primary metabolism that plays a vital role in cellular membrane stabilization, protein synthesis, and the production of secondary metabolites, which have been associated with enhanced antioxidant capacity in edible plants (Karagözler et al., 2008). On the other hand, phytosterols are compounds that are similar in synthesis and structure to cholesterol, s essential components of cellular membranes, sterols play a pivotal role in regulating plant growth, development, and their response to abiotic stress factors. It has been reported that they have beneficial effects on health, exert antioxidant effects in the body, reduce dyslipidemia, and improve insulin resistance (Du et al., 2022).

Antioxidant capacity and inhibitory activity of digestive enzymes assays

Figure 2 presents the results on the ability of goldenberry leaves treated with SA during cultivation to inhibit the oxidative activity of ABTS and DPPH radicals. It was observed that leaves of plants treated with 0.5 and 1.0 mM SA exhibited higher antioxidant capacity compared to the control plants. Additionally, for the DPPH method, there was a statistically significant increase in antioxidant capacity for samples treated with 0.25 mM SA as well. The measurement of antioxidant capacity is of interest in the prevention of oxidation reactions. Antioxidants differ in their chemical structure and may exhibit varying solubility behavior. Polyphenols, for instance, display different solubility degrees depending on factors such as molecular weight and the extent of glycosylation, acylation, or esterification (Aryal et al., 2019). The notable antioxidant capacity of goldenberry leaves can be attributed to their rich content of bioactive compounds, particularly phenolics. These metabolites exhibit exceptional scavenging abilities against free radicals. Extensive research has identified rutin, rosmarinic acid, protocatechuic acid, synaptic acid, ferulic acid, and other phenolic compounds as the primary constituents of *Physalis peruviana* leaves (Ivanova et al., 2019). The presence of these compounds has been found to contribute significantly to the potent antioxidant properties observed in various leafy vegetables, highlighting their substantial dietary and nutritional value (Khanam et al., 2012; Xu et al., 2017).

On the other hand, Table 3 shows the ability of Physalis peruviana leaves treated with SA to inhibit the activity of α-amylase and pancreatic lipase *in vitro*. The results are presented as IC_{50}, where lower values represent a greater inhibition capacity of the enzymes. In general, it was observed that plants treated with 1 mM SA showed the greatest effects, increasing their capacity to inhibit α-amylase and pancreatic lipase activity by up to 35% and 33%, respectively, compared to the control plants. Pancreatic lipase is the enzyme responsible for the majority hydrolysis of dietary triglycerides, accounting for 50-70% of triglyceride hydrolysis. Previous studies have shown that goldenberry extracts could decrease the hydrolysis of triglycerides in the intestine, which could be attributable to the inhibition of

pancreatic lipase by this plant (Kumar & Chauhan, 2021). Alpha-amylase is present in both saliva and pancreatic juice. It plays a crucial role in breaking down alpha-linked polysaccharides, such as starch and glycogen, into glucose and maltose. These breakdown products can rapidly enter the bloodstream. Therefore, inhibiting α-amylase can significantly slow down the digestion process by impeding starch breakdown in the intestine. Consequently, this inhibition can serve as an effective strategy for managing hyperglycemic conditions (Shakoor et al., 2020).

Goldenberry contains some secondary metabolites that have been associated with various health benefits, mainly pyrrolidine alkaloids and flavonoids, such as quercetin, myrecetin, and kaempferol. These compounds have been correlated with the antidiabetic effects of goldenberry. Furthermore, ethanolic extracts of the fruits contain fisalin, citric acid, and vitamin C, which are considered as active principles with antidiabetic properties, these compounds have demonstrated the ability to inhibit the activity of the glycogen-phosphorylase enzyme responsible for the glycogenolysis process (Bernal et al., 2018; Kinasih et al., 2020; Nasser Singab & Youssef, 2014). However, there are not many studies focused on evaluating the nutraceutical properties or metabolite profile of the leaves, a byproduct of goldenberry cultivation, which is also consumed traditionally to a significant extent.

Conclusions

The application of SA did not significantly affect the plant height, pH levels, or titratable acidity of goldenberry plants. However, a slight decrease in the total soluble solids content was observed in SA-treated plants, possibly due to the transport of sugar reserves towards the fruit. The proximate analysis revealed that SA treatment did not alter the fat and ash content of the leaves but led to a slight increase in protein content and a decrease in total carbohydrate content. SA treatment resulted in significant increases in total phenolic compounds, flavonoids, and extractable phenolic compounds in goldenberry leaves. It also showed favorable effects on chlorophyll levels, although higher concentrations of SA tended to decrease chlorophyll content. Additionally, SA treatment increased proline levels and slightly enhanced phytosterol content in the leaves. The SA-treated leaves exhibited higher antioxidant capacity, attributed to their rich content of bioactive compounds, particularly phenolics. Furthermore, SA treatment increased the inhibitory activity of α-amylase and pancreatic lipase, suggesting its potential in managing hyperglycemic conditions. Overall, the study highlights the nutraceutical properties of goldenberry leaves and their potential as a byproduct of goldenberry cultivation. Further research is warranted to explore the metabolite profile and maximize the utilization of goldenberry leaves.

Conflict of interest

Authors declare that they do not have any conflict of interest.

References

Abeynayake, S. W., Panter, S., Chapman, R., Webster, T., Rochfort, S., Mouradov, A., & Spangenberg, G. (2012). Biosynthesis of proanthocyanidins in white clover flowers: cross talk within the flavonoid pathway. *Plant Physiol.*, **158**, 666–678. https://doi.org/10.1104/pp.111.189258

Akhtar, N., Chaudhari, S. K., Arshad, S., Fatima, H., Amjad, M. S., & Saboon. (2019). *Biological Activities and Nutritional Value of Physalis peruviana L. BT - Natural Bio-active Compounds: Volume 1: Production and Applications* (M. S. Akhtar, M. K. Swamy, & U. R. Sinniah (eds.); pp. 587–598). Springer Singapore. https://doi.org/10.1007/978-981-13-7154-7_21

AOAC. (1997). *Association of Official Analytical Chemists International Official Methods of Analysis* (AOAC (ed.); 17th ed.).

Arnao, M. B., Cano, A., Alcolea, J. F., & Acosta, M. (2001). Estimation of free radical-quenching activity of leaf pigment extracts. *Phytochem Anal.*, **12**, 138–143. https://doi.org/10.1002/pca.571

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants*, **8**, 96. https://doi.org/10.3390/plants8040096

Baenas, N., García-Viguera, C., & Moreno, D. A. (2014). Elicitation: a tool for enriching the bioactive composition of foods. *Molecules*, ***19***, 13541–13563. https://doi.org/10.3390/molecules190913541

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, **39,** 205–207. https://doi.org/10.1007/BF00018060

Bernal, C.-A., Castellanos, L., Aragón, D. M., Martínez-Matamoros, D., Jiménez, C., Baena, Y., & Ramos, F. A. (2018). Peruvioses A to F, sucrose esters from the exudate of Physalis peruviana fruit as α-amylase inhibitors. *Carbohydr. Res.*, **461**, 4–10. https://doi.org/https://doi.org/10.1016/j.carres.2018.03.003

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.*, **28**, 25–30. https://doi.org/https://doi.org/10.1016/S0023-6438(95)80008-5

Bustanji, Y., Al-Masri, I. M., Mohammad, M., Hudaib, M., Tawaha, K., Tarazi, H., & Alkhatib, H. S. (2011). Pancreatic lipase inhibition activity of trilactone terpenes of Ginkgo biloba. *J. Enzyme Inhib. Med. Chem.*, **26**, 453–459. https://doi.org/10.3109/14756366.2010.525509

Du, Y., Fu, X., Chu, Y., Wu, P., Liu, Y., Ma, L., Tian, H., & Zhu, B. (2022). Biosynthesis

and the Roles of Plant Sterols in Development and Stress Responses. *Int. J. Mol. Sci.*, **23**. https://doi.org/10.3390/ijms23042332

Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, A. (2015). Phytochemical constituents and biological activities of different extracts of Strobilanthes crispus (L.) Bremek leaves grown in different locations of Malaysia. *BMC Complement Altern. Med.*, **15**, 422. https://doi.org/10.1186/s12906-015-0873-3

Gogoi, M., & Basumatary, M. (2018). Estimation of the chlorophyll concentration in seven Citrus species of Kokrajhar district, BTAD, Assam, India. *Trop. Plant Res.*, **5**, 83–87. https://doi.org/10.22271/tpr.2018.v5.i1.012

Hayat, Q., Hayat, S., Irfan, M., & Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: A review. *Environ. Exp. Bot.*, **68**, 14–25. https://doi.org/10.1016/j.envexpbot.2009.08.005

Ivanova, T., Popova, V., Mazova, N., Stoyanova, A., & Damyanova, S. (2019). Extracts from physalis leaves (Physalis peruviana L.) for prospective application in medicine and cosmetics. *Ukr. Food J.*, **8**, 34–44. https://doi.org/10.24263/2304-974x-2019-8-1-5

Karagözler, A. A., Erdağ, B., Emek, Y. Ç., & Uygun, D. A. (2008). Antioxidant activity and proline content of leaf extracts from Dorystoechas hastata. *Food Chem.*, **111**, 400–407. https://doi.org/https://doi.org/10.1016/j.foodchem.2008.03.089

Karakaya, S., Gözcü, S., Güvenalp, Z., Özbek, H., Yuca, H., Dursunoğlu, B., Kazaz, C., & Kılıç, C. S. (2018). The α-amylase and α-glucosidase inhibitory activities of the dichloromethane extracts and constituents of Ferulago bracteata roots. *Pharm Biol.*, **56**, 18–24. https://doi.org/10.1080/13880209.2017.1414857

Kasali, F. M., Tusiimire, J., Kadima, J. N., Tolo, C. U., Weisheit, A., & Agaba, A. G. (2021). Ethnotherapeutic Uses and Phytochemical Composition of Physalis peruviana L.: An Overview. *Sci. World J.*, 5212348. https://doi.org/10.1155/2021/5212348

Khanam, U. K. S., Oba, S., Yanase, E., & Murakami, Y. (2012). Phenolic acids, flavonoids and total antioxidant capacity of selected leafy vegetables. *J. Funct. Foods*, ***4***, 979–987. https://doi.org/10.1016/j.jff.2012.07.006

Khandaker, L., Masum Akond, A., & Oba, S. (2011). Foliar application of salicylic acid improved the growth, yield and leaf's bioactive compounds in red amaranth (Amaranthus tricolor L.). *Veg. Crop. Res. Bull.*, **74**, 77–86. https://doi.org/10.2478/v10032-011-0006-6

Kim, D.-O., Chun, O. K., Kim, Y. J., Moon, H.-Y., & Lee, C. Y. (2003). Quantification of Polyphenolics and Their Antioxidant Capacity in Fresh Plums. *J. Agric. Food Chem.*, **51**, 6509–6515. https://doi.org/10.1021/jf0343074

Kinasih, L. S., Djamiatun, K., & Al-Baarri, A. N. (2020). Golden Berry (Physalis peruviana) Juice for Reduction of Blood Glucose and Amelioration of Insulin Resistance in Diabetic Rats. *J. Pangan, Gizi dan.*, **15**, 37–44. https://doi.org/10.25182/jgp.2020.15.1.37-44

Koskimäki, J. J., Hokkanen, J., Jaakola, L., Suorsa, M., Tolonen, A., Mattila, S., Pirttilä, A.

M., & Hohtola, A. (2009). Flavonoid biosynthesis and degradation play a role in early defence responses of bilberry (Vaccinium myrtillus) against biotic stress. *Eur. J. Plant Pathol.*, **125**, 629–640. https://doi.org/10.1007/s10658-009-9511-6

Kumar, A., & Chauhan, S. (2021). Pancreatic lipase inhibitors: The road voyaged and successes. *Life Sci.*, **271**, 119115. https://doi.org/10.1016/j.lfs.2021.119115

Li, A., Sun, X., & Liu, L. (2022). Action of Salicylic Acid on Plant Growth. *Front. Plant Sci.*, **13**. https://doi.org/10.3389/fpls.2022.878076

Liu, J., Qiu, G., Liu, C., Li, H., Chen, X., Fu, Q., Lin, Y., & Guo, B. (2022). Salicylic Acid, a Multifaceted Hormone, Combats Abiotic Stresses in Plants. *Life*, **12**. https://doi.org/10.3390/life12060886

Luengwilai, K., Fiehn, O. E., & Beckles, D. M. (2010). Comparison of leaf and fruit metabolism in two tomato (Solanum lycopersicum L.) genotypes varying in total soluble solids. *J. Agric. Food Chem.*, **58**, 11790–11800. https://doi.org/10.1021/jf102562n

Mandal, S., & Gupta, C. K. (2016). Inducing cell wall-bound phenolic compounds by elicitors in eggplant (Solanum melongena). *Appl. Biochem. Microbiol.*, **52**, 650–656. https://doi.org/10.1134/S0003683816060120

Mishra, V., Bacheti, R. K., & Husen, A. (2012). Medicinal Uses of Chlorophyll: a critical overview. w. In: Chlorophyll: Structure, Function and Medicinal Uses, Hua Le and and Elisa Salcedo, Eds., Nova Science Publishers, Inc., Hauppauge, NY 11788 (ISBN 978-1-62100-015-0), pp.177-196. https://www.novapublishers.com/catalog/product_info.php?products_id=22468

Nasser Singab, A., & Youssef, F. S. (2014). Medicinal Plants with Potential Antidiabetic Activity and their Assessment. *Med Aromat Plants*, **03**, 1–12. https://doi.org/10.4172/2167-0412.1000151

Nocetti, D., Núñez, H., Puente, L., Espinosa, A., & Romero, F. (2020). Composition and biological effects of goldenberry byproducts: an overview. *J. Sci. Food Agric.*, **100**, 4335–4346. https://doi.org/10.1002/jsfa.10386

Prado, A. C. P. do, Manion, B. A., Seetharaman, K., Deschamps, F. C., Barrera Arellano, D., & Block, J. M. (2013). Relationship between antioxidant properties and chemical composition of the oil and the shell of pecan nuts [Caryaillinoinensis (Wangenh) C. Koch]. *Ind. Crops Prod.*, **45**, 64–73. https://doi.org/https://doi.org/10.1016/j.indcrop.2012.11.042

Ramadan, M. F. (2020). *Bioactive Phytochemicals of Cape Gooseberry (Physalis peruviana L.) BT - Bioactive Compounds in Underutilized Fruits and Nuts* (H. N. Murthy & V. A. Bapat (eds.); pp. 75–90). Springer International Publishing. https://doi.org/10.1007/978-3-030-30182-8_3

Ramos-Sotelo, H., & Figueroa-Pérez, M. G. (2023). Use of salicylic acid during cultivation of plants as a strategy to improve its metabolite profile and beneficial health effects. *Ital J Food Sci.*, ***35***. https://itjfs.com/index.php/ijfs/article/view/2332

Shakoor, H., Abdelfattah, F., Albadi, K., Adib, M., Kizhakkayil, J., & Platat, C. (2020). Inhibition of Digestive Enzyme and Stimulation of Human Liver Cells (HepG2) Glucose Uptake by Date Seeds Extract. *Evid. -based Complement. Altern. Med.*, 4290702. https://doi.org/10.1155/2020/4290702

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. B. T.-M. in E. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Oxidants and Antioxidants Part A* (Vol. 299, pp. 152–178). Academic Press. https://doi.org/https://doi.org/10.1016/S0076-6879(99)99017-1

Tarchevsky, I. A., Yakovleva, V. G., & Egorova, A. M. (2011). Effects of salicylic acid on protein content and 14C-amino acid incorporation into proteins of pea roots. *Russ. J. Plant Physiol.*, **58**, 580–588. https://doi.org/10.1134/S1021443711040212

Xu, D.-P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.-J., & Li, H.-B. (2017). Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *Int. J. Mol. Sci.*, **18**, 96. https://doi.org/10.3390/ijms18010096

Yıldırım, E., & Dursun, A. (2009). Effect of foliar salicylic acid applications on plant growth and yield of tomato under greenhouse conditions. *Acta Hortic.*, ***807***, 395–400. https://doi.org/10.17660/ActaHortic.2009.807.56

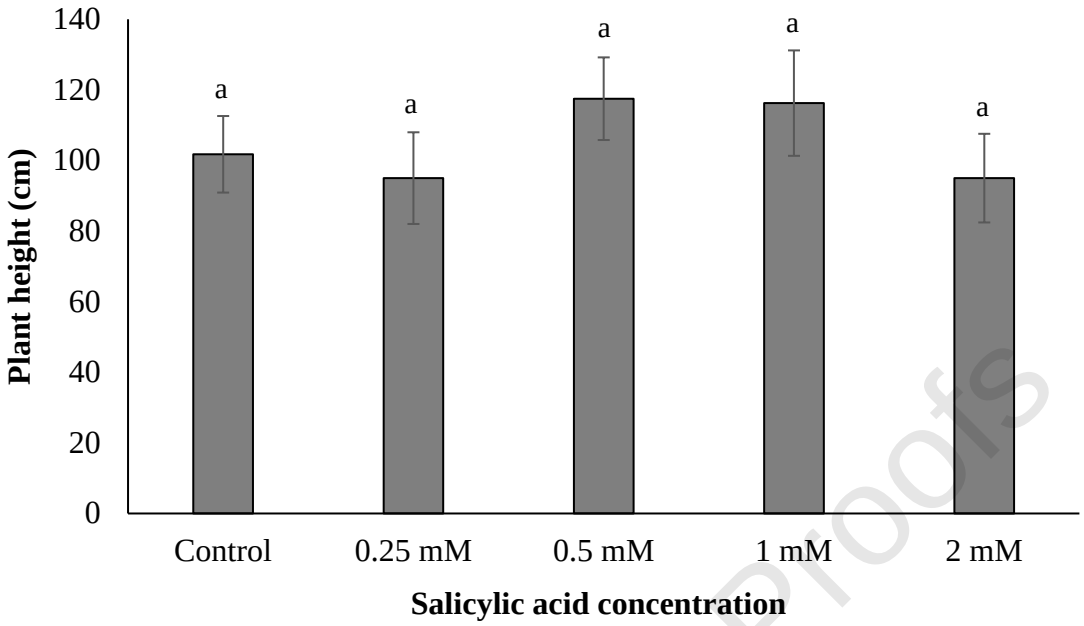

Figure 1. Changes in plant height of *Physalis peruviana* treated with different concentrations of salicylic acid. Results are the average of three independent determinations ± SE. Different letters indicate significant statistical differences between treatments ($p < 0.05$; Tukey's test).

1

Table 1. pH, Total Acidity (TA), Total Soluble Solid Content (TSS) and proximate analysis (% on fresh weight) (mean ± SD) of *Physalis peruviana* treated with different concentrations of salicylic acid.

	Salicylic acid concentration				
	Control	**0.25 mM**	**0.50 mM**	**1.0 mM**	**2.0 mM**
pH	6.12 ± 0.04 [a]	6.03 ± 0.09 [a]	6.18 ± 0.09 [a]	6.15 ± 0.03 [a]	6.10 ± 0.04 [a]
TA	0.12 ± 0.01 [a]	0.18 ± 0.05 [a]	0.16 ± 0.04 [a]	0.17 ± 0.02 [a]	0.19 ± 0.01 [a]
TSS	3.36 ± 0.08 [a]	2.23 ± 0.02 [b]	2.56 ± 0.04 [b]	2.23 ± 0.09 [b]	2.15 ± 0.04 [b]
Moisture	87.98 ± 2.7 [b]	90.53 ± 2.7 [b]	92.30 ± 6.1 [a]	91.48 ± 4.5 [ab]	91.6 ± 6.5 [ab]
Fat	0.41 ± 0.02 [a]	0.43 ± 0.07 [a]	0.40 ± 0.05 [a]	0.42 ± 0.04 [a]	0.46 ± 0.05 [a]
Ash	0.93 ± 0.06 [a]	0.88 ± 0.05 [a]	0.91 ± 0.09 [a]	0.87 ± 0.03 [a]	0.88 ± 0.07 [a]
Protein	1.04 ± 0.06 [b]	1.22 ± 0.16 [ab]	1.35 ± 0.13 [a]	1.17 ± 0.19 [b]	1.48 ± 0.12 [a]
Carbohydrates	6.94 ± 0.76 [a]	4.49 ± 0.72 [b]	4.16 ± 0.50 [b]	4.28± 0.32 [b]	4.17 ± 0.43 [b]

2 Data are mean values ± SD, (n=3); for the same line, means with different letters between columns are significantly different ($p < 0.05$). Statistical
3 analyses performed by Tukey's test.

Table 2 Total, extractable and non-extractable phenolic compounds, chlorophylls, proline and phytosterols contents in leaf extracts of *Physalis peruviana* treated with different concentrations of salicylic acid during cultivation.

Compound	Salicylic acid concentration				
	Control	0.25 mM	0.50 mM	1.0 mM	2.0 mM
Total phenolic compounds [1]	13.51 ± 0.63 [c]	15.43 ± 1.54 [bc]	18.87 ± 0.86 [b]	30.03 ± 2.60 [a]	17.87 ± 2.77 [bc]
Flavonoids [2]	15.10 ± 2.09 [c]	26.38 ± 3.58 [b]	28.35 ± 3.73 [b]	46.29 ± 3.24 [a]	22.02 ± 6.28 [bc]
Hydrolyzable polyphenols [1]	27.36 ± 0.61 [c]	23.58 ± 0.76 [c]	32.44 ± 0.79 [b]	48.16 ± 1.52 [a]	31.49 ± 0.85 [b]
Extractable proanthocyanidins [3]	7.78 ± 0.49 [d]	9.48 ± 0.65 [d]	14.57 ± 0.87 [b]	17.68 ± 0.59 [a]	12.23 ± 0.84 [c]
Non-extractable proanthocyanidins [4]	17.90 ± 0.87 [a]	15.67 ± 1.35 [ab]	14.98 ± 0.62 [b]	11.34 ± 0.34 [c]	16.78 ± 0.64 [a]
Chlorophyll a [5]	11.66 ± 1.08 [b]	12.80 ± 0.58 [b]	11.33 ± 1.78 [b]	18.68 ± 1.58 [a]	18.82 ± 1.07 [a]
Chlorophyll b [5]	12.23 ± 0.48 [c]	11.47 ± 0.68 [cd]	10.13 ± 0.63 [d]	19.83 ± 1.91 [a]	15.01 ± 1.10 [b]
Chlorophyll c [5]	12.08 ± 2.12 [c]	21.12 ± 1.68 [b]	20.87 ± 1.73 [b]	34.91 ± 0.70 [a]	21.57 ± 0.72 [b]
Proline [6]	88.23 ± 5.02 [c]	116.02 ± 8.45 [b]	134.50 ± 10.24 [a]	128.67 ± 9.15 [a]	96.37 ± 8. 63 [bc]
Phytosterols [7]	13.54 ± 0.87 [b]	15.60 ± 1.23 [b]	18.34 ± 4.94 [a]	17.65 ± 1.37 [a]	19.63 ± 1.59 [a]

Data expressed as the mean ± standard desviation, n = 3. Values with different letters on the same line indicate significant statistical difference ($p < 0.05$) using the Tukey test. [1](mg gallic acid equivalents/g dry sample), [2](mg rutin equivalents/g dry sample), [3](mg extractable proanthocyanidins equivalents/g dry sample), [4](mg non-extractable proanthocyandins equivalents/g of dry sample), [5](mg chlorophyll equivalents/g of fresh sample), [6](mg /g of of dry sample), [7](mg β-sitoesterol equivalents /100 g of of dry sample).

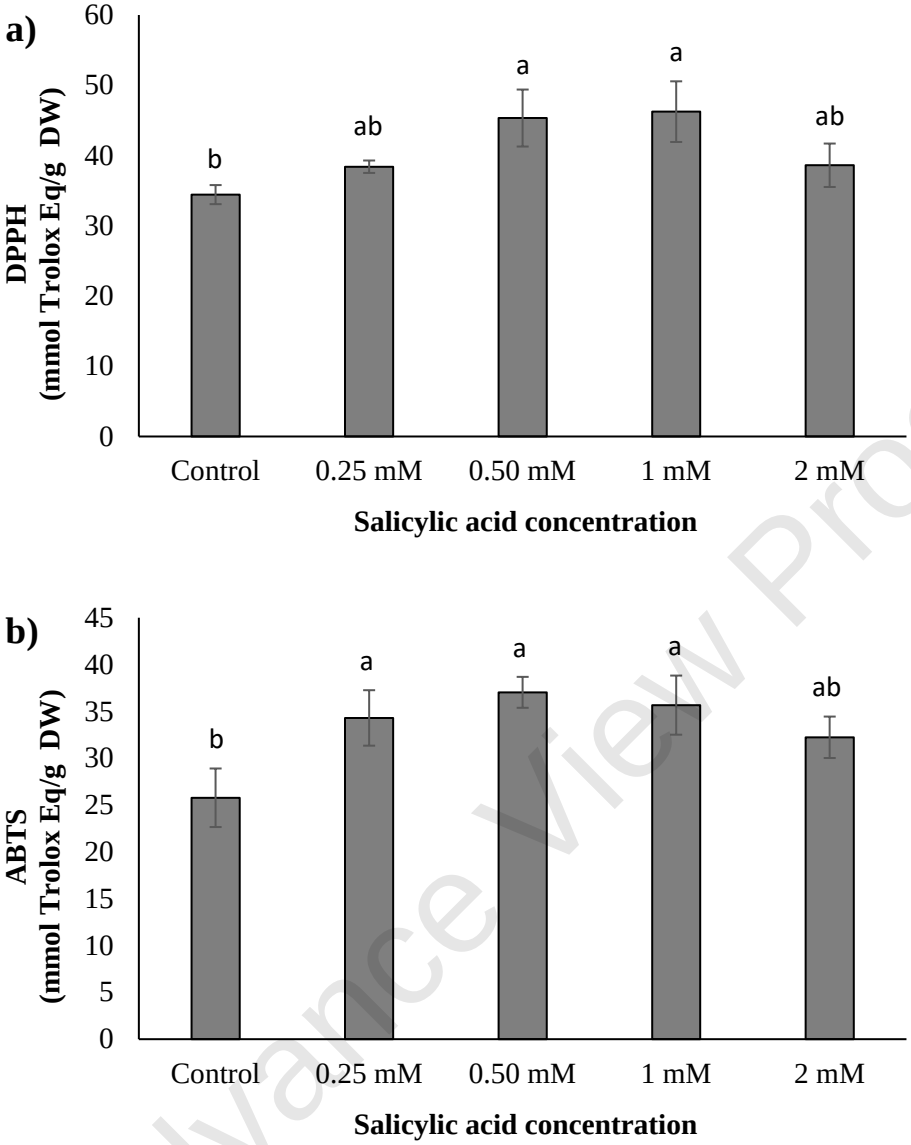

Figure 2. Antioxidant capacity of *Physalis peruviana* leaves treated with chemical elicitors during cultivation. Data are the mean ± SE from three replicates. Different letters (a, b, c, d, e, f) in one measurement indicate statistically significant difference at $p \leq 0.05$ by Tuckey test.

Table 3. Digestive enzyme inhibitory activity of the extracts of *Physalis peruviana* leaves treated with different concentrations of salicylic acid during cultivation.

Salicylic acid concentration	Digestive enzyme inhibitory activity (IC_{50}, µg/ml)	
	α-amylase	**Lipase**
Control	17.37 ± 1.89 [a]	12.44 ± 0.50 [a]
0.25 mM	17.58 ± 2.88 [a]	12.40 ± 1.24 [a]
0.50 mM	13.17 ± 1.56 [ab]	9.88 ± 0.61 [bc]
1.0 mM	11.22 ± 0.87 [b]	8.07 ± 0.54 [c]
2.0 mM	12.35 ± 3.03 [ab]	10.36 ± 0.94 [ab]

Data are the mean ± SE from three replicates. Different letters (a, b, c) in one measurement indicate statistically significant difference at $p \leq 0.05$ by Tuckey test.