

Comparison of Biochemical Compounds in Methanolic Extracts of Stem, Root, and Callus Tissues of San Pedro Cactus (*Echinopsis pachanoi*)

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Abstract

San Pedro cactus (*Echinopsis pachanoi*) is a medicinal and ornamental plant native to the desert regions of Mexico and the Andes. This study aimed to compare the biochemical compounds present in methanolic extracts of stem, root, and callus tissues of San Pedro cactus. The experiment was conducted as a completely randomized design with three replications. The results showed significant differences among the three tissue samples for all measured traits ($P < 0.01$). The stem extract exhibited the highest total alkaloid, total phenol, total flavonoid, and antioxidant activity compared with the root and callus extracts. Total phenol content in the stem extract was three times higher than in the callus and two times higher than in the root. The main alkaloid compounds were concentrated in the stem, with amounts approximately six times higher than in the callus and eight times higher than in the root. Only total protein content was higher in the root extract than in the stem and callus. The number of free peptides and amino acids was higher in the stem and root extracts than in the callus. The lowest amounts of secondary metabolites were observed in the callus extract. These findings suggest that the key effective compounds of San Pedro cactus are primarily formed in differentiated tissues, and callus culture without elicitors is not efficient enough to achieve maximum metabolite production. Therefore, in vitro production of effective compounds in this plant requires the expansion of differentiated tissue cultures such as hairy root, lateral root, and embryogenic callus cultures.

Key message

The stem of San Pedro cactus accumulates the highest levels of alkaloids, phenols, flavonoids, and antioxidants, while callus tissue produces the least; differentiated cultures are needed for efficient metabolite production.

Introduction

The San Pedro cactus (*Echinopsis pachanoi*), belonging to the Cactaceae family, is a robust, spiny, and columnar plant native to the Andes Mountains (Ogunbodede et al., 2010). This species is known for its distinctive biological traits, including antimicrobial, analgesic, and psychoactive effects, as well as a unique biochemical composition featuring specific peptides such as Ep-AMP1, mescaline, bridgexigenins A, B, and C, and pachanols A, B, and C (Aboye et al., 2015; Kinoshita et al., 1995). In recent years, there has been a notable emphasis on exploring plants for the discovery of novel peptides, given that plants are rich sources of diverse amino acid sequences (Cavallini et al., 2011). Within the San Pedro cactus, the discovery of *Echinopsis pachanoi* antimicrobial peptide 1 (Ep-AMP1), the first cystine-knot peptide identified in the Cactaceae family, has garnered attention for its potent antimicrobial properties. Notably, this peptide demonstrates antimicrobial efficacy against bacteria that surpasses that against eukaryotic cells by a factor of 500 (Aboye et al., 2015).

In recent times, there has been a surge of interest in harnessing plant secondary metabolites as promising sources of effective medicinal compounds, driven by concerns over the adverse effects of

synthetic drugs. This has led to a growing emphasis on extracting therapeutic agents from medicinal plants (Dias et al., 2016; Hussain et al., 2012; Wang et al., 2017). Notably, the growth and performance of plants are influenced not only by species and variety but also by ecological factors and regional climate (Hemmati et al., 2020).

Utilizing *in vitro* culture techniques for the extraction of medicinal compounds presents numerous advantages. These methods overcome limitations imposed by seasonal variations, geographical constraints, and environmental factors. They enable optimal and rapid production, establishing a consistent system for both quantity and quality (Ramshi, 2015). Therefore, enhancing our comprehension of the growth patterns of San Pedro cactus in *in vitro* cultures is crucial for determining optimal cultivation stages and harvest timings to maximize biomass and metabolite accumulation (Cabañas-García et al., 2021).

Biotechnology offers a pathway to upscale the production of plant metabolites without harming the environment or resorting to excessive field harvesting. However, there remains a gap in understanding the comparative capacity of wild plants versus laboratory cultures in producing secondary metabolites (Costa et al., 2012). Given the medicinal properties and unique biochemical compounds present in San Pedro cactus, further investigations are warranted to identify these compounds, quantify their distribution across different tissues, and explore the potential for secondary metabolite production in both *in vivo* and *in vitro* settings.

Therefore, the present study was conducted to compare the biochemical compounds present in methanolic extracts of stem, root, and callus tissues of San Pedro cactus, and to evaluate the potential of callus culture for secondary metabolite production.

Material and Methods

This experiment was carried out as a completely randomized design with three replications at the Laboratory of the Department of Horticultural Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Iran. A separate experiment was conducted to investigate the production of San Pedro callus using various hormonal treatments, and the results are currently being prepared for publication (Behnam et al., 2024). Among the treatments tested to enhance callus production, the most effective approach involved using an explant containing the areole and applying a hormonal combination of 2 mg/L 2,4-D and 2 mg/L BA. This optimized treatment was utilized in the current experiment for callus production.

Preparation of plant material

A two-year-old San Pedro cactus plant, approximately 20 cm in height and 5 cm in diameter, was obtained from a mother plant in the "Cactus House" greenhouse located in Semnan City, Iran. The middle segment of the stem from two plants was used to prepare the required samples for the *in vitro* callus

production stages. Additionally, the methanolic extract was prepared from the middle part of the cactus stem, while the root tissue of the same plant was used for extracting the methanolic extract.

Preparation of methanolic extract

To prepare the methanolic extract, 0.5 g of fresh tissue from each plant sample (stem, root, and callus) was weighed. The tissue was then finely ground in a mortar and transferred to a McCartney bottle. Subsequently, 5 mL of 80% methanol was added to the samples, which were then placed on a shaker for 24 h. Afterward, the samples were filtered using filter paper, and the resulting extract was stored in a refrigerator at 4°C for further analysis.

Determination of peptide content and free amino acids

Peptide content and free amino acids were assessed following the method described by Church et al. (1983) with minor modifications, employing the reaction of amine groups with o*-phthalaldehyde (OPA) reagent. In this procedure, 2.5 mL of the methanolic extract was combined with 5 mL of 10% trichloroacetic acid (TCA). After incubating for 10 min at room temperature, the mixture was centrifuged at 5,000 rpm for 15 min. The resulting supernatant was filtered through Whatman No. 1 filter paper and used to determine the peptide content. To quantify the peptide content, 3 mL of the OPA reagent was mixed with 200 µL of the supernatant. Following a 2-min incubation, the absorbance of the sample was measured at 340 nm using a spectrophotometer.

Measurement of total protein

Total protein was measured using the prepared extract and Coomassie Brilliant Blue G-250 reagent in 95% ethanol and 85% orthophosphoric acid (Bradford, 1976). Bovine serum albumin (BSA) was used as the standard protein for drawing the standard curve. For this purpose, the extracted supernatant was added to 2.5 mL of Bradford's reagent, and the tubes were placed in the dark for 15 min after mixing. The absorbance of the samples was read at 595 nm, and the total protein concentration was calculated based on comparison with the prepared standard curve.

Measurement of antioxidant activity by DPPH method

Free radical inhibition was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. First, 1 mL of methanolic extract was mixed with 1 mL of DPPH at a concentration of 0.1 mM. For the control, 1 mL of pure methanol was used as a blank. The samples were placed in the dark for 30 min, and then the absorbance was read at 517 nm using a spectrophotometer (Ebrahimzadeh et al., 2010).

Measurement of antioxidant capacity by total antioxidant method (TAOC)

The total antioxidant capacity was evaluated by the method of Sun et al. (2011). To prepare the TAOC reagent, sulfuric acid (H_2SO_4 , 0.6 M), sodium phosphate (Na_3PO_4 , 28 mM), and ammonium molybdate ($(\text{NH}_4)_2\text{MoO}_4$, 4 mM) were used. Next, 1 mL of TAOC reagent was added to 1 mL of methanolic extract, and after vortexing, the test tubes were incubated for 90 min at 95°C in a water bath. After this period and immediately after cooling the samples, their absorbance was read against the control (all compounds except the extract) at 695 nm using a spectrophotometer. To draw the standard curve, ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$) was used, and the values were expressed as mg ascorbic acid equivalent per g fresh weight (mg AAE/g FW).

Measurement of total phenol

Total phenolic compounds were quantified using the Folin–Ciocalteu colorimetric method (Singleton et al., 1999). Briefly, 0.1 g of fresh tissue (stem, root, or callus) was ground in 5 mL of 95% ethanol and left in the dark for 24 h. Then, 1 mL of 95% ethanol was added to 1 mL of the supernatant, and the solution volume was adjusted to 5 mL with distilled water. Subsequently, 0.5 mL of 10% Folin reagent and 1 mL of 5% sodium carbonate were added. The resulting mixture was kept in the dark for 1 h. Finally, the absorbance of each sample was measured at 765 nm using an Analytik Jena spectrophotometer, and the total phenol content was calculated as mg gallic acid equivalent per g fresh weight (mg GAE/g FW). Gallic acid concentrations ranging from 0 to 500 mg/L were used as standards.

Measurement of total flavonoid content

The total flavonoid content was quantified based on the rutin standard curve (Akkol et al., 2008). One mL of the methanolic extract was mixed with 250 μL of 10% aluminum chloride solution and 250 μL of 1 M potassium acetate. The absorbance of the samples was measured at 415 nm using a spectrophotometer. The total flavonoid content was expressed as mg rutin equivalent per g fresh weight (mg RE/g FW).

Measurement of total alkaloid

Phosphate buffer solution and bromocresol green (BCG) solution were used to measure total alkaloid content. Two stock solutions (acid and base) were needed to prepare the phosphate buffer solution. In this experiment, citric acid was used as the acid and disodium phosphate as the base. The acid and base were prepared at a concentration of 0.2 N in 1 L. To make phosphate buffer solution with pH 7.4, a ratio of 2:1 was used, and the pH was adjusted using a pH meter.

To prepare 0.1 mM BCG solution, 69.8 mg of BCG was first dissolved in 2 N NaOH and then completely dissolved by adding 5 mL of distilled water, and finally the volume was brought to 1,000 mL with distilled water. Then, 5.5 mL of the methanolic extract was poured into a test tube, and 1 mL of 2 N hydrochloric acid (HCl) was added. After resting for 30 min, the solution was filtered using filter paper. Approximately 5 mL of the filtered extract was washed three times with 10 mL of chloroform. After washing, the solution in the test tube separated into two phases. The upper aqueous phase was transferred to another test tube. This acidic solution was neutralized using 0.1 N NaOH. After bringing the pH of the solution to 7, 5 mL of BCG and 5 mL of phosphate buffer were added. Then, a second washing step with chloroform was performed. Ten mL of chloroform was added to the neutralized solution and washed three times. The upper blue phase was removed, and the lower phase, which contained the highest amount of alkaloids, was separated and transferred to another test tube. The alkaloid solution was read using a spectrophotometer at 470 nm. The total alkaloid content was expressed as mg atropine equivalent per g fresh weight (mg AE/g FW).

Data analysis

Statistical analysis was performed using SAS software (version 9.2). Comparison of means was done using Student's t-test at the 5% and 1% probability levels.

Results and Disscusion

The analysis of variance showed highly significant differences ($P < 0.01$) among the methanolic extracts of stem, root, and callus tissues for all measured traits, including total phenol (TPH), total flavonoid (TFL), antioxidant activity (ANT), total antioxidant capacity (TANT), total protein (TP), free peptides and amino acids (FP&AA), and total alkaloid (TA) (Table 1).

Table 1
Analysis of variance (mean squares) for biochemical compounds in methanolic extracts of stem, callus, and root tissues of San Pedro cactus

Sources of variation	(Mean square)							
	df	TPH	TFL	ANT	TANT	TP	FP&AM	TA
Explant	2	1.99**	0.081**	663.639***	3.348**	3.72**	11872469.52**	0.170**
Error	6							
ns, *, and **: No significant, significant at 5 and 1% probability. TPH: Total phenols, TFL: Total flavonoids, ANT: Antioxidant activity, TANT: total antioxidant, TP: Total protein, FP&AM: Free peptide & amino acids, TA: Total alkaloid								

Total phenol and flavonoid content

The stem extract exhibited the highest total phenol content among the three tissues (Fig. 1). Specifically, the phenol content in the stem was approximately three times higher than in the callus and two times higher than in the root. Similarly, the total flavonoid content was significantly higher in the stem compared with the root and callus tissues (Fig. 1).

These results suggest distinct biochemical profiles among different parts of the San Pedro cactus, highlighting the potential for unique functional properties associated with each tissue type. The distinct environmental conditions experienced by root organs, which are sheltered beneath the soil, shield them from the harmful effects of UV-B radiation that aerial organs are exposed to. This difference in exposure results in lower production of phenolic and flavonoid compounds within root cells, serving as a protective mechanism to mitigate potential damage caused by UV-B radiation (Moradi Roodmajani et al., 2020). Additionally, the unique anatomical structure of root cells compared with other plant organs decreases the capacity for metabolite synthesis and storage within root tissues (Beck, 2010). It is worth noting that the levels of flavonoids present in plants are influenced by factors such as plant species, organ type, and developmental stage (Petrussa et al., 2013). Therefore, the contrasting environmental and anatomical conditions in root cells play a crucial role in shaping the biosynthesis and accumulation of phenolic and flavonoid compounds, ultimately influencing the plant's response to UV-B exposure and its overall metabolic profile.

The diversity of chemical compounds in plants is intricately linked to their specific functions, resulting in a unique distribution among different plant organs. For instance, in *Coryphantha macromeris*, a study identified 69 phenolic compounds, with 26 exclusively found in shoot organs, 19 in root organs, and 24 present in both organs (Cabañas-García et al., 2019). This underscores the organ-specific distribution of metabolites within plants. Similarly, examination of fragrant violet ecotypes in distinct regions of Mazandaran revealed substantial differences in phenolic content among leaves, stems, and roots. Consistently, the root organs across all regions exhibited lower phenolic and flavonoid levels compared with aerial organs, emphasizing organ-specific variations in secondary metabolite content (Moradi et al., 2019).

Contrasting results were observed in the research on *Ferula gummosa* by Zeinali et al. (2014), where the highest total phenol content was recorded in roots, in contrast to the present study's findings. The researchers suggested that the specialization of specific plant organs for particular functions may explain the variance in metabolite accumulation. These observations highlight the complex interplay between plant physiology, environmental factors, and organ-specific biochemical processes shaping the composition and distribution of chemical compounds within plants.

Costa et al. (2012) investigated the amount of phenolic compounds present in wild thyme compared with *in vitro* thyme and reported that the amount of phenolic compounds caffeic acid, luteolin, and apigenin in the water-ethanol extract of wild thyme was higher than in *in vitro* culture. The results of the experiment by García-Pérez et al. (2012) regarding *Poliomintha glabrescens* confirm the existence of higher amounts of the phenolic compound luteolin in the wild plant than in *in vitro* culture.

This issue may reflect the effect of laboratory culture conditions, such as reduced light intensity, on the production of secondary metabolites. Another notable investigation delved into *Opuntia ficus-indica*, where the *in vitro* cladode extract showcased significantly higher concentrations of total phenols and kaempferol, a powerful flavonoid recognized for its anticancer properties, in comparison to the field-grown cladode extract. Specifically, the *in vitro* extract contained 156.5 mg/g of total phenols and 2.807 mg/g of kaempferol, while the field-grown counterpart exhibited 86 mg/g of total phenols and 1.304 mg/g of kaempferol (Heikal et al., 2021). These findings offer a conflicting perspective on the matter.

Antioxidant activity and total antioxidant capacity

Polyphenols are garnering increasing global attention due to their antioxidant properties. Antioxidant activity is characterized by a chemical component's ability to absorb and/or impede the creation of free radicals. The consumption of antioxidants plays a vital role in safeguarding health and warding off inflammatory, cardiovascular, neurological (such as Parkinson's and Alzheimer's), and cancerous diseases (Dykes & Rooney, 2007; Salvayre et al., 2016). Consequently, the identification of plant organs rich in antioxidant compounds holds significant importance. The anti-free radical efficacy of phenolic compounds is intricately linked to their molecular structure, with the presence of phenolic hydrogens facilitating the generation of phenoxyl radicals through hydrogen donation, thus enabling phenolic compounds to neutralize free radicals (Abdulhafiz et al., 2020). The assessment of antioxidant activity of San Pedro stem, root, and callus extracts using DPPH and TAOC methods revealed that the stem extract exhibited superior antioxidant effects compared with the root and callus extracts (Fig. 2).

The study comparing biochemical compounds in various plant extracts sheds light on the differences in secondary metabolite production based on growth conditions and tissue types. The analysis of *Alocasia longiloba* extracts revealed that callus extracts exhibited the highest total phenol content and antioxidant activity, while leaf extracts from field-grown plants contained more total flavonoids. This underscores the impact of growth environment and tissue specificity on metabolite composition. Researchers highlighted a positive linear correlation between antioxidant activity and total phenol content, underscoring the significance of phenolic compounds in antioxidant potential (Abdulhafiz et al., 2020). In a similar vein, investigating wild thyme compared with *in vitro* thyme extracts revealed higher antioxidant efficacy in wild specimens, yet *in vitro* cultures displayed elevated levels of rosmarinic acid, suggesting their potential as alternative antioxidant sources (Costa et al., 2012). This aligns with findings reporting higher rosmarinic acid content in *in vitro* thyme cultures compared with wild thyme plants, highlighting the prospective utility of *in vitro* cultures for antioxidant compound production (Pérez-Tortosa et al., 2012; Shan et al., 2005).

Exploring *Opuntia* species, Robles-Martínez et al. (2016) found that callus cultures and cell suspensions exhibited comparable levels of phenolic compounds, flavonoids, and antioxidant activity, surpassing field plant cladodes in antioxidant compound content. The researchers attributed the higher metabolite levels in callus cultures to enhanced growth dynamics, emphasizing the pivotal role of growth phase in secondary metabolite production. A study on *Gentianella austriaca* revealed similar secondary metabolite profiles between tissue culture and *in vivo* samples, with higher xanthone content in natural specimens,

indicating the influence of benzyl adenine on metabolite production in culture medium (Vinterhalter et al., 2008). The research noted a decline in antioxidant activity with increased benzyl adenine concentration, along with altered levels of individual compounds, suggesting a complex interplay between growth stimulants and metabolite production (Janković et al., 2011).

Total protein, free peptides, and amino acids

The analysis of total protein content in different plant organs highlighted varying protein distributions, with notable differences between organs such as root and stem, reflecting distinct metabolic activities in different plant parts (Fig. 3). The root extract exhibited the highest total protein content, surpassing both stem and callus tissues. Research on *Mindium laevigata* indicated higher total protein levels in the stem, possibly indicative of enhanced metabolic processes in this organ (Jafarian et al., 2017). Similarly, investigations on *Costus pictus* underscored differential phytochemical compositions in plant parts, with leaves rich in flavonoids and antioxidant compounds, while the rhizome exhibited higher phenol and protein content (Jarite et al., 2019).

Ceropegia bulbosa plants exhibited the highest protein content compared with their *in vitro* counterparts (Dhir & Shekhawat, 2014). Research on *Acanthophyllum sordidum* indicated that the protein content of the intact plant surpassed that of the callus tissue. Notably, protein levels in the callus decreased but rose significantly during shoot regeneration from callus (Meratan et al., 2009). This study underscores the enhancement and augmentation of secondary metabolite production in differentiated tissues cultured *in vitro*.

While peptide and free amino acid levels in the stem and root showed no significant difference, San Pedro callus displayed the lowest amounts compared with the other two organs (Fig. 3). Additionally, total phenol, flavonoid, and protein content in the callus were lower than in the stem and root. Various species have been reported to require a certain degree of differentiation in callus for successful phytochemical compound production (Misawa, 1994). Moreover, different rates of nitrogen division between plant parts can contribute to varied levels of secondary metabolite accumulation and antioxidant activity, with higher carbon-to-nitrogen ratios indicating increased secondary metabolite production and heightened antioxidant activity (Ibrahim & Jaafar, 2011).

Ng et al. (2016) conducted an experiment exploring secondary metabolite levels in stem extracts and three types of calluses from *Boesenbergia rotunda*. Findings revealed that the embryogenic callus exhibited the highest amino acid and primary metabolite content compared with the stem, dry, and fresh callus types. They suggested this could be due to the elevated requirement for amino acids and primary metabolites during cell differentiation and division in plant regeneration from embryogenic callus. Interestingly, secondary metabolite levels in the stem sample surpassed those in all callus types, with residual secondary metabolites possibly retained from the original explant.

Total alkaloid content

Alkaloids, cyclic compounds containing nitrogen atoms, are vital plant defense mechanisms against herbivores and pathogens, with around 12,000 known alkaloid compounds identified in plants (Gleason & Chollet, 2012). The current research indicates that the San Pedro stem concentrates the majority of alkaloid compounds, nearly six times more than callus tissue and about eight times more than the root (Fig. 4).

The aerial portion of the San Pedro cactus exemplifies the historical traditional use of hallucinogenic substances, with sacred psychedelic plants, notably the San Pedro cactus, playing a crucial role in Andean healing practices for millennia (Paniagua-Zambrana & Bussmann, 2020). San Pedro cactus is renowned for its high alkaloid content, leading to its classification as a drug cactus, with mescaline as its primary hallucinogenic component (Ogunbodede et al., 2010). Tyrosine and phenylalanine amino acids act as precursors to mescaline (Dinis-Oliveira et al., 2019). Notably, while the free amino acid levels in the stem and roots are comparable, they exceed those found in callus tissue. Furthermore, the total alkaloid content in the stem tissue surpasses that of the root, while total protein content in the root exceeds that of the stem. Amino acids serve as common precursors for alkaloid compounds and proteins. Thus, the free amino acids within each tissue likely contribute to the synthesis of the specific metabolites the tissue produces. As suggested by Zeinali et al. (2014), the accumulation of metabolites in each organ may result from specialized functions that are tailored to the final use of that organ.

Alkaloids in cacti are very important in medicine and have been used as painkillers, anticancer agents, and respiratory stimulants (Abdulazeem et al., 2018; Cassels, 2019; Castro-Campos-Pinto et al., 2015). Mescaline inhibits the neuron's cholinergic transmission, blocks the release of acetylcholine, and affects K⁺ conductance. These effects were attributed to mescaline acting as a serotonin 5-HT_{2A} receptor agonist (Castro-Campos-Pinto et al., 2015).

Research has shown that practically the largest amount of mescaline, which is the main alkaloid compound in peyote cactus (*Lophophora williamsii*), is found in its crown and constitutes 15.7% of its total alkaloids (Cassels & Sáez-Briones, 2018). Comparing the amount of alkaloid present in new and old stems of *Lophocereus schottii* showed that not only is there a difference between young and old stems in the same plant, but also the alkaloid present in the epidermis tissue is different from the cortex tissue in the same plant (Kircher, 1969). In the research conducted by Hemmati et al. (2018) on different cultivars and organs of *Catharanthus roseus*, it was observed that the type of alkaloids identified and their amount differ significantly between cultivars and different organs. For example, the lowest amount of vinblastine, which is one of the most important alkaloid compounds of this plant, was observed in the root of the Icy Pink variety. Also, two alkaloids, ajmalicine and vincristine, were not present in the roots. The amount of secondary metabolites in different plant organs is different according to the type of plant and the specialized organ used (Moradi et al., 2019).

On the other hand, secondary metabolites, especially alkaloids, are mainly produced to play a role in the plant's defense system. Both plant growth and defense are fed by compounds synthesized from a common pool of carbon and nitrogen, implying competition for the allocation of carbon and nitrogen to

both metabolisms (Royer et al., 2013). The absence of alkaloid compounds in the callus compared with the stem and root tissue of the plant can be attributed to the allocation of nitrogen and carbon for the synthesis of essential primary metabolites and the development of key structures of the cellular skeleton in the callus.

The findings from the investigation of the presence and concentration of alkaloids, including ajmalicine, catharanthine, and vindoline, in various tissues of *Catharanthus roseus*, such as *in vitro* shoot, *in vitro* root, callus, and leaves from field-grown plants, revealed specific patterns. For instance, ajmalicine was not detected in the callus, and vindoline was absent in the tissue culture root and callus. Furthermore, the quantity of vindoline in the tissue culture stems was approximately half of that found in the leaves of field-grown plants (Hernández-Domínguez et al., 2006). A study on *Datura innoxia* indicated that the alkaloid concentration was 0.1% in the whole plant and 0.01% in the callus tissue (Hiraoka & Tabata, 1974). These results align with our own observations.

Conversely, research on *Cereus peruvianus* cactus demonstrated that the alkaloid production ratio in the callus was nearly double that of the aerial parts of the plant (1.7:1) (Olivera & Machado, 2003). In the case of *in vitro* propagation of *Fritillaria unibracteata* onions, the growth rate was significantly increased by 30 to 50 times compared with natural conditions, with higher levels of alkaloids and other beneficial micronutrients observed in tissue culture onions relative to wild onions (Gao et al., 1999). Similarly, a study on the *in vitro* propagation of *Ceropegia bulbosa* revealed that laboratory-grown plants exhibited higher total antioxidant activity and phenolic content, while natural plants had higher chlorophyll and protein content (Dhir & Shekhawat, 2014).

Several factors influence variations in total alkaloid content, such as cellular development and differentiation, the allocation of carbon and nitrogen resources, plant species, and specialized organs. Notably, the production of alkaloids, crucial for plant defense systems, tends to be higher in plants grown under natural conditions than in isolated and controlled laboratory settings without precursors and elicitors in the tissue culture medium.

According to Bourgaud et al. (2001), the limited presence of secondary metabolites in callus tissue can be ascribed to the emphasis on primary metabolism, such as cellular structure formation and respiration, during active growth phases. Subsequent cessation of growth leads to a decrease in the demand for carbon for primary metabolites, thereby allowing for increased secondary metabolite synthesis. Enzyme activities often become more prominent during growth plateaus rather than the earlier growth phases, suggesting potential biochemical differentiation of cells during growth arrest (Bourgaud et al., 2001). In most instances involving unorganized structures like callus, secondary metabolite production remains minimal (Isah et al., 2018). Moreover, certain levels of differentiation may be necessary for the biosynthesis of secondary metabolites in select plant species (Karuppusamy, 2009). For enhanced metabolite production, methods like root cultures, somatic embryos, and shoot cultures in differentiated tissues are considered superior alternatives in many plants for achieving higher yields of plant metabolites (Ahmad et al., 2013; Murthy et al., 2014).

Conclusion

The stem of San Pedro cactus exhibited the highest concentration of secondary metabolites, including total phenols, flavonoids, and alkaloids, while the root contained the highest total protein content. The callus tissue showed the lowest levels of all measured compounds, likely due to the absence of cell differentiation and the allocation of carbon and nitrogen to primary metabolism. Since alkaloids are the principal metabolites in this plant, the stem appears to be the specialized organ for their production. Therefore, *in vitro* cultures of differentiated tissues, such as hairy root and somatic embryo cultures, combined with the strategic use of elicitors, are recommended to enhance secondary metabolite production in San Pedro cactus.

Abbreviations

Plant/Tissue & General Terms	Measured Traits	Chemical Compounds & Reagents
San Pedro – <i>Echinopsis pachanoi</i>	TPH – Total Phenols	DPPH – 2,2-Diphenyl-1-picrylhydrazyl
Ep-AMP1 – <i>Echinopsis pachanoi</i> Antimicrobial Peptide 1	TFL – Total Flavonoids	TAOC – Total Antioxidant Capacity
2,4-D – 2,4-Dichlorophenoxyacetic acid	ANT – Antioxidant Activity	OPA – *o*-Phthalaldehyde
BA – Benzyl Adenine	TANT – Total Antioxidant Capacity	TCA – Trichloroacetic acid
DNA – Deoxyribonucleic acid	TP – Total Protein	BCG – Bromocresol Green
RNA – Ribonucleic acid	FP&AA / FP&AM – Free Peptides and Amino Acids	BSA – Bovine Serum Albumin
UV-B – Ultraviolet-B radiation	TA – Total Alkaloid	HCl – Hydrochloric acid
pH – Potential of hydrogen	Units	NaOH – Sodium hydroxide
rpm – Revolutions per minute	mg/g FW – Milligram per gram Fresh Weight	H ₂ SO ₄ – Sulfuric acid
nm – Nanometer	mg AAE/g FW – Milligram Ascorbic Acid Equivalent per gram Fresh Weight	Na ₃ PO ₄ – Sodium phosphate
mL – Milliliter	mg GAE/g FW – Milligram Gallic Acid Equivalent per gram Fresh Weight	(NH ₄) ₂ MoO ₄ – Ammonium molybdate
μL – Microliter	mg RE/g FW – Milligram Rutin Equivalent per gram Fresh Weight	C ₆ H ₈ O ₆ – Ascorbic acid
mg – Milligram	mg AE/g FW – Milligram Atropine Equivalent per gram Fresh Weight	GAE – Gallic Acid Equivalent
g – Gram		RE – Rutin Equivalent
h – Hour		AE – Atropine Equivalent
min – Minute		AAE – Ascorbic Acid Equivalent
°C – Degree Celsius		5-HT _{2A} – 5-Hydroxytryptamine 2A receptor
N – Normal (concentration)		
M – Molar		

mM – Millimolar

FW – Fresh Weight

SAS – Statistical Analysis System

df – Degrees of freedom

ns – Not significant

SE – Standard Error

ANOVA – Analysis of Variance

C/N – Carbon to Nitrogen ratio

Declarations

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Ethics declaration

not applicable

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

[HB] contributed to the study conception and design. Material preparation, data collection, and analysis were performed by [HB] and [AGH]. The first draft of the manuscript was written by [HB], and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

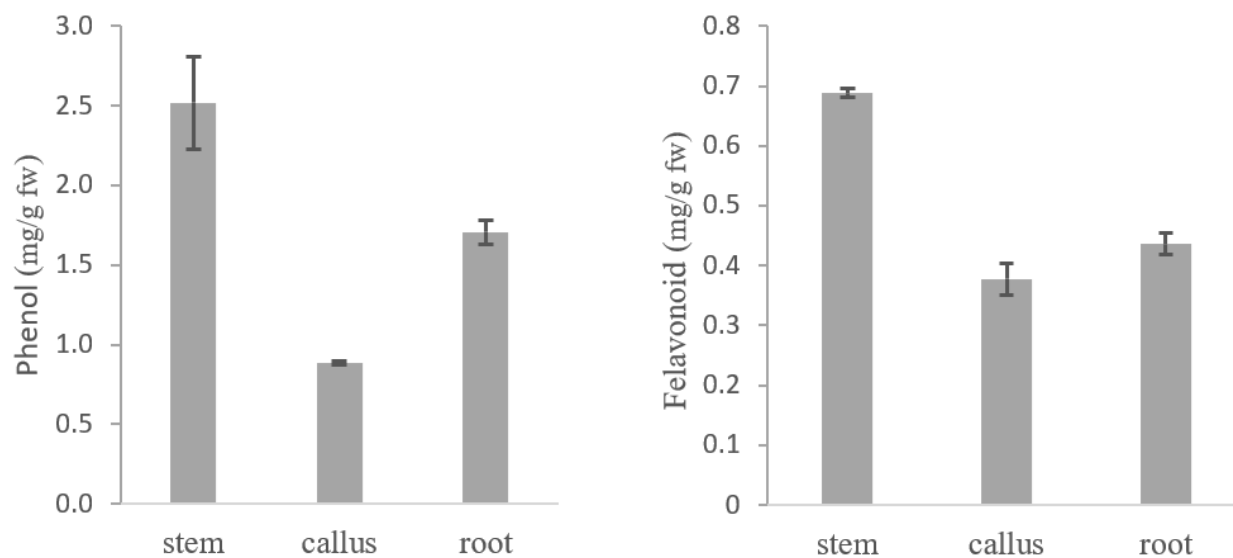


Figure 1

The amount of phenol and flavonoids present in stem, root and callus tissue

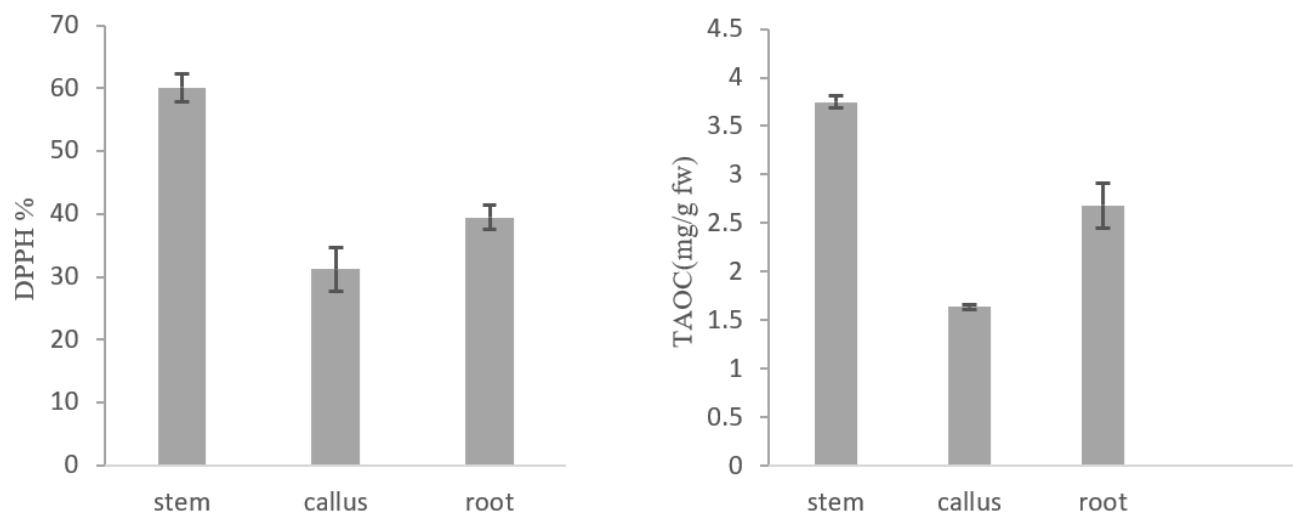


Figure 2

Antioxidant activity (DPPH, left) and total antioxidant capacity (TAOC, right) in stem, callus, and root tissues of San Pedro cactus. Values are means $\pm$ standard error (n = 3)

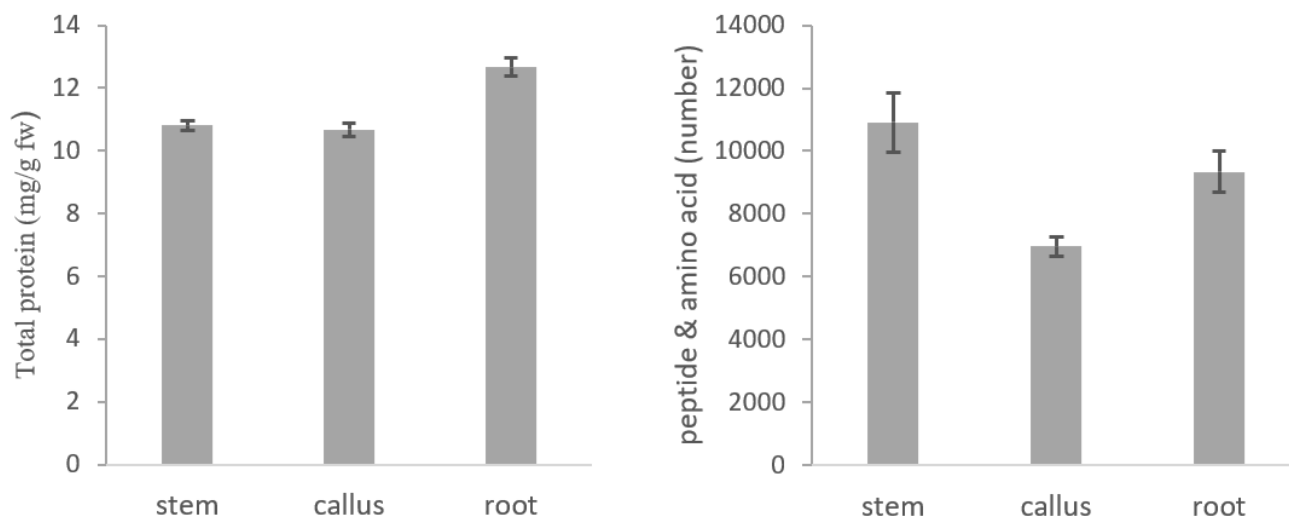


Figure 3

Total protein content (left) and free peptide and amino acid content (right) in stem, callus, and root tissues of San Pedro cactus. Values are means $\pm$ standard error (n = 3).

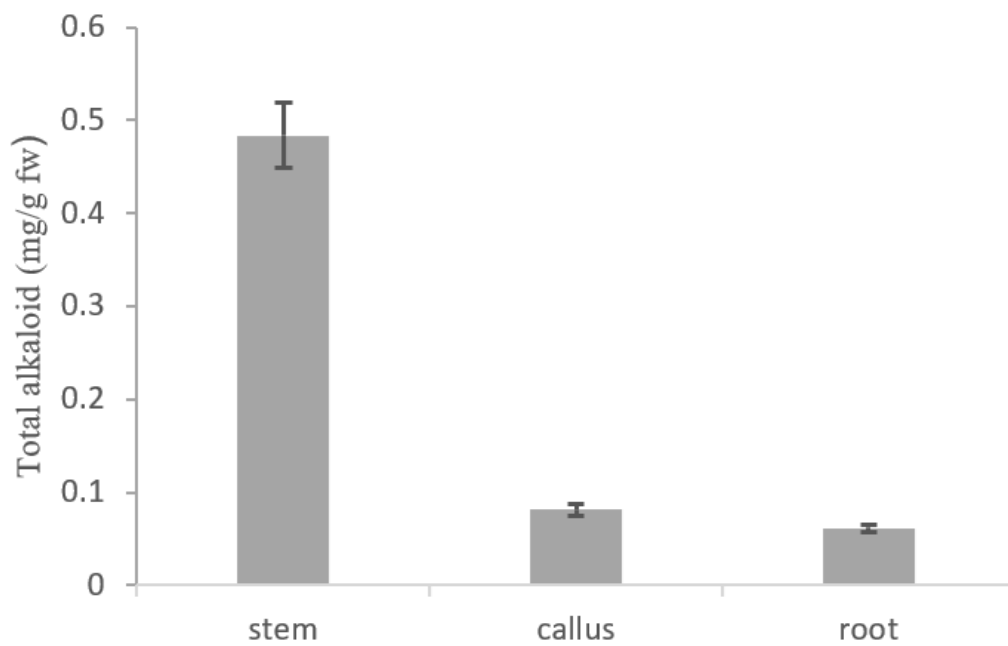


Figure 4

The amount of total alkaloid measured in three tissues of stem, root and callus