Research Paper

Impact of Nutrient Management on Phytochemical Composition in *Agave marginata* L.: Considerations for Toxicological Relevance

Ali Mohammad Rahimi[1], **Mehrab Yadegari**[2*], **Mohammad Moghaddam**[3], **Omid Mehrpour**[4]

1. Department of Medicinal Plants and Agriculture, Faculty of Veterinary Medicine and Agriculture, Shahrekurd Branch, Islamic Azad University, Shahrekurd, Iran.
2. Medical Plant Research and Processing Center, Shahrekurd Branch, Islamic Azad University, Shahrekurd, Iran.
3. Medical Plant Research and Processing Center, Ferdosi University, Mashhad, Iran.
4. Michigan Poison & Drug Information Center, School of Medicine, Wayne State University, Detroit, United States.

Use your device to scan and read the article online

Citation: Rahimi AM, Yadegari M, Moghaddam M, Mehrpour O. Impact of Nutrient Management on Phytochemical Composition in Agave marginata L.: Considerations for Toxicological Relevance. International Journal of Medical Toxicology and Forensic Medicine. 2025; 15(2):E45807. https://doi.org/10.32598/ijmtfm.v15i2.45807

doi https://doi.org/10.32598/ijmtfm.v15i2.45807

Article info:
Received: 17 Jul 2024
First Revision: 20 Jul 2024
Accepted: 14 Sep 2024
Published: 10 Jun 2025

Keywords:

Agave marginata L., Saponins, Hecogenin, Chlorophyll, Micronutrients, Phytochemical optimization, Therapeutic potential, Nutrient management

ABSTRACT

Background: This study aims to investigate the effects of strategic nutrient management, incorporating absorbent nano-superpolymer, growmore fertilizer, iron, and zinc on the growth and phytochemical composition of *Agave marginata* L. in Shiraz City, Iran. Following a conceptual framework for nutrient management, the study primarily aims to enhance the plant's saponin content, a bioactive compound with significant therapeutic potential and potential toxicological risks, while examining other qualitative and quantitative growth parameters.

Methods: A completely randomized design assessed the effects of varying levels of growmore fertilizer, iron, zinc micronutrients, and absorbent nano-superpolymer against a control. Measured parameters included stem height, diameter, fresh and dry weight, and phytochemical concentrations, such as hecogenin, saponins, and chlorophyll to evaluate the effectiveness and safety of the treatments.

Results: The interventions demonstrated statistically significant enhancements in multiple measured traits, particularly saponin concentration, which is critical for the plant's medicinal potential. While the focus remains on the therapeutic benefits of enhanced saponin levels, the study also emphasizes the necessity for toxicological assessments. The results of the first year showed an increase in chlorophyll content with the first level of growmore treatment, further amplified in the second year, indicating a cumulative effect of nutrient management on the plant's phytochemical profile and potential health benefits.

Conclusion: This study highlights the critical role of precise nutrient management in improving the medicinal quality and yield of *A. marginata* L., with a clear positive correlation between targeted fertilization strategies and enhanced phytochemical profiles relevant to medical applications. The results suggest significant potential for *A. marginata* L. in pharmaceutical development, with future research necessary to conduct comprehensive toxicological evaluations to ensure safe therapeutic use.

*** Corresponding Author:**
Mehrab Yadegari, Associated Professor.
Address: *Medical Plant Research and Processing Center, Shahrekurd Branch, Islamic Azad University, Shahrekurd, Iran.*
E-mail: *mehrabyadegari@yahoo.com*

Introduction

The global shift towards herbal medicine has captured the attention of researchers across diverse fields, including agricultural botany, chemistry, biotechnology, pharmacy, and medicine. The increasing prevalence of chronic diseases and the limitations of conventional pharmaceutical treatments have prompted renewed interest in herbal medicines, which are often seen as safer and more compatible with human physiology than synthetic drugs [1-6]. Medical plants, including *Agave marginata* L., have become a significant research topic due to their potential health benefits and industrial applications.

Herbal medicines, derived from natural sources, are perceived as more aligned with the body's physiological processes, often presenting fewer risks and side effects than chemical drugs [7].

However, despite their widespread use in traditional medicine, there remains a critical need for rigorous clinical and pharmacological studies to validate their safety and efficacy. The toxicological effects of plant compounds are particularly concerning in regions like Iran, where medicinal plants are common [8, 9]. This study addresses the toxicological relevance of bioactive compounds, such as saponins and hecogenin, known for their therapeutic potential but also pose toxic risks at elevated concentrations.

Iran is home to a rich diversity of medicinal plants, which are well-suited for cultivation and export due to the country's favorable climate and geography [7]. Historically, Iran has been a center for producing and using medicinal plants. Among these, *A. marginata* L. holds particular significance. The name "Agave" comes from the Greek word "Agavos", meaning "illustrious", about its striking appearance when it flowers. Agave, originally classified in the Amaryllidaceae family, now belongs to the independent family Agavaceae, which includes over 300 perennial species [10, 11].

A. marginata L. thrives in sunny environments and is drought-resistant, making it well-suited to Iran's climate, particularly in Fars Province. The plant's saponins, found in its roots, are known for their ability to lower blood lipids and cholesterol and exhibit anti-inflammatory properties [10, 11]. However, saponins also pose toxicological risks, such as hemolytic activity and gastrointestinal irritation, necessitating comprehensive toxicological assessments. Similarly, hecogenin, another bioactive compound derived from Agave, is used in the pharmaceutical industry to produce cortisone and sex hormones. While these bioactive compounds are valuable in the pharmaceutical context, their toxicological profiles must be carefully evaluated, especially when their concentrations are increased through nutrient management [11].

Nutrient management framework

This study follows a structured nutrient management framework designed to optimize the phytochemical profile of *A. marginata* L. The interventions used, including growmore fertilizer, iron, zinc micronutrients, and absorbent nanopolymers, were selected based on their proven capacity to enhance plant growth and stimulate the production of secondary metabolites, as indicated in earlier research [12]. Each intervention targets key aspects of plant physiology:

Growmore fertilizer provides balanced nitrogen, phosphorus, and potassium (NPK) formulation, essential for overall plant vigor and the activation of biochemical pathways in secondary metabolite production.

Iron plays a vital role in chlorophyll synthesis and photosynthesis, processes closely linked to the plant's ability to generate bioactive compounds, such as saponins and hecogenin.

Zinc supports plant enzyme function, stress tolerance, and defense mechanisms, promoting growth and increased phytochemical concentrations.

Absorbent nano polymers improve water retention in the soil, which is crucial to optimize nutrient uptake and ensure consistent moisture levels, especially in arid climates like Shiraz City, Iran, where this study was conducted.

Combining these carefully selected interventions, this approach aims to maximize the production of bioactive compounds while ensuring sustainable plant growth, with the ultimate goal of balancing therapeutic potential with toxicological safety [13]. These interventions were selected to optimize the production of bioactive compounds, particularly saponins and hecogenin, while minimizing potential toxicological risks. The choice of these specific nutrients is supported by their known roles in enhancing bioactive compound production while mitigating toxicological risks. Growmore fertilizer supplies a balanced combination of macronutrients and micronutrients that are essential to promote plant growth and improve the synthesis of secondary metabolites, such as saponins and hecogenin [14]. Iron is critical in metabolic processes like photosynthesis and respiration, which directly contribute to phytochemical production and reduce oxidative stress to prevent toxicity [15]. On the other hand, zinc stabilizes membrane structures, promotes defense mechanisms, and reduces oxidative stress, further contributing to bioactive compound production and managing toxicity risks [15].

The rationale for using these specific interventions stems from their ability to improve the plant's overall health and its phytochemical yield. Growmore fertilizer and micronutrients, such as iron and zinc enhance plant metabolism, leading to increased production of secondary metabolites, which are responsible for the plant's medicinal properties [16, 17]. The use of absorbent nano polymers aims to improve water retention in the soil, which is crucial for the growth of *A. marginata* L. in arid environments like Iran. By applying these treatments, this study seeks to enhance the plant's therapeutic value and its suitability for large-scale pharmaceutical production.

The toxicological relevance of this study lies in the balance between increasing the therapeutic compounds in *A. marginata* L. and mitigating the associated toxic risks. Saponins, despite their beneficial effects in reducing cholesterol and inflammation, cause hemolytic activity and gastrointestinal irritation when present in high concentrations. Similarly, hecogenin plays a critical role in steroid synthesis, but its safety profile needs thorough evaluation, particularly when its levels are manipulated through nutrient treatments. This study aims to establish safe thresholds for using these bioactive compounds in medicinal applications focusing on the therapeutic potential and toxicological risks.

Necessity and novelty of the study

This study addresses a critical gap in existing research by investigating the impact of nutrient management on both the therapeutic and toxicological aspects of *A. marginata* L., while previous studies have focused on the benefits of bioactive compounds in medicinal plants, few have explored how nutrient interventions can enhance these benefits and increase toxic risks. The novelty of this study lies in its dual focus, not only optimizing phytochemical production but also ensuring that the increased concentrations of these compounds remain within safe limits for pharmaceutical use. This comprehensive approach differentiates it from previous research and highlights its relevance to agricultural and pharmaceutical industries. The study's focus on nutrient management interventions aims not only to enhance the therapeutic potential of *A. marginata* L. but also to explore how chemical manipulation through micronutrient supplementation may influence the toxicological profile of the bioactive compounds produced, such as saponins and hecogenin. This approach is critical to understanding the balance between therapeutic benefits and toxicological risks in pharmaceutical applications.

The primary aim of this study is to assess the impact of nutrient management on the growth and phytochemical composition of *A. marginata* L., with specific objectives:

1) To measure the effects of different growmore fertilizer, iron, and zinc micronutrient levels on plant growth parameters, such as height, stem diameter, fresh weight, and dry weight over two crop seasons; 2) To assess the impact of nutrient treatments on the phytochemical composition, focusing on the saponin and hecogenin concentrations; 3) To identify and quantify the toxicological risks associated with increased concentrations of saponins and hecogenin, using correlations between concentration levels and known toxic effects, such as hemolytic activity.

Materials and Method

Design of the experiment

This research was structured as a completely randomized study with eight treatments and over two years using pot-grown plants in the biology research greenhouse at the School of Agriculture, Shiraz University (Niayesh Town, Bajgah). The study followed a conceptual framework of nutrient management, focusing on enhancing the phytochemical profile of *A. marginata* L., particularly concerning its therapeutic and toxicological implications.

The SAS statistical software, version 9, was employed to analyze the data and charts were created using Excel 2016. The samples were analyzed in the School of Agriculture laboratory at Shiraz University. The experimental results and statistical analyses were evaluated separately to ensure the robustness of the results.

The study spanned the 2019-2020 and 2020-2021 crop seasons. During this time, the *A. marginata* L. plants were grown and maintained in the research center's greenhouse. The physicochemical properties of the plant samples were thoroughly analyzed.

Study variables

The primary characteristics investigated in this study included:

Morphological parameters: Height, stem diameter, fresh weight, and dry weight; Phytochemical parameters: Chlorophyll a content, chlorophyll b content, total chlorophyll content, hecogenin content (key variable), and saponin content (key variable); The relationships between these traits were evaluated using analysis of variance (ANOVA) and correlation coefficient tests.

Measurement of hecogenin

For hecogenin content measurement, one gram of dried *Ag. marginata* leaf powder (dried at 40 ˚C in a vacuum drying oven) was dissolved in 3 mL of methanol (Merck, Germany) and homogenized for 3 minutes. The solution was centrifuged for 5 minutes at 5000 rpm and filtered using a 0.22 µm filter. The hecogenin content was measured at 255 nm using a microplate reader (Citation 5, U.S.A.). The wavelength of 255 nm was selected based on the standard sample scanning from 190–700 nm. Pure hecogenin (Merck, Germany) at 0–5 µg/mL concentrations was used to generate the standard curve.

Measurement of total saponin content

Total saponin content was measured using the Penafil method. A 0.2 g sample was mixed with 3.2 mL of glacial acetic acid/sulfuric acid (1:1) and homogenized for 1 minute. Then, it was heated at 60 ˚C for 30 minutes, which resulted in a purple color, indicating the presence of saponins. After cooling, the total saponin content was measured at 527 nm using a UV-1650 PC spectrophotometer (Shimadzu, Japan). Glacial acetic acid was used as the control, and a standard curve of oleanolic acid (0–4000 µg/mL) was generated to quantify the saponin content.

Measurement of chlorophyll content

Chlorophyll content was measured by homogenizing 0.2 g of *A. marginata* powder in 15 mL of 80% acetone. After centrifugation, the chlorophyll content was measured using a UV-1650 PC spectrophotometer (Shimadzu, Japan) at 663 nm, 645 nm, and 652 nm, with 80% acetone serving as the blank. The chlorophyll content was also measured using a Cary 50 UV-Vis spectrophotometer (Germany) at 646.8 nm, 663.20 nm, and 652 nm. The Equations 1, 2 and 3 were used to calculate pigment volume concentrations:

1. $Chl_a=12.25\ A663.2-2.79\ A646.8$

2. $Chl_b=21.21\ A646.8-5.1\ A663.2$

3. $\text{Total Chl}=Chl_a+Chl_b$

Treatments and conceptual framework

This experiment applied eight different nutrient treatments to assess their effects on the qualitative performance of *A. marginata* L. under the Shiraz climate. The treatments included:

1) Growmore fertilizer (three levels); 2) Fe micronutrients (two levels); 3) Zn micronutrients (one level); 4) Super-absorbent nano polymers (one level); 5) Control treatment (one level).

These interventions were selected based on previous studies demonstrating their effectiveness in improving the phytochemical content of medicinal plants. The goal was to optimize the plant's therapeutic value while minimizing the associated toxicological risks.

Experimental framework

The experiment was structured using a conceptual framework to evaluate the impact of nutrient interventions on multiple variables, including physiological, phytochemical, and morphological traits. The relationships between stem height and diameter, fresh and dry weight of the stem, hecogenin content, saponin content, chlorophyll content, chlorophyll b content, and total chlorophyll content were thoroughly examined. By applying this framework, the study was conducted to determine the role of targeted nutrient management in enhancing both therapeutic and toxicological profiles of *A. marginata* L.

Results

Study context and statistical analysis

This study demonstrated that applying macronutrients and micronutrients significantly increased secondary plant metabolites and active ingredients in *A. marginata* L., which is crucial for both therapeutic benefits and potential toxicological impacts.

Plant growth parameters

Height and fresh weight

The results showed that the height and fresh weight of *A. marginata* plants were significantly affected by nutrient treatments. Figure 1 illustrates the distribution and concentration of both micro and macro elements across the different nutrient treatments, demonstrating their significant impact on the phytochemical composition of *A. marginata* L (Tables 1, 2 and 3).

First year: In the first year, the height of the plants treated with the third level of growmore fertilizer was lower than the control and other treatments. Treatments also significantly impacted plant fresh weight, with the differences statistically significant at the 1% level. Second year: In the second year, plants treated with various levels of growmore fertilizer and micronutrients showed a significant increase in fresh weight compared to the control (30 g), and these differences were statistically significant at the 1% level.

Dry weight

The application of different treatments, excluding the Iranian iron fertilizer and first-level growmore treatment, led to a significant increase in the dry weight of the plants during the second year compared to the control plants (2.91 g). The differences were statistically significant at the 5% level, underscoring the long-term impact of nutrient management on the plant's biomass accumulation.

Phytochemical analysis

Saponin content

The analysis of saponin content indicated a significant increase in the first and second years due to nutrient treatments. However, this increased saponin content, while promising for its therapeutic potential, may elevate the toxicological risks associated with its hemolytic effects. Thus, further studies are required to evaluate safe thresholds for pharmaceutical applications.

First year: The Duncan test revealed that the first-level growmore treatment substantially positively affected saponin content in the first year, with a statistically significant difference at the 1% level (Tables 4, 5 and 6). Second year: In the second year, treatments positively impacted saponin content, reaffirming the long-term effect of nutrient management on phytochemical concentration (Table 7).

Hecogenin content

Hecogenin levels were lower in plants treated with Fe chelate and first-level growmore compared to the control plants (28.95 ppm). This decrease was significant and warrants further investigation to optimize the plant's therapeutic potential while minimizing any possible reductions in hecogenin content due to specific treatments.

Table 1. Test result of soil macro-elements

Row	Laboratory Number	Characteristics	SP (%)	EC (ds/m)	Total Acid Saturation (PH of Paste)	Neutralizing Substances	Organic Carbon	Nitrogen	Phosphorus (ppm)	Potassium (ppm)	Clay	Silt	Sand
1	11653-26318-1	-	47	2.2	7.66	54.67	1.46	0.14	16	544	20.3	45.6	34.1

SP: Saturation percentage; EC: Electrical conductivity.

Table 2. Test result of soil micro-elements

Row	Laboratory Number	Characteristics	Iron (ppm)	Zinc (ppm)	Manganese (ppm)	Copper (ppm)
1	11653-26318-1	-	8.1	2.8	9.7	1.5

Figure 1. Micro and macro elements

Table 3. Test result of agricultural water

Row	Laboratory Number	Characteristics																
		EC (ds/m)	TDS (mg/L)	Acidity	CO_3^{2-} (meq/L)	HCO_3^- (meq/L)	Cl^- (meq/L)	SO_4^{2-} (meq/L)	Anions	Ca^{2+} (meq/L)	Mg^{2+} (meq/L)	Na^+ (meq/L)	K^+ (meq/L)	Cations	SSP (%)	SAR	Total Hardness (ppm)	Alkalinity (ppm)
1	11653-26318-1	785	502	7.58	0	4.5	0.9	2.5	7.9	4	3.5	0.8	0.04	8.34	9	0.41	375	225

Abbreviations: EC: Electrical conductivity; TDS: Total soluble salts; CO_3^{2-}: Carbonate; HCO_3: Bicarbonate; Cl^-: Chloride; SO_4^{2-}: Sulfate; Ca^{2+}: Calcium; Mg^{2+}: Magnesium; Na^+: Sodium; K^+: Potassium; SSP: Soluble sodium percent; SAR: Sodium adsorption ratio.

Table 4. Pearson correlation between some of the traits measured in the 1st year (n=32)

Traits	1	2	3	4	5	6	7	8	9	10
1. Height	1									
2. Diameter	0.369*	1								
3. Fresh weight	0.591**	0.221	1							
4. Dry weight	0.657**	0.117	0.815**	1						
5. Dry matter content	0.256	-0.032	0.012	0.485**	1					
6. Chlorophyll a	-0.220	0.276	-0.363*	-0.295	-0.102	1				
7. Chlorophyll b	-0.442*	-0.020	-0.589**	-0.509**	-0.136	0.809**	1			
8. Total chlorophyll	-0.310	0.183	-0.461**	-0.385*	-0.119	0.977**	0.915**	1		
9. Saponin	-0.224	0.118	-0.230	-0.026	0.212	0.774**	0.739**	0.797**	1	
10. Hecogenine	0.194	-0.140	0.494**	0.478**	0.157	-0.135	-0.241	-0.179	0.225	1

*P<0.05, **P<0.01.

Table 5. Results of ANOVA indicating the effects of treatments on some morphological features of the plant (1st year)

Sources of Changes	df	Mean of Squares				
		Height	Diameter	Fresh Weight	Dry Weight	Dry Matter Content
Treatment	7	296.40**	0.01**	2092.9**	31.71**	12.25**
Error	24	47.31	0.002	69.84	0.66	1.79

*P<0.05, **P<0.01.

Table 6. Results of ANOVA indicating the effects of treatments on chlorophyll, saponin and hecogenin (1st year)

Sources of Changes	df	Mean of Squares				
		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Saponin	Hecogenine
Treatment	7	0.0054**	0.0013**	0.0115**	88692756**	75.36**
Error	24	0.00005	0.00007	0.00006	578366	3.58

*P<0.05, **P<0.01.

Chlorophyll content

The chlorophyll content (chlorophyll a and b) was significantly correlated with saponin levels (P<0.01). A positive correlation was observed between chlorophyll a and total chlorophyll (P<0.01), suggesting that photosynthetic activity may influence the synthesis of bioactive compounds in *A. marginata* (Table 7).

Correlation results

In the second year, a positive correlation was observed between plant height and diameter and between fresh and dry weight, indicating the complementary effects of nutrient treatments on plant morphology and biomass (Table 7).

- **Negative correlations:** A negative correlation was found between plant diameter and dry matter content

Table 7. Pearson correlation between some of the traits measured in the 2nd year

Traits	1	2	3	4	5	6	7	8	9	10
1. Height	1									
2. Diameter	0.395*	1								
3. Fresh weight	0.432*	0.456**	1							
4. Dry weight	0.365*	0.275	0.904**	1						
5. Dry mater content	-0.109	-0.410*	-0.307	0.110	1					
6. Chlorophyll a	-0.275	-0.105	0.051	0.049	-0.005	1				
7. Chlorophyll b	-0.403*	-0.261	-0.018	0.020	0.100	0.917**	1			
8. Total chlorophyll	-0.329	-0.166	0.026	0.039	0.034	0.989**	0.967**	1		
9. Saponin	-0.359*	-0.106	0.192	0.219	-0.030	0.204	0.261	0.230	1	
10. Hecogenine	-0.062	0.040	-0.076	-0.035	0.100	-0.124	-0.182	-0.149	0.245	1

$^{*}P<0.05$, $^{**}P<0.01$.

($P<0.01$), as well as between plant height and chlorophyll b and saponin contents ($P<0.05$).

Positive correlations: A significant positive correlation was observed between fresh and dry weight ($P<0.05$), as well as between chlorophyll a and total chlorophyll content ($P<0.01$) (Table 7), supporting the hypothesis that nutrient treatments promote both biomass and phytochemical yield. This correlation emphasizes the phytochemical yield and raises concerns about the potential amplification of toxic components, such as elevated saponin levels, which warrant toxicological scrutiny.

Discussion

Our results demonstrate that while nutrient management significantly increases the concentrations of bioactive compounds, this chemical manipulation also raises crucial considerations regarding the toxicological potential of these compounds, especially as their therapeutic efficacy increases. Therefore, comprehensive toxicological evaluations are essential to ensure that chemical enhancements do not compromise safety. This study confirms that applying macronutrients and micronutrients, specifically growmore fertilizer, iron, and zinc, can significantly enhance the production of secondary metabolites, such as saponins and hecogenin in *A. marginata* L. These bioactive compounds are crucial for their therapeutic potential, yet their increased concentrations necessitate a toxicological risk evaluation. The results of this study are consistent with international research. For example, studies from Mexico demonstrated that sulfuric acid treatment of Agave leaves for bioethanol production significantly increased yields of bioactive compounds, similar to the increase in saponin content observed in this study [18]. However, unlike the sulfuric acid treatments used in Mexican studies for industrial applications like bioethanol production, our study focused on applying micronutrients, a more sustainable and less invasive method to enhance the plant's medicinal value by boosting bioactive compound production. Likewise, research from Spain on fiber extraction using hydrochloric acid supports the importance of optimizing nutrient treatments to enhance phytochemical output while minimizing toxicological risks [19]. While both studies emphasize the importance of maximizing phytochemical production, our approach leverages nutrient management rather than chemical extraction, aligning better with environmentally sustainable practices and targeting direct medicinal applications. These comparisons demonstrate nutrient management strategies' broader applicability to improve species yield and medicinal value [19, 20].

Therapeutic potential of nutrient management

The application of micronutrients, such as iron and zinc, combined with growmore fertilizer, significantly improved the phytochemical profile of *A. marginata* L. in this study. The growmore fertilizer, iron, and zinc combination were chosen based on their complementary roles in plant metabolism. Growmore provides balanced macronutrients (NPK) essential for overall plant health, while iron and zinc serve as critical micronutrients for enzyme activation, photosynthesis, and secondary metabolite production. Iron is vital for chlorophyll synthesis, ensuring optimal photosynthetic efficiency, while zinc contributes to enzyme regulation and stress tolerance. This combination was expected to enhance overall plant growth and significantly increase the production of bioactive compounds, such as saponins and hecogenin. As demonstrated in similar studies, the increase in saponin levels suggests potential therapeutic applications in conditions, such as hyperlipidemia, inflammation, and wound healing [14]. However, it is critical to balance the efficacy of these bioactive compounds with their toxicological risks. For instance, elevated saponin levels are associated with hemolytic activity and gastrointestinal irritation, particularly at higher concentrations. Establishing a therapeutic window where saponin concentrations are effective yet safe is essential for clinical use. The dual role of saponins and hecogenin as both therapeutic agents and potential toxins underscores the need for further toxicological studies to establish safe dosage levels for pharmaceutical applications [21].

Nutrient optimization and toxicological risks

This study demonstrates the significant impact of nutrient management on phytochemical yield, particularly for saponin and hecogenin content. This study advances current knowledge by demonstrating the effectiveness of a more sustainable nutrient management approach compared to traditional chemical treatments. By focusing on the targeted application of micronutrients like iron and zinc, we provide a scalable and environmentally friendly method for enhancing the phytochemical profile of *A. marginata* L. This method maximizes bioactive compound production and minimizes the need for invasive chemical treatments, which can degrade soil health and negatively impact long-term agricultural sustainability. This study evaluated the effects of individual and combined nutrient applications to identify which intervention produced the most significant improvements in phytochemical yield. Treatments were tested with varying combinations of growmore, iron, and zinc to assess their contributions and potential synergies in enhancing plant growth and bioactive compound production. It was hypothesized that combining all three nutrients would yield the highest concentrations of saponins and hecogenin due to their roles in primary and secondary metabolic processes. Previous studies have shown that foliar application of micronutrient fertilizers enhances secondary metabolite production in medicinal plants, which is consistent with our results on increased saponin and hecogenin concentrations in Agavespecies [14]. However, the toxicological risks of elevated saponin and hecogenin levels are significant. Studies indicate that compounds, such as hecogenin, found in high quantities in Agave species, can induce DNA damage, though this may be reversible through cellular repair mechanisms . Additionally, high levels of saponins cause hemolysis and gastrointestinal disturbances if not properly managed [22].

Further studies indicate that hecogenin, found abundantly in Agave species, exhibits cytotoxic effects, particularly at higher concentrations, which may induce DNA damage in cells. However, this damage appears reversible under certain conditions. Hecogenin has been widely studied for its pharmacological benefits, including anti-inflammatory, anticancer, and antiulcerogenic activities. However, its mechanism of action and potential toxicological effects at varying doses require further investigation, especially in pharmaceutical applications [23].

In particular, studies on steroidal saponins from Agave species have shown that while they offer significant anti-inflammatory and immunoregulatory properties, they can also induce cytotoxic effects on cancer cells, underscoring the need for precise dosage control.

Incorporating toxicological assessments from the initial stages of pharmaceutical development is crucial to ensure that enhancing phytochemical content does not inadvertently increase toxicity [23]. As demonstrated in various studies, incorporating toxicological assessments during early pharmaceutical development stages can help prevent potential safety risks associated with elevated saponin concentrations, such as hemolysis and gastrointestinal irritation [22]. For instance, saponins have shown promise in reducing blood lipids and anti-inflammatory effects, but their hemolytic activity can pose significant risks when used inappropriately. Therefore, comprehensive toxicological assessments should accompany any therapeutic exploration, particularly as the use of *A. marginata* L. in pharmaceutical contexts grows [12, 13, 17, 24, 25].

Correlation of growth and phytochemical content

This study's positive correlation between chlorophyll content and saponin levels suggests that photosynthetic activity is critical in enhancing bioactive compounds' production. It was expected that treatments with higher levels of iron would lead to increased chlorophyll content, correlating with enhanced photosynthesis and greater saponin production. Similarly, zinc was anticipated to boost the plant's stress response and metabolic efficiency, resulting in improved phytochemical output.

As a balanced source of NPK, growmore fertilizer was predicted to promote overall plant growth and health, indirectly supporting the biosynthesis of secondary metabolites like saponins and hecogenin. This result is consistent with studies showing how micronutrient interventions, such as the application of iron and zinc, enhance photosynthetic efficiency and secondary metabolite production in plants [14]. However, unlike previous studies that relied on more invasive treatments, such as acid extractions, our nutrient management approach supports optimal plant growth and phytochemical yield more sustainably, which is critical for pharmaceutical applications.

This correlation highlights the need for precision in nutrient management strategies that support optimal plant growth and maximize phytochemical yields. These results are significant for the development of *A. marginata* L. as a sustainable source of bioactive compounds for pharmaceutical purposes, especially in regions with arid climates [12, 13, 22, 26].

Further precision in nutrient management ensures that strategies optimize plant growth and enhance phytochemical yields. These efforts are particularly significant for developing *A. marginata* L. as a viable source of bioactive compounds in pharmaceutical applications, especially in regions with arid climates [22, 24, 26, 27, 28-31].

Future research directions

Further research is needed to elucidate the molecular mechanisms involved in the biosynthesis of saponins and hecogenin in *A. marginata* L. Such studies provide deeper insights into optimizing cultivation practices to maximize therapeutic efficacy and safety. In addition, future investigations should focus on more comprehensive toxicological assessments to ensure that these bioactive compounds can be used safely in therapeutic contexts.

Based on these results, we recommend incorporating precision nutrient management strategies in the large-scale cultivation of *A. marginata* L. Specifically, growmore, iron, and zinc should be fine-tuned to achieve the desired balance between maximizing phytochemical production and maintaining plant health. By implementing this targeted approach, cultivators can enhance Agave's medicinal value while contributing to sustainable agricultural practices, particularly in arid regions where nutrient optimization is crucial.

Also, it is required to develop sustainable agricultural practices, such as precision irrigation and nutrient management systems, to support the large-scale cultivation of *A. marginata* L. in arid regions. These efforts will enhance the plant's medicinal and commercial potential while contributing to environmental conservation and sustainable agriculture. In parallel, future research should prioritize toxicological assessments of key bioactive compounds like saponins and hecogenin to establish safe dosage ranges for pharmaceutical applications.

Conclusion

This study highlights the significant role of nutrient management in enhancing the growth and phytochemical profile of *A. marginata* L., particularly increasing saponin and hecogenin content. While these compounds offer therapeutic potential, their elevated levels require careful toxicological evaluation to ensure safe use.

To advance the field, future research should focus on in vitro and in vivo studies to determine safe dosage levels and pharmacokinetic assessments to evaluate long-term safety. Additionally, molecular techniques, such as CRISPR-Cas9 can further elucidate the biosynthetic pathways of these compounds, enabling more targeted production strategies.

Optimizing nutrient interventions, such as precise fertigation and micronutrient applications, can enhance plant health and bioactive yields, especially in arid regions. Sustainable practices, combined with ongoing collaboration between researchers and policymakers, will help unlock the full medicinal and commercial potential of *A. marginata* L.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

The research was extracted from doctoral dissertation under a cooperation agreement with the Department of Food, Science, and Technology at the School of Agriculture, Shiraz University.

Authors' contributions

Designing the study: Ali Mohammad Rahimi, Mehrab Yadegari, and Mohammad Moghaddam; Writing the draft and revising the manuscript: Ali Mohammad Rahimi, Mehrab Yadegari, and Mohammad Moghaddam; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

References

[1] Rezaei M, Javadmoosavi SY, Mansouri B, Azadi NA, Mehrpour O, Nakhaee S. Thyroid dysfunction: How concentration of toxic and essential elements contribute to risk of hypothyroidism, hyperthyroidism, and thyroid cancer. Environmental Science and Pollution Research. 2019; 26(35):35787-96. [DOI:10.1007/s11356-019-06632-7] [PMID]

[2] Karrari P, Mehrpour O, Balali-Mood M. Iranian Crystal: A misunderstanding of the crystal-meth. Journal of Research in Medical Sciences. 2012; 17(2):203-4. [PMID]

[3] Mehrpour O, Keyler D, Shadnia S. Comment on Aluminum and zinc phosphide poisoning. Clinical Toxicology. 2009; 47(8):838-9. [DOI:10.1080/15563650903203684] [PMID]

[4] Hemmati M, Zohoori E, Mehrpour O, Karamian M, Asghari S, Zarban A, et al. Anti-atherogenic potential of jujube, saffron and barberry: anti-diabetic and antioxidant actions. EXCLI Journal. 2015; 14:908-15. [PMID]

[5] Mehrpour O, Amouzeshi A, Dadpour B, Oghabian Z, Zamani N, Amini S, et al. Successful treatment of cardiogenic shock with an intraaortic balloon pump following aluminium phosphide poisoning. Archives of Industrial Hygiene and Toxicology. 2014; 65(1):121-6. [DOI:10.2478/10004-1254-65-2014-2393] [PMID]

[6] Nakhaee S, Amirabadizadeh A, Brent J, Mehrpour O. Impact of chronic lead exposure on liver and kidney function and haematologic parameters. Basic & Clinical Pharmacology & Toxicology. 2019; 124(5):621-8. [DOI:10.1111/bcpt.13179] [PMID]

[7] Omidbeigi R. [Production and processing of medicinal plants (Persian)]. Mashhad: Astan Quds Pub; 2005. [Link]

[8] Mortezaeinejad F. [An introduction to phytohormone (Persian)]. Isfahan: Islamic Azad University of Isfahan (Khorasgan branch) ; 2014. [Link]

[9] Mozaffarian V. [Identification of Medicinal and Aromatic Plants of Iran (Persian)]. Tehran: Farhang Moaser Pub; 2012. [Link]

[10] Brickell C, Cathey HM. The American Horticultural Society AZ encyclopedia of garden plants. New York: DK Pub; 2004. [Link]

[11] Ahmadzadeh M, Rostami AK. [Aloe Vera (Persian)]. Tehran: Ayizh Pub; 2016. [Link]

[12] Yadegari M. Effects of Zn, Fe, Mn and Cu foliar application on essential oils and morpho-physiological traits of lemon balm (Melissa officinalis L.). Health & Environmental Research Online (HERO). 2017; 20(2):485-95. [Link]

[13] Yadegari, M. Irrigation periods and Fe, Zn foliar application on agronomic characters of Borago officinalis, Calendula officinalis, Thymus vulgaris and Alyssum desertorum. Communications in Soil Science and Plant Analysis. 2017; 48(3):307-15. [DOI:10.1080/00103624.2016.1269796]

[14] Puente-Garza CA, García-Lara S, Gutiérrez-Uribe JA. Enhancement of saponins and flavonols by micropropagation of Agave salmiana. Industrial Crops and Products. 2017; 105:225-30. [DOI:10.1016/j.indcrop.2017.05.014]

[15] Rout GR., Sahoo S. Role of iron in plant growth and metabolism. Review in Agricultural Science. 2015; 3:1-24. [Link]

[16] Khoshgoftarmanesh AH. [Advanced topics in plant nutrition (Persian)]. Isfahan: Isfahan University of Technology Publication Center; 2011. [Link]

[17] Marschner H. Mineral nutrition of higher plants. San Diego: Academic Press; 1995. [Link]

[18] Santos-Zea L, Rosas-Pérez AM, Leal-Díaz AM, Gutiérrez-Uribe JA. Variability in saponin content, cancer antiproliferative activity and physicochemical properties of concentrated agave sap. Journal of Food Science. 2016; 81(8):H2069-75. [DOI:10.1111/1750-3841.13376]

[19] Hernández-Hernández HM, Chanona-Pérez JJ, Vega A, Ligero P, Mendoza-Pérez JA, Calderón-Domínguez G, et al. Acetosolv treatment of fibers from waste agave leaves: Influence of process variables and microstructural study. Industrial Crops and Products. 2016; 86:163-72. [DOI:10.1016/j.indcrop.2016.03.043]

[20] Rijal D, Vancov T, McIntosh S, Ashwath N, Stanley GA. Process options for conversion of Agave tequilana leaves into bioethanol. Industrial Crops and Products. 2016; 84:263-72. [DOI:10.1016/j.indcrop.2016.02.011]

[21] Sosa-Herrera MG, Martínez-Padilla LP, Delgado-Reyes VA, Torres-Robledo A. Effect of agave fructans on bulk and surface properties of sodium caseinate in aqueous media. Food Hydrocolloids. 2016; 60:199-205. [DOI:10.1016/j.foodhyd.2016.03.033]

[22] Sidana J, Singh B, Sharma OP. Saponins of agave: Chemistry and bioactivity. Phytochemistry. 2016; 130:22-46. [DOI:10.1016/j.phytochem.2016.06.010] [PMID]

[23] Ingawale DK. Saponins and sapogenins of agave with respect to diverse pharmacological role of hecogenin. International Journal of Pharmacy and Pharmaceutical Sciences. 2020; 12(2):1-7. [Link]

[24] Yadegari M. Effects of manganese and copper on essential oil composition of lemon balm (Melissa Officinalis L.). Bangladesh Journal of Botany. 2016; 45(1):257-60. [Link]

[25] Ram V, Misra S, Upadhyay R. Effect of sulphur, zinc and biofertilizers on quality characteristics in mungbean. Indian Journal of Pulses Research. 2002; 15(2):139-41. [Link]

[26] Rubio-Piña J, Quiroz-Moreno A, Sánchez-Teyer LF. A quantitative PCR approach for determining the ribosomal DNA copy number in the genome of Agave tequila Weber. Electronic Journal of Biotechnology. 2016; 22:9-15. [DOI:10.1016/j.ejbt.2016.05.002]

[27] Amiri N, Yadegari M, Hamedi B. Essential oil composition of cirsium arvense L. produced in different climate and soil properties. Records of Natural Products. 2018; 12(3):251-62. [DOI:10.25135/rnp.27.17.06.043]

[28] Alimohammadi M, Yadegari M, Shirmardi HA. Effect of elevation and phenological stages on essential oil composition of Stachys. Turkish Journal of Biochemistry. 2017; 42(6):647-56. [DOI:10.1515/tjb-2016-0267]

[29] Reaisi Z, Yadegari M, Shirmardia HA. Effects of Phenological Stage and Elevation on Phytochemical Characteristics of Essential Oil of Teucrium polium L. and Teucrium orientale L. International Journal of Horticultural Science and Technology. 2019; 6(1):89-99. [DOI:10.22059/ijhst.2019.275327.276]

[30] Nasiri Y, Zehtab-Salmasi S, Nasrullahzadeh S, Najafi N, Ghassemi-Golezani K. Effects of foliar application of micronutrients (Fe and Zn) on flower yield and essential oil of chamomile (Matricaria chamomilla L.). Journal of Medicinal Plants Research. 2010; 4(17):1733-7. [Link]

[31] Said-Al Ahl H, Said-Al Ahl H, Mahmoud, AA. Effect of zinc and/or iron foliar application on growth and essential oil of sweet basil (Ocimum basilicum L.) under salt stress. Ozean Journal of Applied Sciences. 2010; 3(1):97-111. [Link]