Bionatura
International Journal of Biotechnology and Life Sciences
ISSN 1390-9355
Vol. 10 No. 2. 2025
https://revistabionatura.org/

Evaluación de la fitoquímica y el poder antimicrobiano del extracto obtenido de hojas de *Agave cocui* Trelease

Evaluation of the Phytochemistry and Antimicrobial Power of the Extract Obtained from Agave cocui Trelease Trelease

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Abstract

Agave cocui Trelease is an agavaceous plant widely distributed in arid and semi-arid regions of Venezuela. Its leaves are rich in fibers and secondary metabolites, which have been shown to participate in the plant's defense mechanisms and have a range of applications in medicine and agriculture. Due to the limited available information regarding the phytochemical behavior of this species, extracts obtained from leaf maceration were studied using solvents of different polarities, including ethanol, hexane, chloroform, and water. A phytochemical screening detected the presence of metabolites such as saponins, flavonoids, phenolic compounds, anthraquinones, leucoanthocyanins, coumarins, and tannins. The extracts were evaluated for antifungal and antibacterial activity, which were positive for the ethanolic, aqueous, and saponin extracts, as evidenced by the inhibition halos formed at extract concentrations of 0,125 mg/mL, 0,250 mg/mL, and 0,50 mg/mL. The obtained inhibition halos ranged between 10 ± 0,20 and 14,50 ± 0,50 mm for antifungal activity and between 8,23 ± 0,25 and 14,77 ± 0,25 mm for antibacterial activity. These halos allowed determination of the minimum inhibitory concentration (MIC) needed for both activities, with values ranging from 15,04 mg/mL to 28 mg/mL for antifungal activity and 33 mg/mL to 14,7 mg/mL for antibacterial activity. These results indicate that although the extracts exhibited a high MIC, they successfully inhibited the test microorganisms Fusarium solani and Escherichia coli.

Keywords: Agave cocui Trelease; metabolitos; fitoquímica, inhibition halo

Introduction

Since its origin, humans have maintained a close relationship with natural resources, including plants, which have been used not only for food and construction but also to heal and alleviate diseases. To date, around 50,000 plant species have been reported to have medicinal uses, representing approximately 10% of all known plant species [(1)]. Natural compounds derived from plants have been, and will continue to be, immensely important as sources of therapeutic agents and models for the design, semi-synthesis, and synthesis of various drugs for the treatment of human and animal diseases. With the growing interest in developing herbal-based medicines with fewer adverse effects, there are increasing opportunities to explore the therapeutic and other biological aspects of previously unexplored natural elements. [(2)]

Based on the aforementioned, phytochemistry emerges as a scientific discipline dedicated to the study of chemical compounds produced by plants, particularly those derived from secondary metabolism. These secondary metabolites fulfill essential ecological functions, such as defense against herbivores, attraction of pollinators, and adaptation to adverse environmental conditions. Additionally, many of these compounds possess pharmacological properties, which has driven their research in medicine and biotechnology [(3)]. The secondary metabolism of higher plants has enabled the synthesis of a vast diversity of compounds with therapeutic potential. These metabolites have been key in the development of drugs, antibiotics, insecticides, and herbicides, contributing significantly to biotechnology and modern medicine. Despite their importance, it is estimated that less than 10 % of plant species have been thoroughly studied to assess their biological activity. [(3)]

The resistance of phytopathogenic fungi to synthetic fungicides has driven the search for natural alternatives with antifungal activity. Various plant extracts have demonstrated efficacy against agricultural pathogens, both in in vitro assays and field applications, offering a sustainable option for disease management in crops [(4).]. Microbial resistance to conventional antibiotics has driven the search for new therapeutic alternatives. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have emphasized the importance of developing new antimicrobial products, highlighting that the plant kingdom offers a promising source of molecules with pharmacological potential. Phytochemical research has led to the identification of compounds with antibiotic and antifungal activity, contributing to the development of more effective and sustainable treatments. [(4)]

The Cocui agave (Agave cocui Trelease) is an agave species widely distributed in the arid, semi-arid, and rocky outcrop regions of Venezuela and Colombia [(5)], Its primary use is liquor production, for which only its core (piña) is utilized, while its leaves—rich in fibers and secondary metabolites—are discarded. (6). In the state of Falcón, specifically in Pecaya, Sucre municipality, the presence of *Agave cocui Trelease* is well known. Commonly referred to as "penca," it is primarily used for the production of

alcoholic beverages such as *pecayero* or *cocuy*, utilizing only the plant's core (*piña*), while its leaves—rich in fibers and secondary metabolites—are discarded.

Due to the limited available information on this plant, this research conducted a phytochemical study to identify its secondary metabolites and assess its antifungal and antimicrobial activity as a scientific contribution. Based on the above, the objective of this study was to evaluate the phytochemistry and antimicrobial potential of the crude ethanolic extract and its fractions obtained from *Agave cocui Trelease* leaves, providing an alternative for pharmaceutical and agricultural applications through the development of new drugs and insecticides, while promoting the use of an organic waste that is abundantly available in the study area.

Materials and methods

Collection and Treatment of Plant Material: Leaf samples from the plant species were collected from young plants that showed no apparent signs of disease or wilting. The leaves were dried in a circulating air oven at 50°C for 48 hours. The material was then processed using a blade sieve mill.

Extraction by Maceration: A total of 40 g of the processed material (leaves) was macerated at room temperature for 24 hours using ethanol, hexane, chloroform, and water as extraction solvents

Identification of Secondary Metabolites: A total of 25 mL of the filtrate obtained from maceration was taken to determine the main secondary metabolites present in each extract. The metabolites to be identified and the methods used are presented in Table 1.

Table 1. Methods for the Determination of Secondary Metabolites

Analysis	Method
Flavonoids	Shinoda Reaction
Saponins	Foam Presence
Alkaloids	Dragendorff and Meyer Reaction
Coumarins	Reaction with KI and NH_3
Tannins	Gelatin Reaction
Phenolic Compounds	$FeCl_3$ Reaction
Anthraquinones	Borntrager Reaction
Leucoanthocyanins	Rosenheim Reaction

Preparation of Saponin Extract

A total of 10 g of dried sample was mixed with 150 mL of water and heated on a hot plate for 1 hour at 320 °C. After resting, the mixture was filtered to obtain an initial extract. The remaining residue was then

treated with 100 mL of ethanol and boiled again at 300 °C for 1 hour. After resting, the solution was filtered into the same Erlenmeyer flask containing the first extract. The combined extracts were transferred to a beaker and placed in an oven at 70 °C until dried, yielding a crude saponin extract weighing 2 g/ml. This crude extract was then diluted in 100 mL of distilled water to obtain the final saponin extract, with a concentration of 2 g of saponins per 100 mL. [7]

Inoculum Preparation

Potato Dextrose Agar (PDA) culture medium was prepared following the methodology outlined in the Culture Media Manual (8). *Fusarium solani* fungal strains were obtained from the strain collection of the Laboratory for Research and Academic Support in Health Sciences (LIADSA - UNEFM). These strains were inoculated into test tubes containing PDA medium set at an inclined angle and incubated at 25 °C for 48 hours to facilitate growth.

Evaluation of Extracts for Antifungal Activity

The antifungal activity evaluation was conducted using the paper disc diffusion technique. Solutions of the obtained extracts were prepared in mg/L. Filter paper discs were impregnated with the different extracts for 24 hours before being used in the assay.

Microorganism Activation

Before conducting the antibacterial activity assay, the E. coli bacteria were activated to ensure proper growth in the corresponding agar medium. To achieve this, an inoculum of the microorganism—preserved in the LIADSA laboratory at room temperature—was transferred to 25 mL of sterile BHI broth. The solution was homogenized and kept in darkness under constant agitation for 24 hours.

Inoculum Preparation

Approximately 3 to 5 colonies were taken and inoculated into tubes containing nutrient broth. The mixture was vortexed until reaching a concentration of 1.5×10^8 cells/mL, corresponding to McFarland tube 0.5. Subsequently, Petri dishes containing Mueller-Hinton (MH) agar (Oxoid) were inoculated.

Evaluation of Extracts for Antibacterial Activity

The diffusion method was used, employing sterile cotton swabs. Additionally, 6 mm sterile qualitative Whatman paper discs were aseptically placed on Mueller-Hinton (MH) agar. These discs were impregnated with 10 µL of each extract, which had been previously dissolved in dimethyl sulfoxide (DMSO) to achieve a final concentration of 30 mg/mL.

Results

Identification of Secondary Metabolites in Different Solvents

Table 2 presents the identification of secondary metabolites found in Agave cocui. The ethanolic extract exhibited the presence of saponins, tannins, leucoanthocyanins, flavonoids, phenolic compounds, and anthraquinones. The aqueous extract contained the same metabolites as the ethanolic extract, except for leucoanthocyanins, which were present in lower quantities. In organic solvents such as hexane, only tannins and coumarins were detected, while the chloroform extract showed a low presence of coumarins.

Table 2. Secondary Metabolites Present in Agave Cocui Trelease Extracts

Secondary Metabolites	Ethanolic Extract	Hexanoic Extract	Chloroform Extract	Aqueous Extract
Saponins	+++	-	-	+++
Tannins	+	++	-	++
Leucoanthocyanins	++	-	-	-
Alkaloids	-	-	-	-
Flavonoids	+++	-	-	+
Phenolic Compounds	+++	-	-	++
Anthraquinones	+++	-	-	+
Coumarins	-	+	+	-

+Low presence. ++ Moderate presence. +++ Abundant presence. – No presence.

Evaluation of the Antifungal Activity of the Extract

The antifungal activity of each extract obtained from the studied plant species was determined. The results of the antifungal activity are presented in Table 3. The evaluated extracts demonstrated effectiveness in inhibiting the growth of the Fusarium solani strain. The ethanolic extract exhibited antifungal activity at concentrations of 0,125 mg/mL, 0,250 mg/mL, and 0,5 mg/mL, as did the aqueous extract, due to their high content of secondary metabolites.

Table 3. Antifungal Activity of Extracts Obtained from the Plant Species

Extractos	Concentration of Extracts Used (mg/mL) for *Fusarium solani*			
	0.0 (control)	**0.125**	**0.250**	**0.5**

Ethanolic	-	+	+	+
Hexanoic	-	-	-	-
Chloroformic	-	-	-	-
Aqueous	-	+	+	+
Saponinic	-	-	+	+

Exhibits activity. - Does not exhibit activity

The chloroformic and hexanoic extracts did not exhibit antifungal activity at any of the evaluated concentrations, which is attributed to the absence of secondary metabolites. The hexanoic extract contained tannins and coumarins, while the chloroformic extract showed only a small quantity of coumarins, insufficient for inhibition. This limited presence is likely due to the low polarity of the solvents used. On the other hand, the saponinic extract demonstrated antifungal activity at higher concentrations (0,250 mg/mL and 0,5 mg/mL), as saponins are secondary metabolites known to act as protective or inductive agents.

Table 4 illustrates how Fusarium solani responded to the evaluated extracts, with the ethanolic extract proving the most effective across all concentrations. This is explained by the high content of secondary metabolites detected in Agave cocui Trelease. The ethanolic and aqueous extracts contained at least five secondary metabolites: tannins, flavonoids, phenolic compounds, anthraquinones, and leucoanthocyanins, in addition to saponins. In contrast, the saponinic extract contained only saponins, suggesting that the presence of this metabolite directly influenced the antifungal activity observed in the different extracts. Furthermore, the saponin concentration obtained was particularly relevant, as it reinforced the notion that the antifungal activity of the ethanolic and aqueous extracts is largely due to their high saponin content. Once the antifungal activity was evaluated, it was determined that only the extracts with the highest presence of secondary metabolites exhibited inhibition halos, confirming their effectiveness.

Table 4. Inhibition Halos Reported in the Evaluation of Antifungal Activity

Extracts	Inhibition Halos Obtained (mm)					
	0.125	**DE**	**0.250**	**DE**	**0.5**	**DE**
Ethanolic	**10,43**	**±0,40**	**12,83**	**±0,73**	**14,50**	**±0,50**
Aqueou	**11,10**	**±0,36**	**11,77**	**±0,25**	**13,83**	**±0,35**
Saponinic	**-**	**-**	**10**	**±0,20**	**12,10**	**±0,36**

presents the inhibition halos observed in the antifungal activity of the extracts at each evaluated concentration. The largest halo measured 14,50 ± 0,50 mm at a concentration of 0,50 mg/mL, while the smallest halo was 10 ± 0,20 mm at 0,250 mg/mL. Comparing the antifungal activity of the three different extracts of Agave cocui Trelease at the three concentrations, the ethanolic extract exhibited the highest activity, with inhibition values of 10,43 ± 0,40 mm, 12,83 ± 0,73 mm, and 14,50 ± 0,50 mm. These values were consistently higher across all concentrations when compared to the aqueous and saponinic extracts.

Antimicrobial Activity

"Secondary metabolites in plants play a crucial role in their adaptation and defense mechanisms. Additionally, they exhibit extensive chemical diversity, making them valuable for applications in the pharmaceutical, cosmetic, and agricultural industries."(9), The antibacterial activity of each extract obtained from the studied plant species was determined. The results are presented in Table 5.

Table 5. Antibacterial Activity of Extracts Obtained from the Plant Species

Extractos	Concentration of Extracts Used (mg/mL) for E. coli			
	0.0 (control)	**0.125**	**0.250**	**0.5**
Ethanolic	**-**	+	+	+
Hexanoic	**-**	-	-	-
Chloroformic	**-**	-	-	-
Aqueous	**-**	-	+	+
Saponinic	**-**	-	+	+

+ Exhibits activity. - Does not exhibit activity

Table 5 presents the antibacterial activity of the evaluated extracts. Among the five studied extracts, three exhibited activity. The ethanolic extract demonstrated antibacterial effectiveness across all evaluated concentrations. Similar to the antifungal activity, the hexanoic and chloroformic extracts did not show antibacterial activity at any tested concentration, which is attributed to the absence of secondary metabolites.

Regarding the aqueous and saponinic extracts, antibacterial activity was observed at concentrations higher than 0,125 mg/ml. The aqueous extract exhibited this activity due to its significant content of secondary metabolites, including saponins, tannins, phenolic compounds, anthraquinones, and flavonoids. Meanwhile, the saponinic extract demonstrated effectiveness, likely due to the versatile bioactivities of saponins.

Table 6 presents the inhibition halos obtained from the extracts that exhibited antibacterial activity against E. coli, ranging from 8,23 ± 0,25 mm to 14,77 ± 0,25 mm. Comparing the antibacterial activity of the three extracts of Agave cocui Trelease at the three evaluated concentrations, the ethanolic extract showed the highest activity, with inhibition values of 10,43 ± 0,40 mm, 12,37 ± 0,46 mm, and 14,77 ± 0,25 mm. In contrast, the aqueous and saponinic extracts exhibited activity at only two of the three concentrations. The aqueous extract recorded inhibition values of 10,77 ± 0,25 mm and 13,43 ± 0,40 mm, while the saponinic extract showed halos of 8,23 ± 0,25 mm and 11,77 ± 0,25 mm.

Table 6. Inhibition Halos Reported in the Evaluation of Antibacterial Activity

Extracts	Inhibition Halos Obtained (mm)					
	0,125	**DE**	**0,250**	**DE**	**0,125**	**DE**
Ethanolic	**10,43**	**±0,40**	**12,37**	**±0,46**	**14,77**	**±0,25**
Aqueou	**-**	**-**	**10,77**	**±0,25**	**13,43**	**±0,40**
Saponinic	**-**	**-**	**8,23**	**±0,25**	**11,77**	**±0,25**

At the conclusion of the antibacterial activity evaluation, it was observed that only the extracts with the highest secondary metabolite content were effective in inhibiting *E. coli*. In these extracts, as well as in the saponin concentrate, inhibition halos formed, confirming their positive antibacterial activity. Similar to the antifungal activity results, the saponin concentrate yielded significant findings. It indicated that the antibacterial activity observed in the other two active extracts is directly related to their saponin content.

Tabla 7. CMI de las actividades evaluadas.

Extracts	Minimum Inhibitory Concentration (MIC) in mg/mL. Antifungal Activity					
	0,125	**MIC**	**0,250**	**MIC**	**0,5**	**MIC**
Ethanolic.	**10,43**	**26,8**	**12,83**	**20,1**	**14,50**	**15,04**
Aqueou	**11,10**	**25**	**11,77**	**23**	**13,83**	**17,3**
Saponinic	**-**	**-**	**10**	**28**	**12,10**	**22,1**
Extracts	Minimum Inhibitory Concentration (MIC) in mg/mL Antibacterial Activity					
	0,125	**MIC**	**0,250**	**MIC**	**0,5**	**MIC**

Ethanolic	**10,43**	**26,8**	**12,37**	**21,4**	**14,77**	**14,7**
Aqueou	**-**	**-**	**10,77**	**25,8**	**13,43**	**18,4**
Saponinic	**-**	**-**	**8,23**	**33**	**11,77**	**23**

Table 7 presents the minimum inhibitory concentrations (MICs), which were determined based on the inhibition halos obtained for each extract at different concentrations. The data show that the larger the inhibition halo, the lower the concentration required to inhibit microorganisms (*Fusarium solani* and *E. coli*). Additionally, the ethanolic extract exhibited the lowest MIC for both antifungal and antibacterial activity, a result attributed to its high secondary metabolite content.

Discussion

The study on Agave cocui Trelease revealed that the ethanolic and aqueous extracts are the richest in secondary metabolites, including saponins, flavonoids, phenolic compounds, and anthraquinones. This indicates that the extract is soluble in solvents of medium polarity, such as ethanol. The presence of secondary metabolites in Agave cocui Trelease aligns with reports on other Agave species, such as Agave americana and Agave angustifolia, where the presence of flavonoids, terpenes, saponins, tannins, cardiotonic glucosides, and steroids has been documented in alcoholic extracts. [(10)]

The absence of metabolites such as saponins in extracts like chloroform and water is due to their higher solubility in polar solvents, such as water and ethanol, which is a direct result of their chemical structure. These molecules contain both a hydrophilic (polar) region and a hydrophobic (non-polar) region, allowing them to interact with polar solvents through hydrogen bonds and dipole-dipole forces [(11)]. The presence of coumarins in solvents such as chloroform and hexane is due to their limited solubility in polar solvents like water, where their solubility is approximately 0,02 %. However, they are easily soluble in alcohols (such as methanol) and other organic solvents like chloroform, ether, and oils. [(12)]

The ethanolic and aqueous extracts were effective against Fusarium solani, whereas the hexanoic and chloroformic extracts showed no activity, which is attributed to the absence of secondary metabolites with antimicrobial properties. This finding aligns with previous reports on Agave scabra, where aqueous and ethanolic extracts inhibited the growth of Fusarium sp. by 16,6 % and 15 %, respectively [(13)]. After evaluating the antifungal activity of the studied extracts, it was determined that only those with the

highest presence of secondary metabolites exhibited inhibition halos, confirming their antifungal effectiveness

The inhibition halos for the antifungal activity of Agave cocui extracts ranged from 8,23 mm to 14,77 mm. These values are lower than those reported for Agave scabra Salm Dyck, which exhibited inhibition halos ranging from 10,3 mm to 17,3 mm [(14)]. The minimum inhibitory concentrations (MICs) for Agave cocui extracts ranged from 15,04 mg/mL to 28 mg/mL against Fusarium solani. These values were higher than those reported for Agave aspérrima extracts, which successfully inhibited fungal growth. Among Agave aspérrima extracts, methanolic extracts from leaves and flowers exhibited the highest activity, with MICs between 0,95 ± 0,37 mg/mL and 1 ± 0,5 mg/mL. In contrast, extracts from A. americana, A. lechuguilla, and A. tequilana did not demonstrate fungal growth inhibition. [(15)]

Similarly, in the antibacterial activity evaluation, the ethanolic, aqueous, and saponinic extracts inhibited the growth of Escherichia coli, whereas the hexanoic and chloroformic extracts showed no effect. The efficacy of the ethanolic extract is attributed to the presence of flavonoids and phenolic compounds, which influence the metabolic pathways of E. coli, contributing to its inhibition ([16]). The aqueous, ethanolic, and saponinic extracts of Agave cocui exhibited antibacterial activity against the Gram-negative bacterium E. coli. This finding is consistent with studies on aqueous and methanolic extracts from Agave sisalana leaves, which have demonstrated similar effects. Likewise, this aligns with research on Agave attenuata Salm., where leaf extracts obtained using different solvents exhibited antimicrobial activity against B. subtilis, P. multocida, Aspergillus niger, A. flavus, S. aureus, and *E. coli*. [(17)]

The saponin concentrate obtained was of great importance, as it demonstrated that a significant portion of the activity observed in the ethanolic and aqueous extracts is due to their high saponin content. This saponinic extract is of considerable interest, given that saponins have exhibited various biological activities, including antimicrobial and cytotoxic effects. This is attributed to their structural composition, where a sugar chain attached to carbon-3 enables them to form complexes with sterols in cell membranes, resulting in the creation of large pores and ultimately causing cell lysis. [(18)]

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The analysis revealed inhibition halos ranging from 8,23 mm to 14,77 mm. These values are lower than those reported for Agave americana, which exhibited halos in the range of 19 mm to 23 mm for methanolic extracts [19]. The ethanolic extract exhibited the highest effectiveness across all evaluated concentrations, which is attributed to its high concentration of flavonoids and phenolic compounds. The aqueous and saponinic extracts also demonstrated activity, although to a lesser extent. The saponinic extract's effectiveness is influenced by the ability of saponins to alter cell membrane permeability.

The MICs obtained for the three extracts of Agave cocui were lower than those observed for the hydroalcoholic extract of Agave angustifolia against Escherichia coli and Staphylococcus epidermidis, which reported a MIC of 60 mg/ml^{-1} [20]. However, this differs from reports on Agave spp., where the extract showed no activity against Escherichia coli. This variation may be attributed to differences in the phytochemical composition of both species [10], however, similar studies conducted on A. americana L. observed higher activity of leaf extracts against the Gram-negative bacteria E. coli and K. pneumoniae compared to their inhibitory effect on S. aureus. *(21)*

Conclusions

The study on Agave cocui Trelease highlights the richness of its ethanolic and aqueous extracts in key secondary metabolites, such as saponins, flavonoids, and phenolic compounds. The differential solubility of these metabolites in various solvents confirms patterns observed in other Agave species, reinforcing their bioactive potential. In terms of antimicrobial activity, both ethanolic and aqueous extracts demonstrated antifungal and antibacterial efficacy, with flavonoids and saponins playing an essential role in inhibiting Fusarium solani and Escherichia coli. Comparisons with other Agave species emphasize variations in potency and spectrum of action, which can be attributed to phytochemical differences.

Furthermore, the saponin concentrate obtained provides significant contributions to antimicrobial activity, as its mechanism of action involves interactions with cellular membranes. The formation of inhibition halos and the determination of minimum inhibitory concentrations (MICs) reflect the effectiveness of the extracts against selected pathogens, with results that are comparable and, in some cases, lower than those reported for other Agave species. This study reinforces the importance of Agave cocui Trelease as a potential source of bioactive compounds with antimicrobial applications, supporting future research aimed at optimizing its biotechnological and health-related uses

Author Contributions: Conceptualization, Jose Ojeda and; methodology, Indira Garcia; formal analysis, Maria Moreno; investigation, Martin Rodriguez.; resources, Osman Castro.; data curation, Indira Garcia.; writing—original draft preparation, Jose Ojeda; writing—review and editing, Maria

Moreno; visualization, Martin Rodriguez.; supervision, Osman Castro. All authors have read and agreed to the published version of the manuscript."

Funding: This research did not receive external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

Acknowledgments: Research and Teaching Support Laboratory for Health Sciences (LIADSA) at the National Experimental University “Francisco de Miranda.”

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|Received: [18 marzo 2024] |Accepted: [24 mayo 2025] Published: [15 junio 2025] |
Citation: Ojeda, J ; Moreno, M; Gracias, I; Rodríguez, M; Castro, O. Evaluación de la fitoquímica y el poder antimicrobiano del extracto obtenido de hojas de Agave cocui Trelease. Bionatura, 2025. Volumen 10, (No 2). 10.70373/RB/2025.10.02.1
Peer review information: Bionatura thanks the anonymous reviewers for their contribution to the peer review of this work using https://reviewerlocator.webofscience.com/