Evaluation of the antimicrobial properties of natural extracts of *Ganoderma lucidum*

Evaluación de las propiedades antimicrobianas de extractos naturales de *Ganoderma lucidum*

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Keywords

Ethanol extract; ganoderic acid; triterpenoids; polysaccharides; bioactive compounds.

Abstract

There is a growing importance of alternative anticancer and anti-inflammatory treatments. Current interest lies in natural medicine and secondary metabolites present in plants, particularly focusing on triterpenes. *Ganoderma lucidum* is a fungal species of significant commercial interest due to its therapeutic and medicinal properties resulting from secondary metabolites such as polysaccharides and triterpenes. These compounds exhibit antitumor activity and bolster the immune system. The study aimed to assess the antimicrobial activity of *G. lucidum* extracts, both commercial and wild. The content of ganoderic acids in all extracts showed a slight difference in concentration between commercial and wild extracts. However, no bacterial inhibition halos were observed in any of the strains used. The presence of varying concentrations of ganoderic acids among treatments underscores the importance of optimizing and standardizing a comprehensive strategy for extracting secondary metabolites, focusing on producing high-quality supplements and pharmaceutical products. Furthermore, preserving the stability of the obtained triterpenes is necessary due to their importance in the medicinal properties of the fungus.

Palabras clave

Extracto etanólico; ácido ganodérico; triterpenoides; polisacáridos; compuestos bioactivos.

Resumen

Existe una creciente importancia de los tratamientos alternativos anticancerígenos y antiinflamatorios. El interés actual radica en la medicina natural y los metabolitos secundarios presentes en plantas, con un enfoque particular en los triterpenos. *Ganoderma lucidum* es una especie fúngica de gran interés comercial por sus propiedades terapéuticas y medicinales debido a la presencia de metabolitos secundarios como polisacáridos y triterpenos, que presentan actividad antimicrobiana. El objetivo del estudio fue la evaluación de la actividad antimicrobiana de extractos de *G. lucidum*, tanto comerciales como silvestres. El contenido de ácidos ganodéricos en todos los extractos tienen una leve diferencia en la concentración entre los extractos comerciales y silvestres. Sin embargo, no se obtuvieron halos de inhibición bacteriana en ninguna de las cepas utilizadas. La presencia de diferentes concentraciones de ácidos ganodéricos entre los tratamientos, resalta la importancia de optimizar y estandarizar una estrategia integral de extracción de metabolitos secundarios, con un enfoque dirigido hacia la producción de suplementos y productos farmacéuticos de alta calidad. Además, es necesario preservar la estabilidad de los triterpenos obtenidos, dada su importancia en las propiedades medicinales del hongo.

Introduction

The fungus *Ganoderma lucidum* (commonly known as Reishi) is naturally distributed in Central America, characterized by a basidiocarp that grows near tree trunks and is renowned in medicinal fields for treating diseases. Polysaccharides and triterpenes are their primary components with significant physiological activity [1]. There exists substantial potential as an antiviral agent due to its capacity to enhance immune defenses through ganoderic acids (GA), ganodermanondiol,

lucidumol, and ganodermanontriol. Commonly employed extraction methods include hot water extraction, ultrasonic bath, maceration, and solvent-based extraction [2]. Quantification of different metabolites is primarily conducted using HPTLC techniques [3].

The key secondary metabolites of *G. lucidum* are triterpenes, which belong to the subgroup of terpenes [4]. These exhibit antimicrobial activity by disrupting specific sites on the bacterial plasma membrane [5]. Therefore, this study aimed to evaluate the antimicrobial activity of both commercial and wild natural extracts of *G. lucidum,* in addition to obtaining *Ganoderma* profiles (GA) for their potential use in the pharmaceutical industry.

Materials and Methods

Collection of Fungi

The wild mushrooms were collected from the green areas of the Omar Dengo Campus of the National University, located in Heredia, Costa Rica (latitude 10°00 '02.08" N, longitude 84°06 '34.61" W) in September 2023. On the other hand, the commercial powdered extract was obtained from a Costa Rican distributor. The experiments were conducted at the Teaching Biotechnology Laboratory (LABID), the Phytochemistry Laboratory (LAFIT), and the Microbial Biotechnology Laboratory (LABIMI) of the same university.

Extraction Process

The fungus was lyophilized, ground, and sieved to obtain particles smaller than 1 mm [7]. On the other hand, for the extract preparation, 2 g of mushroom and 200 mg of the commercial sample were weighed and dissolved in 15 mL and 2 mL of 95% ethanol:water (7:3), respectively [3]. The wild extract was obtained through sonication in an ultrasonic bath (40 KHz) for 5 minutes. The supernatant obtained was passed through a 0.45 µm filter using a syringe. The process was carried out in triplicate until a volume of 15 mL was obtained. Subsequently, the *G. lucidum* extract was concentrated using the Büchi Rotavapor R-200 equipment with a water bath at 40°C [8]. Once the solvent was evaporated, the solids were weighed and dissolved in 95% ethanol and water at a 1:1 ratio to achieve a final concentration of 100 mg/mL for both extracts.

Determination of terpenes by HPTLC

The Camag Autosampler ATS4, Derivatizer, and CAMAG TLC Visualizer 3 equipment were employed. The selected mobile phase consisted of a mixture of dichloromethane:ethyl acetate:cyclohexane: formic acid:ethanol (8:3:9:0.8:0.5) [8]. Aluminum-backed plates with a stationary phase composed of Silica gel 60 F254, measuring 20 x 10 cm, were used in the procedure. For band visualization, the plates were exposed to wavelengths of 366 nm and 254 nm. Additionally, the following reference standards were incorporated: ganoderic acid AG-B, AG-D, and AG-F from the commercial house Sigma Aldrich, with the purpose of identifying the secondary metabolites of interest.

Antimicrobial assessment

The fungal extract was used to evaluate its antimicrobial inhibition capacity by forming inhibition zones. To achieve this, bacterial strains of *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris* were cultured with a concentration of 0.5 on the McFarland scale. These strains were transferred to test tubes containing 0.85% saline solution. Subsequently, 50 mL Falcon tubes were prepared with 25 mL of Muller-Hinton agar and 1 mL of each bacterial strain. After pouring the agar into Petri dishes, wells were created for the *Ganoderma* extracts and Streptomycin/ Penicillin (500 µg/mL) as a positive control, dispensing 50 µL in the wells. Finally, the Petri dishes

were incubated for 24 hours at 35°C for observation and measurement of the inhibition zones [5]. The collected data underwent statistical analysis using a mixed linear model with Poisson distribution, specifically designed to address the problem of inflated zero counts [6], and these were analyzed using the R Studio program (version 4.2.2).

Results

In Figure 1, the HPTLC chromatography for GA is shown, confirming the presence of terpenoids in all extracts obtained from *G. lucidum*. The band of GA from the wild ethanol extract (GA Wild *Ganoderma*) is observed with less intensity compared to the bands of the commercial extract. However, the concentrated sample of the wild extract (GA Wild *Ganoderma* 5:1) displays improved GA bands and a greater variety of compounds.

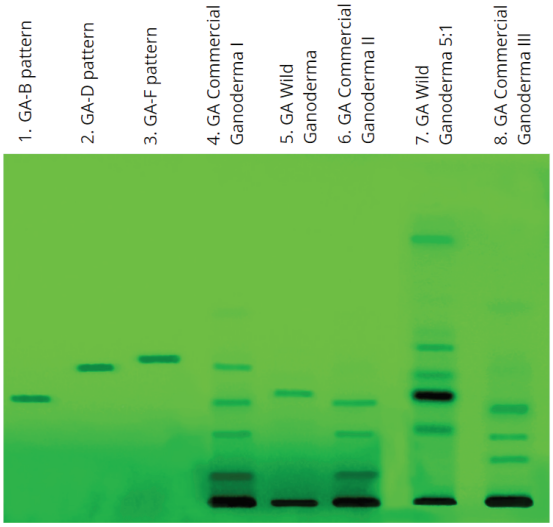

Figure 1. Profiles of ganoderic acid were analyzed from commercial and wild *G. lucidum* extracts using HPTLC. Band profile: Lane 1, GA-B standard. Lane 2, GA-D. Lane 3, GA-F. Lane 4, Commercial *Ganoderma* 1. Lane 5, Unconcentrated Wild *Ganoderma*. Lane 6, Duplicate Commercial *Ganoderma*. Lane 7, Concentrated Wild *Ganoderma*. Lane 8, Triplicate Commercial *Ganoderma*

In the evaluation of the antimicrobial effect, no bacterial inhibition halos were detected in any of the strains used for both extracts ($F:17.45$; $df:2$; $p<0.05$), despite the presence of ganoderic acids in the samples (see Figure 2).

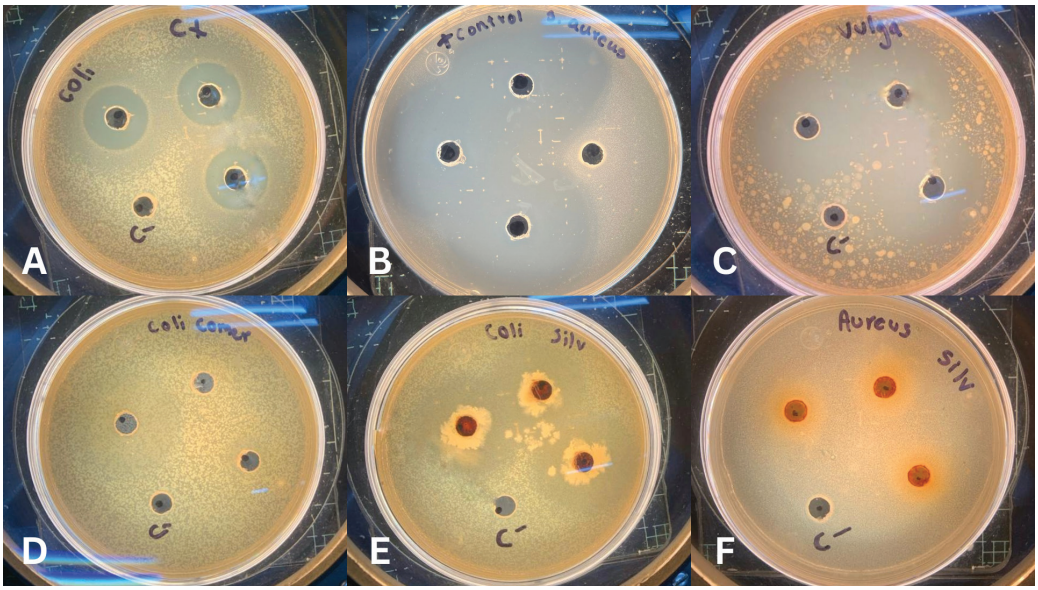

Figure 2. Antimicrobial tests of ethanolic extracts. Three positive controls with antibiotics are presented for three different strains: (A) *E. coli*, (B) *S. aureus*, and (C) *P. vulgaris*. In addition, plates with (D) commercial extract in *E. coli*, (E) wild extract in *E. coli* and (F) wild extract in *S. aureus* are shown, with a comparison to the positive controls

Discussion

In the chromatographic analysis, the absence of GA-F in the extracts indicates a lack of similarity with the reference pattern [9]. These differences are related to the generation of GA, specifically in the lanosterol biosynthesis process, which is influenced by structural changes through acetylation, methylation, and hydroxylation reactions [10]. On the other hand, the proper composition of GA is influenced by the substrate in which the fruiting body is found, due to the presence of fundamental enzymes that catalyze triterpene differentiation reactions, which are specific to certain substrates [11].

In the antibacterial tests conducted, the lack of bacterial inhibition could be related to the solvent used or the extraction time [7, 12]. Another potential extract type is the acetonic extracts, which have been reported to possess antimicrobial activity [13]. Additionally, the contents of antimicrobial compounds vary according to the culture conditions as well as the growth stage of *G. lucidum* [14]. In this case, to obtain high-quality extracts, it is recommended to employ specific culture media and adjust the culture conditions to maximize the yield of *G. lucidum* [15].

The stability of terpenes is of utmost importance due to their fundamental role in the medicinal properties of the fungus. The degradation of triterpenoids can compromise the quality and efficacy of the extracts. It is noteworthy that many triterpenes have demonstrated a wide range of biological activities, emphasizing the need to preserve their stability for application [16].

The need for optimization and standardization of bioactive extraction significantly impacts obtaining high-quality extracts and has the potential to drive the production of high-quality supplements and pharmaceutical products. This optimization is crucial for both public health benefits and the country's economic development [17]. Despite the recent disclosure of data related to the genome and transcriptome of *G. lucidum*, there are still areas of knowledge that need exploration, including its potential in the antimicrobial field [18].

Conclusion

There was no antimicrobial activity in both the commercial and wild extracts, as they did not reveal inhibitory effects on bacterial growth. It is suggested that factors such as time and the extraction solvent were not suitable for the specific growth conditions of the fungus. Finally, the optimization and standardization of extraction methods allow for producing high-quality extracts with potential applications in the pharmaceutical industry.

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