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Evaluation of garlic extracts antifungal potential against causative fungi of dermatomycosis in Chile

Evaluación del potencial antifúngico de extractos de ajos frente a hongos causantes de dermatomicosis en Chile

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Abstract

Background: Several studies on garlic have shown that due to its bioactive components, it exhibits antimicrobial properties. The most prevalent dermatomycoses are those caused by dermatophytes and yeasts of the genus *Candida*. In recent years, an increase in recurrent and recalcitrant cases has been reported due to strains of dermatophytes resistant to terbinafine, a considered first-line antifungal. **Objective:** The objective of this work was to determine the in-vitro antifungal effect of extracts from Chinese garlic (fresh variety) and *Chilote* garlic (fresh and black varieties) against the two primary fungi that cause superficial mycoses: *Trichophyton rubrum* and *Candida albicans*. **Methods:** The hydroalcoholic method obtained Fresh garlic extracts with subsequent freeze-drying. Decreasing concentrations of each extract were tested. **Results:** Both fresh *Chilote* garlic and its black variety showed antifungal effectiveness at different concentrations on both analyzed fungal agents. In contrast, white garlic of Chinese origin did not show effectiveness against *C. albicans* at any concentration tested. **Conclusions:** Black garlic, compared to fresh garlic, does not release a robust characteristic odor or flavor, which would open a point of interest as a natural alternative to the antifungals currently used.

Keywords: black garlic; Chilote; *Allium sativum*; *Allium ampeloprasum*

Resumen

Introducción: Numerosos estudios sobre el ajo han demostrado que, debido a sus componentes bioactivos exhibe propiedades antimicrobianas. Las dermatomicosis de mayor prevalencia son las producidas por dermatofitos y por levaduras del género *Candida*. En los últimos años, se ha reportado un aumento en casos recurrentes y recalcitrantes dado por cepas de dermatofitos resistentes a terbinafina, antifúngico considerado de primera línea. **Objetivo:** El objetivo del presente trabajo fue determinar el efecto antifúngico *in-vitro* de extractos provenientes de ajo chino (variedad fresca) y ajo Chilote (variedades fresca y negra), frente a los dos principales hongos causantes de micosis superficiales: *Trichophyton rubrum* y *Candida albicans*. **Métodos:** Los extractos de ajo fresco se realizaron con un método hidroalcohólico con posterior liofilización. Se testearon concentraciones decrecientes de cada extracto. **Resultados:** Se observó que tanto el ajo Chilote fresco como su variedad negra, poseen efectividad antifúngica para ambos agentes analizados, pero en diferentes concentraciones, mientras que el ajo blanco de origen chino no mostró efectividad frente a *C. albicans* en ninguna concentración testeada. **Conclusión:** El ajo negro en comparación al ajo fresco, no libera un fuerte olor ni sabor característicos, lo que abriría un punto de interés como alternativa natural a los antifúngicos actualmente empleados.

Palabras clave: ajo negro; Chilote; *Allium sativum; A. ampeloprasum*

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Introduction

Garlic (*Allium sativum*) is a plant belonging to the family of Liliaceae and an ingredient in traditional cuisine worldwide. It is a very ancient species whose cultivation was first described in China around 4,000 BC. Currently, the central producing countries are China and India (Bhatwalkar et al., 2021). The characteristic odor and flavor of

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garlic explain its use as a flavoring in food, but another, perhaps older, use has been in medicine. Several studies have shown that due to its bioactive components, it exhibits antimicrobial, immunomodulatory, and anti-inflammatory properties (Bar *et al*., 2022; Khounganian *et al*., 2023). Allicin and ajoene are two of garlic's most active biological compounds (Ledezma & Apitz-Castro, 2006; Li *et al*., 2022). A catalytic reaction between alliin and alliinase generates allicin. Since alliin is found in the cell cytoplasm and alliinase in the vacuole, a cell membrane rupture is necessary for this enzymatic reaction to occur. Therefore, grinding garlic bulbs allows releasing volatile and olfactory sulfur compounds in a more significant proportion (Madariaga *et al*., 2020).

In Chile, Chinese garlic is mainly produced, exported, and domestically consumed (Flaño, 2013). Chilote garlic or elephant garlic (*Allium ampeloprasum*) is a species that presents a much more robust appearance than *A. sativum*, with larger leaves, a giant bulb, and about six bulbils. The cultivation of this vegetable has been historically in the hands of small producers; however, currently, a segment of medium and large farmers dedicate themselves to its cultivation and allocate their production significantly to external markets and agribusiness (Catalan, 2011). Black garlic is fresh garlic that has been fermented for a given time at high temperatures and humidity. The process changes the product's organoleptic characteristics, giving it a sweet, chewy, and gelatinous flavor. Compared to fresh garlic, black garlic does not release a strong flavor and odor due to the reduced content of allicin (Kimura *et al*., 2017). This process not only modifies the nutrients and sensory attributes but also improves bioactivity (Ahmed & Wang, 2021). The antifungal activity of garlic products was first established in 1936. Since then, several studies have verified this property in different components, especially in organosulfur compounds (Ledezma & Apitz-Castro, 2006; Li *et al*., 2022; Li *et al*., 2023). Among fungal infections, the most prevalent dermatomycoses are those caused by dermatophytes (ringworm) and yeasts of the Candida genus. In recent years, in addition to an increase in the number of cases of dermatophytosis in some parts of the world, there has been a reported increase in recurrent and refractory cases due to strains resistant to terbinafine, an antifungal widely used as a first-line agent against many species of dermatophytes (Siopi *et al*., 2021; Uhrlass *et al*., 2022). The objective of this study is to determine the in-vitro antifungal effect of extracts from Chinese garlic (fresh variety) and Chilote garlic (fresh and black varieties) against the two primary pathogens causing superficial mycoses: *Trichophyton rubrum* and *Candida albicans*.

Materials and Methods

Source and preparation of garlic extracts

Chinese garlic cloves from the Frumerc S.A. brand were purchased from a commercial store. Elephant garlic cloves and their black variety were purchased from the Melimei Agricultural company in Manao Bay, Ancud, Chiloé. Fresh garlic was extracted using the hydroalcoholic method (ethanol/water, 80:20), with subsequent freeze-drying. Black garlic was tested by using vegetable capsules without additives.

Trichophyton rubrum ATCC 28188 and *Candida albicans* CBS 562 were used as reference strains.

Culture media

Antifungal activity was tested by a modified macro dilution method in Sabouraud dextrose agar, as previously described (Yamada *et al.,* 2017; Bidaud *et al*., 2023). Four decreasing concentrations of the garlic extracts (6.4, 3.2, 1.6, and 0.8 mg/L) were prepared and poured into sterile plates. In addition, plates without extracts were prepared for positive and sterility control.

Terbinafine was tested on *T. rubrum* at 0.25, 0.06, 0.03, and 0.004 µg/ml, whereas fluconazole was tested on *C. albicans* at 1, 0.5, 0.25, 0.12 µg/ml.

Inoculum

Trichophyton rubrum strain was cultured on Sabouraud dextrose agar diluted 1:10 and incubated at 25°C for ten days to allow adequate sporulation. *Candida albicans* strain was cultured for 24 hours at the same temperature. Inoculum was prepared in 0.9% physiological saline at a concentration of 1 McFarland. Each test plate was inoculated by embedding a swab in the fungal suspension, removing excess serum along the walls of the tube, and touching the surface of the agar at four different points, starting with the control plates without extracts. Each test was performed in triplicate.

Test interpretation

Positive growth was defined as the presence of macroscopic colonies compared to those developed on the positive control plate over the same period. The minimum inhibitory concentration (MIC) was defined as the concentration at which fungal growth was absent.

Results

The results are summarized in Table 1 and shown in Figure 1 and Figure 2. Antifungal activity test performed with Chinese garlic showed the inhibition of *T. rubrum* growth at concentrations ranging from 6.4 to 1.6 mg/L. On the contrary, *C. albicans* growth

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was not inhibited at any concentration regardless of the inoculum. *Chilote* fresh garlic showed total inhibition for *T. rubrum* at all extract concentrations. For *C. albicans*, it showed inhibition at concentrations ranging from 6.4 to 1.6 mg/L.

Regarding *Chilote* black garlic, inhibition was observed for *T. rubrum* at 3.2 and 6.4 mg/L and for *C. albicans* at 6.4 mg/L.

As a reference endpoint, the MIC of terbinafine for *T. rubrum* was 0.06 µg/mL, and that of fluconazole for *C. albicans* was 0.5 µg/mL.

Table 1: Antifungal activity of the three garlic extracts tested in the present study.

Extract concentration (mg/L)	Chinese fresh garlic		*Chilote* fresh garlic		*Chilote* black garlic	
	Candida albicans	*Trichophyton rubrum*	*Candida albicans*	*Trichophyton rubrum*	*Candida albicans*	*Trichophyton rubrum*
0.8	+++	+++	+++	-	+++	+++
1.6	+++	-	-	-	+++	+
3.2	+++	-	-	-	+++	-
6.4	+++	-	-	-	-	-

+++, ++, + : growth compared to the positive control; - : absence of growth

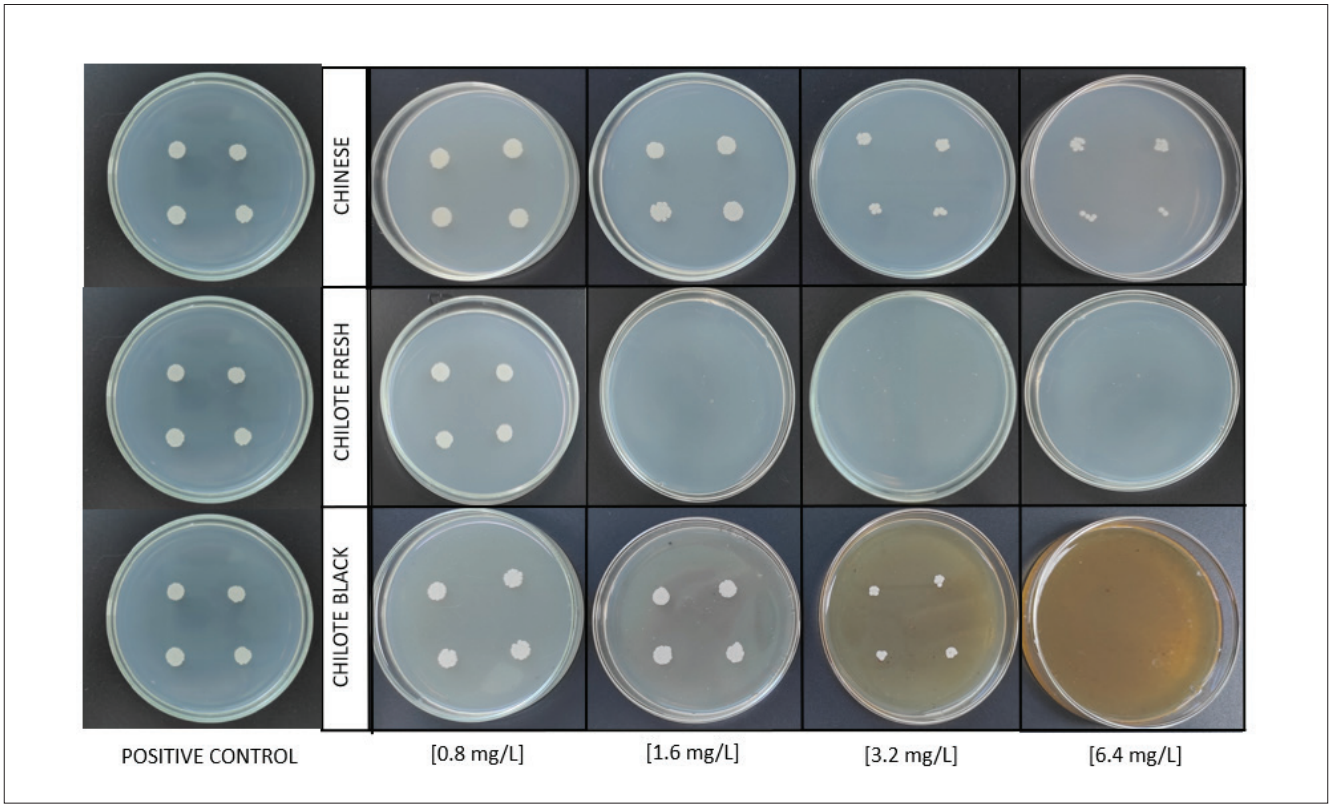

Figure 1: Growth of *Candida albicans* in the three types of garlic compared to the positive control.

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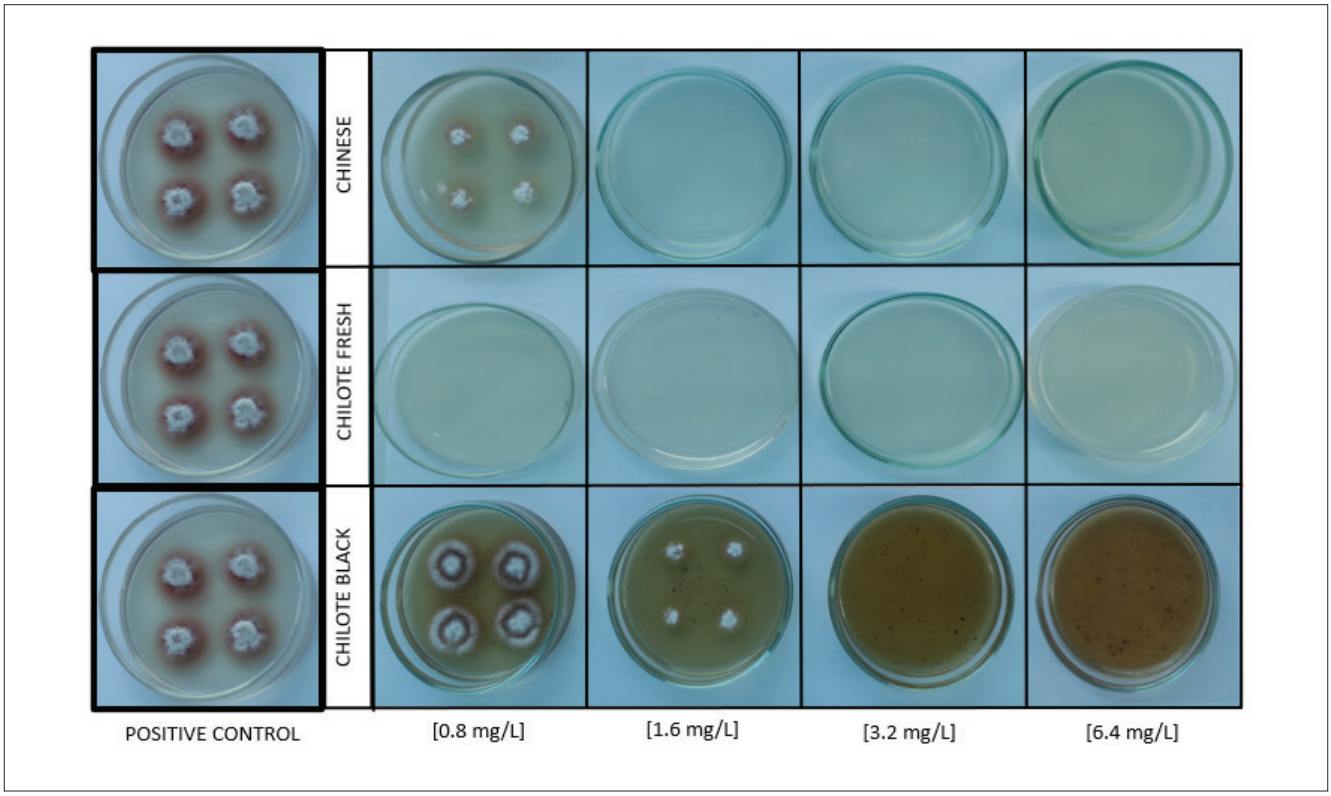

Figure 2: Growth of *Trichophyton rubrum* in the three types of garlic compared to the positive control.

Discussion

Several publications in the literature discuss the antimicrobial properties of garlic, most of them focusing on fresh garlic and referring to ajoene and allicin (Ledezma & Apitz-Castro, 2006; Li *et al.*, 2022; Khounganian *et al.*, 2023).

The method of preparing garlic extract could affect its antifungal capacity, as reported by Daniel *et al*. (2018), who conducted tests against *Fusarium* and *Rhizopus* using both aqueous-based and alcoholic-aqueous-based extracts, observing a lower MIC in the latter. Yetgin *et al*. (2018) compared the antimicrobial activity between Turkish and Chinese garlic on different bacterial strains and *C. albicans*. Turkish garlic was more effective than Chinese garlic. They concluded that these differences are not related to the quantity or volume of garlic tested per trial but directly to its components. Post-harvest treatments, transitioning to black variety, or powdering also influence the chemical properties of garlic. Lyophilization may decrease the amounts of allicin and increase other sulfur compounds with interesting biological properties, such as diallyl sulfide (DAS) or, in the case of black garlic, where up to six-fold higher amounts of S-allylcysteine (SAC) can be found compared to fresh garlic (Sunanta *et al.*, 2023). In our study, we observed that *Chilote* white garlic was superior to Chinese garlic as it showed complete inhibition for *T. rubrum* at all concentrations and for *C. albicans* from 1.6 mg/L. *Chilote* black garlic also inhibited both agents completely, but at a higher concentration than the *Chilote* white. Chinese white garlic showed no effect on *C. albicans* at any concentration but only for *T. rubrum*, similar to *Chilote* white garlic. This result differs from that published by Khounganian *et al.* (2023), who reported in their comparative study between white garlic extract, onion, lemon juice, and the inhibitory effect of garlic against *C. albicans* through agar diffusion. However, they did not use extracts at different concentrations.

In clinical case studies, the effectiveness of ajoene in topical cream for short-term treatment of athlete's foot has been demonstrated. It was tested in 34 patients, showing clinical and mycological cure without recurrence at 90 days post-treatment (Ledezma *et al.*, 1996). Similar results were obtained in onychomycosis caused by *C. albicans*, *C. parapsilosis*, and *C. krusei*, although in a few patients (n=8) (Lemus *et al.*, 2004). Another publication evaluating the effectiveness of ajoene versus terbinafine in tinea corporis and cruris demonstrated that 30 days after treatment, the cure rate was 77% and 75% for the ajoene and terbinafine groups, respectively. Sixty days after treatment, the cure rate was 73% and 71% (Ledezma *et al.*, 1999). In a comparative study between white and black garlic,

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black garlic showed the highest antimicrobial activity when tested by MIC against bacterial strains. Additionally, they conclude that innovative products like black garlic may present enhanced bioactive properties. Therefore, they suggest studying it in more detail to use better this product potential (Botas *et al.*, 2019).

Black garlic, compared to fresh garlic, does not release a robust characteristic odor or flavor due to the reduced content of allicin, which converts into antioxidant compounds such as bioactive alkaloids and flavonoid compounds during the aging process (Kimura *et al.*, 2017), opening novel perspectives for developing some form of topical therapy as a natural alternative to currently employed antifungals as first-line treatments for superficial mycoses.

Conclusions

We showed that both *Chilote* white garlic and its black variety have antifungal effectiveness at different concentrations for both analyzed fungal agents. In contrast, white garlic of Chinese origin did not show effectiveness against *C. albicans* at any concentration tested.

Recognitions

Authors contributions:

Conceptualization: Peggy Vieille Oyarzo Rodrigo; Cruz Choappa. **Data curation:** Peggy Vieille Oyarzo**. Formal analysis:** Peggy Vieille Oyarzo.

Research: Melissa Noguera Gahona Peggy Vieille Oyarzo**. Methodology:** Peggy Vieille Oyarzo. **Project Management:** Peggy Vieille Oyarzo. **Resources:** Melissa Noguera Gahona; Peggy Vieille Oyarzo. **Supervision:** Rodrigo Cruz Choappa. **Validation:** Melissa Noguera Gahona. **Visualization:** Peggy Vieille Oyarzo. **Writing – Original Draft:** Melissa Noguera Gahona; Rodrigo Cruz Choappa; Massimo Cogliati (translator). **Writing – Revision y Edition:** Melissa Noguera Gahona; Rodrigo Cruz Choappa; Massimo Cogliati (translator).

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