JOURNAL OF THE Selva Andina Research Society
Official Journal of the Selva Andina Research Society.

Research Note

ISSN 2072-9294 (print edition)
JSARS
ISSN 2072-9308 (online edition)

In vitro inhibition of *Candida* spp., strains by wild mountain mint essential oil: A traditional medicinal plant in the high Andean regions of Peru

Inhibición in vitro de cepas de *Candida* spp., mediante el aceite esencial de muña silvestre: Una planta tradicional, de uso medicinal en zonas alto andinas del Perú

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Article Data

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Keywords:

Antifungal,
phytochemicals,
inhibition,
in vitro,
wild mountain mint,
medicinal plants.

J. Selva Andina Res. Soc.
2024; 15(2):90-99.
Article ID: 164/JSARS/2023

Article history

Received February 2023.
Returned June 2023.
Accepted May 2024.
Available online August 2024.

Edited by:
Selva Andina Research Society

Palabras clave:

Antifúngico,
fitoquímicos,
inhibición,
in vitro,
muña silvestre,
plantas medicinales.

Abstract

Medicinal plants have been used for centuries by various cultures as a source of treatment and prevention for diseases. Ethnobotanical and scientific studies of "muña" have gained significance due to its bioactive potential against fungi of public health importance. The study was conducted in Puno, Peru (longitude: 15° 50' 15" W, latitude: 70° 01' 18" S, altitude: 4047 meters above sea level), with the aim of evaluating the in-vitro inhibition of wild mountain mint essential oil on *Candida* spp., strains. An experimental in-vitro study was carried out, where 90 inhibitory halos were evaluated at different concentrations using the agar dilution method. The experimental groups consisted of concentrations of mountain mint at: 25, 50, 100, 150, 200, and 250 % (experimental groups), a Fluconazole experimental group (positive group), and a distilled water experimental group (negative group). The experimental groups presented inhibitory halos at 25 % (3.5±1.5 mm), 50 % (11.1±0.6 mm), 100 % (15.8±0.7 mm), 150 % (19.1±0.7 mm), 200 % (24.1±0.5 mm), 250 % (29.3±0.6 mm), fluconazole (25.5±0.6 mm), and for distilled water, no inhibitory halos were observed, as it did not have any inhibitory phytochemical components in its composition. It was observed that the concentration at 250 % presented a superior inhibitory halo compared to the experimental groups and Fluconazole, this is explained by the higher concentration of secondary metabolites present in a higher concentration.

Resumen

Las plantas medicinales han sido utilizadas durante siglos por diversas culturas como fuente de tratamiento y prevención de enfermedades, estudios etnobotánicas y científicos de la muña, adquieren relevancia debido a su potencial bioactivo contra hongos de importancia en la salud pública. El estudio se realizó en Puno-Perú (longitud: 15° 50' 15´´ O, latitud: 70° 01' 18´´ S, y altitud: 4047 msnm), con el objetivo de evaluar la inhibición in vitro del aceite esencial de la muña silvestre sobre cepas de *Candida* spp. Se realizó un estudio experimental in vitro, se evaluaron 90 halos inhibitorios a diferentes concentraciones, mediante el método de dilución en agar. Los grupos experimentales fueron concentraciones de muña al: 25, 50, 100, 150, 200 y 250 % (Grupos experimentales) un grupo experimental fluconazol (grupo positivo) y un grupo experimental agua destilada (grupo negativo). Los grupos experimentales presentaron halos inhibitorios que fueron al 25 % (3.5±1.5 mm), 50 % (11.1±0.6 mm), 100 % (15.8±0.7 mm), 150 % (19.1±0.7 mm), 200 % (24.1±0.5 mm), 250 % (29.3±0.6 mm), fluconazol (25.5±0.6 mm) y para agua destilada, no se observó halos inhibitorios, al no tener ningún componente fitoquímico inhibidor en su composición. Se observó que la concentración al 250 % presento un halo inhibitorio superior en comparación a los grupos experimentales y el fluconazol, esto se explica por la mayor concentración de metabolitos secundarios presentes en una concentración superior.

Introduction

Throughout history, fungi have been the least investigated and addressed pathogenic microorganisms by public health programs, both nationally and globally, in contrast to other infectious agents affecting humans[1]. Invasive fungal infections represent a complex challenge on a global scale, especially due to their high incidence and the high mortality rate they entail[2]. They are of fungal origin caused by opportunistic yeasts belonging to the genus *Candida*, which are very common nowadays[3].

Candidiasis represents the most common morbimortality factor in the world, studies point out its high prevalence and incidence rate, being *Candida albicans* the most recurrent invasive species[4], as well as *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei* and other *Candida* spp.[5-7], most people carry species of the *Candida* genus, as healthy carriers, however, this microorganism is considered commensal and not pathogenic[8].

The main representative of the genus and species is *C. albicans*, which presents a variety of pathological conditions, superficial mucosal infections are the most common due to its high presence[9], very frequently causing involvement of specific organs in patients with chronic conditions that place it in depressive conditions. Oropharyngeal infections attributed to *Candida* contribute significantly to the morbidity associated with HIV infection, with oral infection in this type of patient[10-12]. The various risk factors for infection are crucial because of their prevalence, which increases due to factors such as prolonged hospital stay, use of intensive care units (ICU), surgical interventions, catheterization, immunosuppressive treatment and states of immune suppression[5].

Interest in the therapeutic use of antifungals arose due to the constant presence of infections reported over the years, however, the range of available antifungal treatments are restricted and progress in the development of new drugs has been slow, therefore, the exploration of alternative pharmacological options that present reduced rates of resistance and minimal side effects remains a significant challenge[13]. Ancestral natural medicine possesses a vast knowledge of pharmaceutical riches, with diverse preventive health benefits, derived from complex and historical-cultural knowledge[14].

Muña, an aromatic plant species native to the Andes of South America, distributed between 2500 and 3500 meters above sea level, between temperate and cold climates of the central, northern and southern High Andean zones of Peru[15], is traditionally used as a natural medicine, for its essential oil (EO), due to its composition with antioxidant, antibacterial and antifungal properties, these properties being recognized[16], Studies on the use of muña on various *Candida* isolates have justified its efficient capacity, showing positive results[17-19] however, the literature points out the broad antifungal activity of various vegetative species, such as lemon verbena, oregano, chamomile, lemon balm, rosemary, cinnamon, clove[20-25], indicating their broad antifungal activities and great inhibitory efficacy on *Candida*.

Although there are no clinical reports on the use of EO from pineapple, ethnopharmacological and botanical knowledge can be of great help[15]. Its broad antimicrobial capacity is of great importance and public interest; evaluating in vitro inhibition provides important knowledge that lays the foundations for the use of traditional and modern medicine.

Materials and methods

Place of study. The research work was carried out in the high Andean lake city of Puno, district, province, department of Puno in the extreme south of Peru (longitude: 15° 50' 15" W, latitude: 70° 01' 18" S, altitude: 3827 masl), a region of vast natural wild growth of muña.

Figure 1 Steam injection entrained distillation process

Obtaining and preparation of plant material. The plant material (muña) used in the present study was obtained from woody shrubby plants of natural growth in wild form, in phenological state of vegetative growth (juvenile) in flowering periods, with the help of a commercial sickle 60 kg of the shrubby plant (gross weight) were harvested, selecting impurities and storing them in sacks of agricultural harvest, for its transfer to the laboratory of operations and unitary processes of the Universidad Nacional del Altiplano, only leaves were disintegrated for later drying without solar action, obtaining a final weight of 20 kg (net weight). The leaves were subjected to the process of distillation by dragging with steam injection, reaching a boiling point of 84° C, separating the water and oil as steam, condensing in a refrigeration system and separating the oil from the water by difference of densities in a decantation pear (Figure 1), the whole process lasted a period of 2.5 h, obtaining 75 mL of EO, finally it was conserved in a dark amber flask well closed in a fresh, dry place, protected from light, heat and humidity.

Figure 2 Isolated strains of *Candida* spp.

Experiment design. The *Candida* spp. strains used for the in vitro inhibition of the study were isolated from the post-practicum work tables of each laboratory of the School of Biological Sciences, using a sterile swab soaked in saline solution (Figure 2). The samples were cultured in glass Petri dishes with Sabouraud glucose agar (ASD) modified with the addition of chloramphenicol and incubated at 37° C for 72 h. In vitro inhibition was evaluated by observing the formation of inhibitory halos (HI) on Mueller-Hilton agar, seeded with *Candida* spp. using the dilution technique on modified agar, 50 µL of the different concentrations were deposited in wells (6 mm in diameter). Different concentrations 25, 50, 100, 150, 200 and 250 %, experimental groups (EG) where (25, 50, 100, 150, 200 and 250 µL respectively were the OE and 1000 µL of distilled water for each concentration), as well as the GE fluconazole of 25 µg GE positive (GE+) and the distilled water GE negative (GE-), after an incubation period at 37° C for 72 h, the reading of the quantitative results (mm) and evaluation of each EG under study was made.

Inferential statistical analysis. The data obtained by in vitro inhibition were subjected to an analysis of variance (ANOVA) and for the multiple comparison of means, the Tukey test (p = 0.05) was performed with the SAS statistical program version 3.6 (Basic Edition).

Results

The HI achieved by wild pineapple EO against *Candida* spp. strains tended to increase with respect to the concentration. On the other hand, the in vitro inhibition of wild pineapple EO at 250 % over a period of 72 h was superior to that of the other SGs and to that of SG+ (Table 1 and Figure 3).

Table 1 Variation of inhibitory halos of wild pineapple EO against *Candida* spp. strains.

Experimental groups (%)	N°	Media (mm)	DE (±)	ANOVA	Tukey
25	15	3.5	1.5		A
50	15	11.1	0.6		B
100	15	15.8	0.7	<.0001	C
150	15	19.1	0.7		D
200	15	24.1	0.5		E
250	15	29.3	0.6		F
Fluconazol	15	25.5	0.6		G

GE+: Positive experimental group, N°: Number of replicates, SD (±): Standard deviation, mm: Millimeters, Different letters between rows (GE) indicate significant difference. Tukey (p < 0.05) for each GE.

The different concentrations of wild pineapple EO show a varied susceptibility to *Candida* spp. strains, directly related to the increase in concentration. The concentration of wild pineapple EO at 250 % showed the highest HI and the highest in vitro susceptibility to *Candida* spp. strains and to GE+ (Table 1 and Figure 4).

Figure 3 Inhibitory halos (positive and negative group)

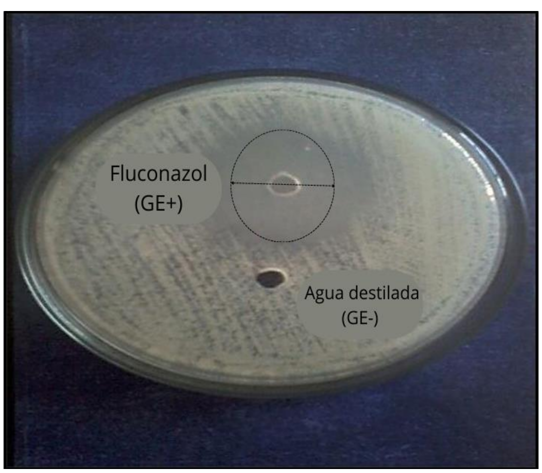
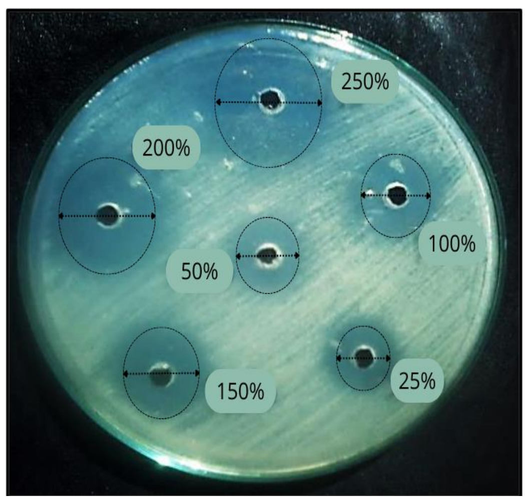


Figure 4 Inhibitory haloes at different concentrations



When comparing the HI between the concentrations of wild pineapple EO at 25, 50, 100, 150, 200, and 250 %, GE+ and GE-, within 72 h, it could be observed that there was a significant difference (p < .0001) between each GE.

Discussion

Studies indicate the in vitro inhibitory effect of pineapple EO on various mycotic microorganism isolates; depending on its concentration, efficacy varies[26-27], its optimum activity is reflected at high concentrations[19,28], equaling the action of antifungal agents such as fluconazole[29], in effect, the HI will be proportional to the concentration applied, possibly explained by the action of monoterpenes such as pulegone, menthone, limonene and myrcene[18,30].

The higher HI with larger diameters obtained, compared to the other concentrations, indicate a greater antifungal inhibition[31-33], the evaluation of HI based on the size of its diameter, provides a more detailed interpretation and evaluation, reason for the qualification of halos of 30 mm in diameter, after the application of 100 % pineapple oil, as sensitive[17,18].

The application of EO, independent of the microorganisms, in the formation of HI will always be proportional to the concentration used, and HI of up to 19 mm in diameter can be observed in Streptococcus mutans and *Lactobacillus acidophilus*[28,34]. In the same context, studies on the extraction and use of EOs from various plant species have a broad antifungal capacity on various isolates of *Candida*, such as *Schinus molle*, *Cymbopogon citratus* (lemon verbena), *Origanum vulgare* (oregano), *Matricaria chamonilla* (chamomile), *Melissa officinalis* (lemon balm), *Rormarinus officinalis* (rosemary), *Cinnamomun zeylanicum* (cinnamon), *Syzygium. aromaticum* (clove)[20-26,35] and species of the *Baccharis* genus[36], reaffirming their innate inhibitory effect.

It is necessary to emphasize that the application of pineapple EO suggests a wide inhibitory activity, thus, they also point out the antibacterial capacity in vitro, inhibiting the development of *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Salmonella* sp, and *Staphylococcus epidermidis*[34,37-41], increasing their efficacy in proportion to the concentration[42] , possibly due to the composition of active principles such as monoterpenes, alcohols, ketones and terpenic oxides, which have the capacity to inhibit microbial growth[43], in addition to carvacrol and thymol, recognized oxygenated monoterpenes with antibacterial activity[41].

Finally, the study suggests the in vitro inhibitory capacity of wild pineapple EO on *Candida* spp. strains, applied at different concentrations, with superior HI in relation to fluconazole. The EO extracted from wild pineapple offers an attractive option as a complementary therapy against fungal infections, due to its potential to combat antimicrobial resistance. The bioactive compounds present in pineapple, corroborated by previous studies, have shown a remarkable capacity to inhibit the growth of microorganisms, which may be especially relevant in a context where resistance to antifungals is a growing problem, the use of pineapple as part of a comprehensive therapeutic approach makes it a valuable option in the fight against fungal infections resistant to conventional treatments. In conclusion, knowing the in vitro inhibition of pineapple EO against microorganisms is fundamental to evaluate its therapeutic potential, optimize its use in the treatment of infectious diseases and better understand the underlying mechanisms of its antimicrobial activity.

Source of financing

The investigation was assumed by the researcher.

Conflicts of interest

There is no conflict of interest in the investigation.

Acknowledgments

To the Universidad Nacional del Altiplano, School of Biological Sciences, Microbiology and Clinical Laboratory Program.

Ethical considerations

The research complied with the requirements established by the Universidad Nacional del Altiplano through the Vice Rector's Office for Research and the PILAR Platform (Platform for University Research Integrated to Academic Work with Accountability).

Research limitations

There were no limitations to the research.

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