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Anti-yeast activity of the essential oil of *Peumus boldus* Mol. from the coastal drylands of central Chile against strains that cause uropharyngeal and vulvovaginal candiadiasis

[Actividad antilevaduriforme del aceite esencial de *Peumus boldus* Mol. del secano costero de la zona central de Chile contra cepas causantes de candiadiasis urofaringia y vulvovaginal]

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Abstract: The aim of this study was to investigate the chemical composition and antifungal activity of the essential oil extracted from leaves of *Peumus boldus* of the coastal drylands of Chile. The essential oil was characterized by gas chromatography-mass spectrometry. The antifungal activity was evaluated by a microdilution assay against five strains of *Candida* of clinical importance. *P. boldus* essential oil had a yield of 0.32% and nineteen compounds were identified in the analysis, mostly ascaridol (49.95%), o-cymene (13.45%) and eucalyptol (9.71%). All yeast strains tested were sensitive to *P. boldus* essential oil, with *C. guillermondi* being the most sensitive, with a minimum inhibitory concentration (MIC) value of 8 µg/mL, twice lower than the MIC value of 16 µg/mL exhibited by fluconazole and itraconazole. These results suggest that *P. boldus* essential oil could be used as natural antifungal agents in free form or in a formulation against pathogenic yeasts.

Keywords: Essential oil; *Peumus boldus*; *Candida sp*.; Ascaridol; *Candida guillermondi*

Resumen: El objetivo de este estudio fue investigar la composición química y la actividad antifúngica del aceite esencial extraído de hojas de *Peumus boldus* del secano costero de Chile. El aceite esencial se caracterizó por cromatografía de gases acoplada a espectrometría de masas. La actividad antifúngica se evaluó mediante un ensayo de microdilución frente a cinco cepas de *Candida* de importancia clínica. El aceite esencial de *P. boldus* se obtuvo con un rendimiento del 0,32% y en su análisis se identificaron diecinueve compuestos, mayoritariamente ascaridol (49,95%), o-cimeno (13,45%) y eucaliptol (9,71%). Todas las cepas de levadura analizadas fueron sensibles al aceite esencial de *P. boldus*, siendo *C. guillermondi* la más sensible, con un valor de concentración inhibitoria mínima (CIM) de 8 µg/mL, dos veces inferior al valor de CIM de 16 µg/mL exhibido por fluconazol e itraconazol. Estos resultados sugieren que el aceite esencial de *P. boldus* podría utilizarse como agente antifúngico natural en forma libre o en una formulación contra levaduras patógenas.

Palabras clave: Essential oil; *Peumus boldus*; *Candida sp*.; Ascaridol; *Candida guillermondi*.

INTRODUCTION

In recent decades, fungal diseases have increased considerably and have become a major public health problem causing about 2 million deaths per year (Kainz *et al.*, 2020). This increase is attributed to several factors, on the one hand, to the considerable increase in the world population susceptible to fungal infections, such as the elderly, neonates or patients with debilitating chronic diseases, as well as immunosuppressed individuals, such as AIDS patients, patients undergoing invasive surgery or transplants and those undergoing pro-longed antibiotic or chemotherapy therapies (Gómez & Escandón, 2023). Likewise, patients subjected to long periods of hospitalization increase the risk of nosocomial (hospital-acquired) infections caused by fungi (Sigi *et al.*, 2023). These diseases are caused by opportunistic fungi that are usually found as members of the resident human microbiota or as saprophytes in the environment. With the host defenses altered, they cause infections ranging from skin/mucosal involvement to life threatening systemic disease (Talapko *et al.*, 2021). The most common opportunistic infections are mainly caused by *Candida* species (Márquez *et al.*, 2017). Candidiasis is infection by *Candida* species (most often *C. albicans*, *C. glabrata*, *C. tropicalis* and *C parapsilosis*), manifested by mucocutaneous lesions, candidemia (bloodstream infection), and sometimes focal infection of multiple sites (Schikora-Tamarit & Gabaldón, 2024). Symptoms depend on the site of infection and include dysphagia, skin and mucosal lesions, blindness, vulvovaginal symptoms, fever, renal shutdown, and disseminated intravascular coagulation (Linhares *et al.*, 2001; Kauffman *et al.*, 2011; Yano *et al.*, 2019). For invasive candidiasis and candidemia, the use of drugs is currently limited to three distinct chemical classes: echinocandins (caspofungin or micafungin), polyenes (amphotericin B) and azoles (ketoconazole or fluconazole) (Houšť *et al.*, 2020). Some compounds are also applied against certain localized mycoses (terbinafine, griseofulvin and ciclopirox). However, their efficacy is often weak, as they do not kill the cells (fungicidal action), but only stop their growth (fungistatic) (Fink *et al.*, 2022). Of this range of compounds, the azoles are the ones with comparatively higher efficacy and the possibility of oral administration (Campoy & Adrio, 2017), leading to a prolonged therapeutic and prophylactic application in high-risk patients, generating the acquisition of antifungal resistance against this family of compounds with the direct increase of less susceptible strains (Fisher *et al.*, 2022). The observed antifungal resistance of *Candida* yeasts has led the U.S. Centers for Disease Control and Prevention (CDC) to classify these pathogens as “serious threats”, elevating them to the same threat level as other antibiotic-resistant microorganisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Shinu *et al.*, 2022). Based on this background, it is necessary to look for alternatives to stop this exponential growth shown by diseases and infections caused by *Candida*-type yeasts. As a viable alternative to the use of essential oils, plant-based antifungals compared to synthetic chemicals are their varied methods of application including inhalation, ingestion, massage and skin applications and the numerous reports that have highlighted their health effects, such as their antioxidant, antiviral, anti-inflammatory, antitumor and antifungal properties (Abd Rashed *et al.*, 2021).

Peumus boldus Mol. is the only species of the monotypic genus *Peumus*, of the Monimiaceas family. This tree is endemic to Chile, which grows in a Mediterranean climate, characterized by a summer season with low water availability in the soil, high solar radiation and high temperatures (Peña-Rojas *et al.*, 2018). Its leaves, which have a strong aroma, are used for medicinal purposes due to their proven therapeutic action as a digestive stimulant, diuretic and others (Moesbach & Ruiz, 2023). This species has numerous pharmacological and phytochemical studies in which they have reported antimicrobial, insecticidal, antiparasitic, antioxidant, and anticancer activity of its essential oil and extracts (Abouelela *et al.*, 2023). In addition, the antifungal activity of the concentrated essential oil of *P. boldus* has been studied and the percentage inhibition of mycelial growth of a wide range of economically important phytopathogenic fungi including *Pythium irregular* (19%), *Fusarium oxysporum* (25.2%), *Rhizoctonia solani* (26%) and *Phragmidium violaceum* (45%), (Bittner *et al.*, 2009). In turn, another study demonstrated the antifungal power of *P. boldus* essential oil at concentrations of 1.5 μL/mL, achieving the inhibition of about 90% of the growth of phytopathogenic strains of *Aspergillus flavus* and *A. parasiticus* (Passone & Etcheverry, 2014). However, to date no anti*candida* activity of its essential oil has been reported. The objective of this study was to determine the chemical composition and evaluate the antifungal activity of the essential oil of *P. boldus* collected in the coastal drylands of central Chile against five clinically important *Candida sp*. commonly associated with nosocomial infections.

MATERIAL AND METHODS

General

All chemical reagents purchased (Merck, Darmstadt, Germany or Aldrich, St. Louis, MO, USA) were of the highest commercially available purity and were used without previous purification.

Plant material

Leaves *P. boldus* were collected in January 2024 in a dry coastal area known as “Las Dichas” (33°17′27″S 71°29′59″W), Casablanca, Chile. A voucher specimen (Pb-010124) was deposited at the laboratory “LPNSO”, Universidad de Playa Ancha, Valparaíso, Chile.

Extraction of essential oil

Fresh plant material (500 g) were submitted to hydrodistillation with 1 L of distilled water for 4 h in a Clevenger-type apparatus, following which the essential oil layer was separated, dried over anhydrous sodium sulfate and was stored for some days at −20°C in amber glass bottles. The yield of fresh oil was determined as the quotient of the weight of oil collected and the dry weight of plant material extracted.

Chemical composition of the essential oil

The *P. boldus* essential oil was diluted with dichloromethane, and 1 μL of the sample was analyzed using a GC-MS/MS, Thermo Scientific (GC: model Trace 1300 and MS: model TSQ8000Evo) operating in EI mode at 70 eV, equipped with a splitless injector (250°C). The transfer line temperature was 200°C. Helium was used as a carrier gas at a rate of 1.2 mL/min, and the capillary column used was a Rtx-5 ms (60 m × 0.25 mm i.d., film thickness 0.25 μm). The temperature program was 40°C (5 min) to 300°C (5 min) at a rate of 5°C/min. The chemical composition of the oil was identified by comparing its spectra with an NIST20 library and confirmed by comparison of their retention index with those reported in the literature (Adams, 2017),

In vitro anti-candida activity assay

The microdilution method (Brito *et al.*, 2022) was used for the assessment of *in vitro* antifungal activity of the essential oil against *Candida parapsilosis* 22019, *C. tropicalis* 9841, *C. lusitaniae* 2305, *C. guilliermondii* 2204 and *C. albicans* 10935. flucanazol, itraconazol and amphotericin B were used as positive control, since it is a clinically used antimycotic drugs. The final concentrations of the essential oil and controls ranged from 256 to 0.25 μg/mL. The MIC determination was performed according to the CLSI reference protocol M27-A2 for yeasts (MIC_{80} value). Inoculated plates were incubated at 37°C in the dark and MIC values determined spectrophotometrically (microplate reader, SpectraMax, Winooski, VT, USA) as an optical density at a wavelength of 540 nm after 24 h and 48 h. MIC was defined as the concentration causing 80% reduction of growth of the strain compared to control growth. All the measurements were obtained from three independent experiments, each preformed in triplicate.

Statistical analysis

Data on the inhibitory effects of essential oil and controls on the growth of *Candida* species were subjected to analysis of variance (ANOVA) on all data with a Tukey's post hoc test ($p<0.05$), using the STATISTICA 7.0 program.

RESULTS AND DISCUSSION

Chemical composition

The extraction yield of essential oil was evaluated according to the biomass of fresh leaves, whose value reached 0.32% for the light yellow oil of *P. boldus*. The results of the chemical analysis of the essential oil of *P. boldus* oil is detailed in Table No. 1.

Table No. 1
Essential oil composition of *P. boldus*

RT [a]	Components	% Area [b]	RI [c]	RI [d]	Identification
11.08	*o*-Cymene	13.45	1028	1029	**RI, MS, Co-I**
11.21	ß-Phellandrene	1.15	1031	1032	**RI, SM**
11.28	Eucalyptol	9.71	1034	1034	**RI, MS, Co-I**
13.00	Fenchone	1.09	1094	1097	**RI, MS**
13.98	p-Menth-2-en-1-ol	0.55	1104	1108	**RI, MS**
14.54	Isopinocarveol	2.77	1134	1136	**RI, MS**
14.70	(−)-Camphor	1.97	1141	1141	**RI, MS, Co-I**
15.24	Pinocarvone	1.66	1148	1148	**RI, MS**

15.66	Terpinen-4-ol	2.91	1162	1162	**RI, MS, Co-I**
16.06	Terpineol	0.76	1189	1189	**RI, MS, Co-I**
16.23	Myrtenal	0.55	1193	1191	**RI, MS**
17.50	Ascaridole	49.95	1238	1238	**RI, MS**
19.33	Isoascaridole	6.61	1302	1300	**RI, MS**
21.86	Methyleugenol	1.05	1401	1401	**RI, MS, Co-I**
22.33	Caryophyllene	0.62	1407	1407	**RI, MS, Co-I**
24.27	Bicyclogermacrene	0.65	1493	1495	**RI, MS**
26.26	(-)-Spathulenol	0.70	1572	1573	**RI, MS**
26.87	Globulol	0.10	1581	1583	**RI, MS**
28.75	Germacratrien-1-ol	1.01	1680	1681	**RI, MS**
	Monoterpenes	14.60			
	oxygenated monoterpenes	78.53			
11.21	Phenylpropanoids	1.05			
11.28	Sesquiterpenes	1.27			
13.00	oxygenated sesquiterpenes	1.81			
13.98	Total	97.26			

[a] RT: Retention time; [b] Surface area of GC peak; [c] RI: Experimental retention index for non-polar column [d] RI: bibliographic retention index for non-polar column. MS Mass spectra, Co-I: Co-injection of standard

Nineteen compounds were identified in the essential oil of *P. boldus*, which corresponded to 97.26% of the total oil analyzed and the main components are ascaridole (49.95%), *o*-cymene (13.45%) and eucalyptol (9.71%) (See Figure No. 1).

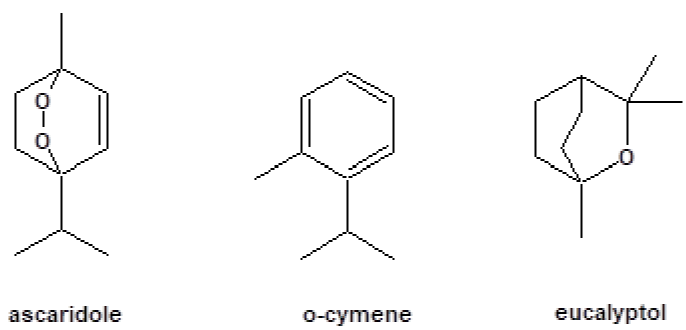

Figure No. 1
Structure of the major compounds present in the essential oil of *P. boldus*

Our results on *P. boldus* essential oil from fresh leaves collected in central Chile are consistent with other studies, identifying ascaridole and *o*-cymene as the most abundant constituents (the content of each of them in the essential oil was often higher than 40% (Bittner *et al.*, 2009) and 10% (Pavela *et al.*, 2019), respectively; however, our report is the first in which eucalyptol, an oxygenated alcohol-type monoterpene, is identified as the third most abundant compound, exceeding 9% of the oil composition. This variation in essential oil content and chemical composition would be given as a protective response to various factors such as adverse environmental and geographic conditions typical of the coastal drylands of central Chile, including poor soils with water scarcity and high solar radiation, which affect its chemical response (Seleiman *et al.*, 2021).

Anti-yeast Activity

The MICs calculated at 48 h for the essential oil extracted from fresh *P. boldus* leaves are shown in Table No. 2.

Table No. 2
MIC_{80} values (µg/mL) of essential oil obtained from leaves of *P. boldus*

Strain	*Essential oil*	*Fluconazole*	*Itraconazole*	*Amphotericin B*
C. parapsilosis	16	2	0.5	>256
C. tropicalis	32	4	2	>256
C. lusitaniae	128	8	4	**R**
C. guilliermondii	8	16	16	**R**
C. albicans	64	2	16	256

R: Resistant yeast strain

In the present study, the anti-*candida* activity of *P. boldus* essential oil was demonstrated against all the microorganisms tested, registering MIC values at 48 h of 8-128 µg/mL. The best values were recorded against *C. guilliermondii* (8 µg/mL) at 48 h activity is more potent than all controls, followed by *C. parapsilosis* (16 µg/mL) and *C. tropicalis* (32 µg/mL) which is superior to amphotericin B® (>256 µg/mL) and lower than the other controls. In the case of *C. albicans*, the essential oil presented a MIC value of 64 µg/mL four times lower than that shown by amphotericin B and a significantly higher value than that shown by both azoles. A similar phenomenon was observed for *C. lusitaniae*, an amphotericin B-resistant strain, which showed a higher sensitivity to the oil (128 µg/mL) but lower than that shown by fluconazole (8 µg/mL) and itraconazole (4 µg/mL).

Several studies have shown that the efficacy of essential oil as antimicrobials is closely related to their chemical composition (Pérez-Zamora *et al.*, 2018). In this context, it is claimed that, due to their complex composition (mainly composed of low molecular weight hydrocarbons and their oxygenated derivatives), the antifungal activity of oils is not related to a single, specific mechanism of action, but is the result of the effect of the different compounds present and depends directly on their partitioning characteristics (Nazzaro *et al.*, 2017). They are able to pass through the cell wall, altering and degrading the membrane, causing leakage of cell contents, coagulation of the cytoplasm, depletion of protons and lysis are consequences of these induced changes in membrane structure and function. The oxygenated terpenic compounds are often considered to be primarily responsible for this effect due to their ability to modify membrane permeability by chemical reaction with the amino and hy-droxylamine groups of membrane proteins (Palmeira-de-Oliveira *et al.*, 2009).

The activity is due to the action of its main components, such as the oxygenated terpene eucalyptol, which has shown a potent antifungal activity, being found mainly in the essential oil of *Eucalyptus urophylla* with antifungal activity against *Aspergillus niger* and *F. oxysporum* (Pujiarti *et al.*, 2017) and in individual tests against *C. albicans* and *C. dubliniensis* (Martínez-Pabón & Ortega-Cuadros, 2020). In addition, monoterpenes such as terpinen-4-ol, which has a free hydroxyl group, are a probable mediator of the *in vitro* and *in vivo* activity of the essential oil of *Melaleuca alternifolia* (tea tree oil) against vaginal infections caused by *C. albicans* (Mondello *et al.*, 2006). In addition, it is well known that terpinen-4-ol and terpineol have shown activity against a number of plant pathogenic fungi of the genus *Fusarium* sp. and *Penicillium* sp. (Morcia *et al.*, 2012).

Isopinocarveol can also contribute, since the antimicrobial activity of the essential oil of *Apium graveolens* leaves has been demonstrated *in vitro* and *in silico*, in whose oil this compound is found above 10% of relative abundance (Foudah et al, 2021). In recent studies it has been shown that another minor compound with over 2% abundance in the oil, camphor, could cause the destruction of the cytomembrane, improving the permeability of the cytomembrane and releasing intracellular macromolecules, such as nucleic acids and proteins from strains of *Fusarium*, one of the main pathogenic fungi, which infects crops and causes large economic losses (Kong *et al*., 2022). However, the action of the oils is also given by the synergism that can occur between all the molecules present, since the potent antifungal activity of the essential oil of the Brazilian plant *Chenopodium ambrosioides* L. has been described, which has a high percentage of ascaridole and a low concentration of molecules with free hydroxyls as has been identified in the oil of *P. boldus* (Jardim *et al.*, 2008).

CONCLUSIONS

Consequently, *P. boldus* oil proved to be a natural alternative against all tested yeasts and proved to be more potent than the drug amphotericin B and comparable to azoles in the reduction of the main clinical strains associated with nosocomial and candiadiasis causing fungal infections. Future studies will evaluate the antifungal potential of *P. boldus* oil in *in vivo* and nanoformulated models with the aim of decreasing its concentration and increasing its bioavailability, along with purification of the majority compounds to mechanistically understand the biological activity of this essential oil.

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