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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Chemical composition and antifungal potential of Brazilian propolis against *Candida* spp.



Composition chimique et potentiel antifongique de la propolis brésilienne contre Candida spp.

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Summary Propolis is known to have biological properties against numerous microorganisms of clinical interest. This study aimed to determine the chemical composition and antifungal activity of Brazilian propolis (types 3 and 13) against *Candida* spp. and their effects on the morphology of preformed and mature *Candida* biofilms. Samples of propolis (3 and 13) collected by *Apis mellifera* honeybees were obtained from different regions in Brazil. Ethanolic extracts of propolis (EEP) were prepared, fractionated and submitted to chemical analysis by GC/MS. The extracts and their hexane, dichloromethane and ethyl acetate fractions were tested for their ability to inhibit *Candida* spp. (*C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. kruzei*, *C. tropicalis* and *C. parapsilosis*) by determination of the minimum inhibitory and fungicidal concentrations (MIC/MFC). Additionally, their effects on morphology of preformed and mature biofilms were observed by scanning electron microscopy. The phenolic compounds *p*-coumaric acid, caffeic acid phenethyl ester (CAPE), kaempferol and quercetin were identified in the EEP-3 and its bioactive dichloromethane fraction; and isoflavonoids such as medicarpin, vestitol and

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MOTS CLÉS

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formononetin were found in the EEP-13, and triterpenes in its bioactive hexane fraction. The EEP-3 and EEP-13 and their bioactive fractions showed MIC values ranging from 0.2 to 125 $\mu\text{g}/\text{mL}$ and MFC values between 125 and 500 $\mu\text{g}/\text{mL}$. The EEP and fractions were predominantly fungistatic agents. All extracts and fractions disrupted biofilm structures at 500 $\mu\text{g}/\text{mL}$ and amorphous areas with cell damage were clearly observed in preformed and mature biofilms. Propolis types 3 and 13 have strong anti-*Candida* activity and should be considered as promising candidates to treat oral and systemic candidiasis.

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Résumé Propolis est connue pour avoir des propriétés biologiques contre un certain nombre de micro-organismes d'intérêt clinique. Cette étude visait à déterminer la composition chimique et l'activité antifongique de la propolis brésilienne (types 3 et 13) contre *Candida* spp. et leurs effets sur la morphologie de biofilms préformés et matures de *Candida*. Des échantillons de la propolis (3 et 13) recueillies par des abeilles *Apis mellifera* ont été obtenus à partir de différentes régions du Brésil. Des extraits éthanoliques de la propolis (EEP) ont été préparés, fractionnés et soumis à une analyse chimique par GC/MS. Les extraits et leurs fractions hexane, dichlorométhane et d'acétate d'éthyle ont été testés pour leur capacité à inhiber *Candida* spp. (*C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. kruzei*, *C. tropicalis* et *C. parapsilosis*) par détermination de la concentration minimale inhibitrice et fongicide (CMI/CMF). En outre, leurs effets sur la morphologie des biofilms préformés et matures ont été observés par microscopie électronique à balayage. Les composés phénoliques *p*-coumarique acide, ester phénéthylique de l'acide caféique (CAPE), la quercétine et le kaempférol ont été identifiées dans le EEP-3 et fraction dichlorométhane bioactif ; et les isoflavonoïdes tels que medicarpin, la formononétine et vestitol ont été trouvés dans le EEP-13, et les triterpènes dans sa fraction d'hexane bioactive. Les EEP-3 et EEP-13 et leurs fractions bioactives ont montré des valeurs CMI allant de 0,2 à 125 $\mu\text{g}/\text{mL}$ et des valeurs CMF entre 125 et 500 $\mu\text{g}/\text{mL}$. L'EEP et fractions étaient principalement des agents fongistatiques. Tous les extraits et fractions perturbaient la structure des biofilms à 500 $\mu\text{g}/\text{mL}$ et des zones amorphes avec des dommages aux cellules ont été clairement observées dans les biofilms préformés et matures. Les propolis 3 et 13 ont une activité anti-*Candida* forte et devraient être considérées comme des candidats prometteurs pour traiter la candidose buccale et systémique.

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Introduction

Propolis is a chemically complex resinous mixture collected by *Apis mellifera* bees from different parts of plants [38]. The botanical origin and chemical composition of propolis may vary based on the specific climate and flora of each region [25]. In nature, bees use propolis to assemble their hives and also as a physical barrier against predators, moisture and wind, due to its resinous, waxy nature [38]. Throughout the centuries, mankind has benefited from the high medicinal, functional and economic value of propolis collected worldwide, which also has a cultural value due to its use in mummification rituals by the ancient Egyptians [4,30].

Brazilian propolis are commonly classified into 13 types based on their physicochemical properties, such as color, texture and chemical profile, as well as on geographic origin [1,28]. Our research group has been extensively studying the type 3 from southern Brazil [19] and the type 13 from Maceio City, Alagoas state, northeastern Brazil [1,5,6,33]. Previously, we demonstrated that these types of propolis and some of their isolated compounds have antibacterial [1,14,23], anti-caries [6,15,22], anti-inflammatory [5], antioxidant [1,7] and antiproliferative [7] effects. There are also reports by other authors on propolis extracts and derivatives being active against yeasts (*Candida* spp. and *Saccharomyces*

cerevisiae) [25,37] and dermatophytes (*Trichophyton* spp.) [34]. The major presence of flavonoids in the chemical composition of several samples of propolis, including the types 3 and 13, may explain their promising antifungal properties [11].

The toxicity of current antifungal drugs, their side effects, and microbial resistance have encouraged the discovery of novel chemical molecules with potent biological activity against fungal biofilms, particularly those of clinical interest in the medical setting. In this study, we determined the chemical composition and antifungal activity of Brazilian propolis (types 3 and 13) against *Candida* species potentially involved in the development of oral candidiasis or catheter-related infections. The effects of hydroalcoholic extracts of these types of propolis and their bioactive fractions against the structure of preformed and mature *Candida* biofilms were investigated.

Material and methods

Propolis samples and extract fractionation

Samples of crude Brazilian propolis collected by *Apis mellifera* honeybees were obtained from different regions in

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