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Original article

The essential oil of *Allium sativum* as an alternative agent against *Candida* isolated from dental prostheses

Alejandro Mendoza-Juache, Saray Aranda-Romo, Josué R. Bermeo-Escalona, Araceli Gómez-Hernández, Amaury Pozos-Guillén, Luis Octavio Sánchez-Vargas*

Facultad de Estomatología Universidad Autónoma de San Luis Potosí, Mexico

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ABSTRACT

Background: The colonization of the surfaces of dental prostheses by *Candida albicans* is associated with the development of denture stomatitis. In this context, the use of fluconazole has been proposed, but its disadvantage is microbial resistance. Meanwhile, the oil of *Allium sativum* has shown an effect in controlling biofilm formation by *C. albicans*.
Aims: The objective of this study was to determine the antifungal activities of the essential oil of *A. sativum* and fluconazole against clinical isolates of *Candida* species obtained from rigid, acrylic-based partial or total dentures and to compare these agents' effects on both biofilm and planktonic cells.
Methods: A total of 48 clinical isolates obtained from the acrylic surface of partial or complete dentures were examined, and the following species were identified: *C. albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida krusei*. For each isolate, the antifungal activities of the essential oil of *A. sativum* and fluconazole against both biofilm and planktonic cells were evaluated using the Clinical & Laboratory Standards Institute (CLSI) M27-A3 method. The isolates were also evaluated by semiquantitative XTT reduction.
Results: All planktonic *Candida* isolates were susceptible to the essential oil of *A. sativum*, whereas 4.2% were resistant to fluconazole. Regarding susceptibilities in biofilms, 43.8% of biofilms were resistant to *A. sativum* oil, and 91.7% were resistant to fluconazole.
Conclusions: All planktonic cells of the different *Candida* species tested are susceptible to <1 mg/ml *A. sativum* oil, and the majority are susceptible to fluconazole. Susceptibility decreases in biofilm cells, with increased resistance to fluconazole compared with *A. sativum* oil. The essential oil of *A. sativum* is thus active against clinical isolates of *Candida* species obtained from dentures, with effects on both biofilm and planktonic cells *in vitro*.

Aceite esencial de *Allium sativum* como tratamiento alternativo contra aislamientos de *Candida* procedentes de prótesis dentales

Palabras clave:
Candida albicans
Candida glabrata
Aceite esencial de *Allium sativum*
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Prótesis dental
Cepas clínicas orales

RESUMEN

Antecedentes: La colonización por parte de *Candida albicans* de las superficies de las prótesis dentales se asocia con el desarrollo de estomatitis. Se ha propuesto el uso de fluconazol, pero su desventaja es la resistencia microbiana. El aceite de *Allium sativum* ha mostrado su efectividad al controlar la formación de biopelícula de objetivos.
Objetivos: Determinar la sensibilidad de cepas clínicas de especies de *Candida*, obtenidas de prótesis dentales parciales o totales rígidas de base acrílica, al aceite esencial de *A. sativum* y comparar su efecto en células planctónicas y en biopelícula.
Métodos: Se incluyeron 48 cepas clínicas de la superficie acrílica de prótesis dentales totales o parciales, identificadas entre las siguientes especies: *C. albicans, Candida glabrata, Candida tropicalis y Candida krusei*. Se evaluó la sensibilidad de cada una al aceite esencial de *A. sativum* y al fluconazol mediante la metodología M27-A3 del CLSI, tanto sobre células planctónicas como en biopelícula, y mediante el método semicuantitativo de la reducción de XTT en el último caso.

* Corresponding author.
E-mail addresses: lo.sanchezvargas@gmail.com, cdlosv@gmail.com (L.O. Sánchez-Vargas).

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Resultados: Todas las cepas planctónicas de *Candida* fueron sensibles al aceite esencial de *A.sativum*, mientras que el 4,2% fue resistente al fluconazol. En cuanto a su sensibilidad en biopelícula, el 43,8% fue resistente a *A. sativum* y el 91,7% lo fue al fluconazol.
Conclusiones: Todas las cepas en forma planctónica de las diferentes especies de *Candida* fueron sensibles a concentraciones inferiores a 1 mg/ml del aceite esencial de *A. sativum* y en menor proporción a fluconazol. La sensibilidad disminuyó en las células en biopelícula, con mayor resistencia al fluconazol en comparación con el aceite esencial de *A. sativum*. Por tanto, el aceite esencial de *A. sativum* es activo frente a cepas clínicas de diferentes especies de *Candida*, obtenidas de dentaduras, con efectos en biopelícula y células planctónicas *in vitro*.

The incidence and prevalence of fungal infections have increased considerably in recent years. The main fungal cause of these infections is the genus *Candida*,[20] comprising 17 species of medical interest that have been associated with infections in humans, and particularly *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata*.[10,33]

Wearing complete dentures is a risk factor because these prostheses can promote *Candida* biofilm formation and oral candidiasis.[31] *Candida* infections are commonly associated with biofilms on both the mucosa and the plastic surfaces of indwelling devices. These biofilms consist of matrix-enclosed micro-colonies of yeast, hyphae, and pseudohyphae arranged in a complex structure[18] and are inherently resistant to antifungals, so affected devices generally need to be removed.[34,35]

Candida pathogenicity has been attributed to several virulence factors, including adhesion to host cells or medical devices; biofilm formation; and the secretion of hydrolytic enzymes such as proteases, phospholipases, and hemolysins.[14,42] Among clinical *Candida* strains, biofilm formation is variable and depends on the species.[25,28]

Colonization of and biofilm formation on the surfaces of dental prostheses comprise important risk factors for the development of denture stomatitis because they support diverse microbial species, promoting the mechanisms that confer resistance and increase pathogenicity in *Candida* in particular. In fact, Bilhan et al. (in 2009) and De la Rosa et al. (in 2012) reported a significant association between the presence of *C. albicans* on prosthetic surfaces and the development of denture stomatitis.[5,17]

Regarding the use and care of dental prostheses, hygienic techniques and the use of medicines, antiseptics, and nanomaterials, among other approaches, have been proposed to control biofilm development and possible secondary conditions associated with the microorganisms present in these prostheses.[21,38] However, among the disadvantages of these proposed approaches are the emergence of strains with antimicrobial resistance and alterations in the prosthetic structure. Therefore, studies of alternative treatments that offer the fewest secondary effects are required. Alternative medicine includes several natural compounds that are effective against certain pathogenic species of interest in dentistry, as in other medical fields.[27,30]

The essential oil of *Allium sativum* comprises one of the natural alternatives most robustly proven to exhibit activity against diverse microorganisms. Previous works report that allicin is one of the active components of *A. sativum* oil and exerts a significant effect in inhibiting the growth of *Pseudomonas aeruginosa* biofilms.[30] In 2011, Khodavandi et al. reported that the oil of *A. sativum* has a similar effect in controlling biofilms of *C. albicans* American Type Culture Collection (ATCC) strains in comparison with fluconazole.[27]

The purpose of the present study was to determine the antifungal activity of the essential oil of *A. sativum* and to compare it with that of fluconazole in the context of clinical isolates of *Candida* species obtained from dental prostheses.

Materials and methods

Study design and clinical isolates

A cross-sectional study approved by the institutional bioethics committee was conducted. A total of 56 patients aged ≥40 years with total or partial bilateral, rigid, acrylic-based dental prostheses (16 maxillary, 12 mandibular and 28 both maxillary/mandibular) and with a minimum of 6 months of use were included in our study. When the patients had both types of prostheses (maxillary/mandibular), only one sample was taken. All of them had requested diagnostic dental care during the period of July to December 2014.

Patients with flexible dental prostheses were excluded. Those who agreed to participate were given a brief explanation of the protocol and were then asked for authorization by signing an informed consent form.

The patients were asked to remove their dental prosthesis from their oral cavity in order to undergo a clinical examination of the oral mucosa with artificial light and a dental mirror. Any pathological lesions observed were registered. If a patient was diagnosed with sub-prosthetic stomatitis, exfoliative cytology was performed, and the sample was stained using the Gram technique to discern whether yeast, hyphae, and/or pseudohyphae were present. The cytology result was reported to the patient, and the case was then followed until remission.

Samples were taken from the internal surfaces of the acrylic prosthesis basis with a sterile swab (Protec®; México, D.F., México); in particular, after the prosthesis was removed, scraping of all support surfaces that were in contact with the palate and the alveolar ridges was immediately performed. After being obtained, the swabs were suspended in 500 μL of 0.9% saline solution (PiSA, Jalisco, Mexico) and were then immediately taken to the microbiology laboratory for processing.

Microbiological assessment and identification of isolates

Each sample collected was mixed for 20 s with a vortex, after which 100 μl of the suspension were plated on CHROMagar *Candida* (CHROMagar®; Paris, France) and incubated at 36 °C for 2 days. To ensure that seemingly negative samples were in fact negative, these samples were incubated for an additional 7-day period at a temperature of 30 ± 1 °C. Using chromogenic cultures, presumptive identification of *Candida* species was performed according to the colorimetric characteristics described by the manufacturer of the agar for each species (colonies green in color = *C. albicans*, mauve in color = *C. glabrata*, pink in color with a curly texture = *C. krusei*, and blue in color = *C. tropicalis*). The use of this medium allowed the separation of two or more strains from the overgrowth of different species from the same sample. Cultures positive for one or more species and purified cultures were reseeded and purified on Sabouraud glucose

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