Enhancement of the Antifungal Activity of Antimicrobial Drugs by *Eugenia uniflora* L.

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ABSTRACT Candidiasis is the most frequent infection by opportunistic fungi such as *Candida albicans*, *Candida tropicalis*, and *Candida krusei*. Ethanol extract from *Eugenia uniflora* was assayed, for its antifungal activity, either alone or combined with four selected chemotherapeutic antimicrobial agents, including amphotericin B, mebendazole, nistatin, and metronidazole against these strains. The obtained results indicated that the association of the extract of *E. uniflora* to metronidazole showed a potential antifungal activity against *C. tropicalis*. However, no synergistic activity against the other strains was observed, as observed when the extract was associated with the other, not enhancing their antifungal activity.

KEY WORDS: • *antifungal activity* • *Candida sp.* • *citotoxicity* • *Eugenia uniflora* • *modifying activity*

DEVELOPING COUNTRIES WITH TRADITIONAL use of the biodiversity as medicine, including Brazil, still suffer with the so-called “neglected diseases”,¹ which are treated by traditional communities with plant natural products. Brazil features the largest biodiversity in the world.² However, only 8% have been studied in search for bioactive compounds.³

Candidiasis is the most frequent infection caused by opportunistic fungi. The main species associated with this disease are *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, and *Candida krusei*. The clinical features of candidiasis are quite diverse, varying between mucosal colonization to the invasion of several internal organs.⁴ These yeasts remain in the microbiota, becoming pathogenic in cases such as congenital or acquired immunodeficiency and immunosuppression.⁵ Several natural products have been studied extensively in the search for alternative treatments for these fungi, including *Himantanthus articulatus*, *Mentha longifolia*, *Malva sylvestris*, and *Psidium guajava*.^{6–8}

The effects of all natural products can be limited by their toxicity. Evaluating the toxicity of active substances is one of the most important steps for the utilization of these compounds in animal models. The drugs currently utilized

against Chagas disease and candidiasis feature high toxicity, affecting host tissues.⁹

Eugenia uniflora is often used as food and medicine in folk medicine due to antimicrobial and other biological activities.^{10,11} Known in Brazil as *pitanga*, this plant has been studied due its antioxidant, hypotensive, photosensitizing, and antibiotic modulatory activities.^{12–15} Several phytoconstituents of *E. uniflora* have been isolated, such as flavonoids myricitrin, quercetin and quercitrin 3- rhamnoside, as well as steroids, mono- and triterpenoid compounds, tannins, anthraquinones, phenols, cineol, and essential oils.^{16,17}

Thus, due to the social and economic importance of candidiasis as neglected diseases and the medicinal use of this fruit in ethnomedicine, this work evaluated the antifungal and cytotoxic activities of *Eugenia uniflora*.

Leaves of *E. uniflora* were collected during the rainy season (April, 2008) in the municipality of Crato, Ceará State, Brazil. The plant material was identified by Dr. Arlene Pessoa, and a voucher specimen was deposited with identification number #3106 at the “Dárdano de Andrade Lima” Herbarium of the Regional University of Cariri (URCA).

A total of 200 g of leaves were dried and powdered at room temperature. The powdered material was extracted by maceration using 1 L of 95% ethanol as solvent at room temperature. The mixture was allowed to stand for 72 h at room temperature. The extract was then filtered and concentrated under vacuum in a rotary evaporator (60°C and 760 mm/Hg of temperature and pressure).¹⁸ Each 200 g of aerial parts yield 5.6 g of extract. The *E. uniflora* ethanol extract (EEEU) was diluted using DMSO.

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The fungal strains utilized in the assays were *C. albicans* ATCC 40227, *C. krusei* ATCC 40147, and *C. tropicalis* ATCC 13803. The strains were obtained from the Clinical Mycology Laboratory, UFPB, Brazil. All strains were maintained in heart infusion agar slants (HIA; Difco, Chicago, USA), and before the assays, the cells were grown for 24 h at 37°C in brain heart infusion (BHI; Difco).

The antifungal drugs amphotericin B (Sigma Co., St. Louis, MO, USA), mebendazole (Lasa-Pharmaceutical Industries Ltda., Campinas, São Paulo, Brazil), nystatin (Laboratório Teuto Brasileiro S/A, Anápolis, São Paulo, Brazil), and metronidazole (Prati, Donaduzzi & Cia Ltda., Curitiba, Paraná, Brazil) were prepared following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).¹⁹

The minimal inhibitory concentration (MIC) was determined using 10% BHI by the microdilution method and suspensions with 10⁵ CFU/ml and an extract concentration ranging between 1024–8 µg/mL.²⁰ The MIC is defined as the lowest concentration at which no microbial growth is observed. For evaluation of the extracts as modulators of resistance to antifungals, a subinhibitory concentration (MIC/8) was mixed with the antifungal drug assayed, in which, the concentration varied between 1024–0.5 µg/mL. The plates were incubated for 24 h at 37°C.

The antifungal and modulatory activity of EEEU is shown in Table 1. The MIC was >1024 µg/mL, which did not demonstrate clinical relevance of the possible use of EEEU as an antifungal drug.²¹ However, an interesting potentiation of the antifungal activity was demonstrated when EEEU was associated with metronidazole against the *C. tropicalis* strain, lowering the MIC of this antifungal drug fourfold.

Holetz *et al.*¹⁰ tested *E. uniflora* against the yeasts *C. albicans*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*, and demonstrated a substantial clinical activity against these yeasts, except for *C. albicans*. Other reports using the essential oil of *E. uniflora* demonstrated activity against dermatophytes.²² These results could indicate that the phytoconstituents extracted with ethanol do not show activity against the strains tested, or that plants from different regions could show different activities. Extracts of other plants, such as *H. articulatus*, *M. longifolia*, *M. sylvestris*, and *P. guajava*,^{6–8} have been tested against yeasts of the genus *Candida* and represent a better alternative to the treatment of candidosis. However, this is the first report

demonstrating the potentiation of the activity of an antifungal drug when combined with *E. uniflora* extract. The potentiating effect of the extracts of *E. uniflora* and of other plants has been demonstrated against multidrug-resistant bacteria.^{23–26}

This modulatory strategy is called herbal shotgun or synergistic multieffect targeting and refers to the utilization of plants and drugs in an approach using mono- or multi-extract combinations, which can affect not only a single target, but various targets, where the different therapeutic components collaborate in a synergistic agonistic manner. This approach is not only meant for combinations of extracts; combinations between natural products or extracts and synthetic products or antibiotics are also possible.^{27–28}

Our results indicate that *E. uniflora* (and the family Myrtaceae in general) could be a source of nutraceuticals with an antifungal-modifying activity, representing an interesting alternative to combat infectious diseases such as candidiasis. This plant appears to be promising in the development of therapies, mainly due to the low toxicity *in vitro*, which allows us to proceed with *in vivo* studies for drug evaluation.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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TABLE 1. MINIMUM INHIBITORY CONCENTRATION AND MODULATORY ACTIVITY OF *EUGENIA UNIFLORA* COMBINED WITH ANTIFUNGAL DRUGS AGAINST THE YEAST STRAINS

Extract/ antifungal	C. albicans		C. krusei		C. tropicalis	
	Alone	+EEEU	Alone	+EEEU	Alone	+EEEU
EEEU	>1024	-	>1024	-	>1024	-
Anphotericin B	>1024	>1024	>1024	>1024	>1024	>1024
Mebendazol	>1024	>1024	>1024	>1024	>1024	>1024
Nistatin	>1024	>1024	>1024	>1024	>1024	>1024
Metronidazol	64	64	>1024	>1024	128	32

EEEU, *Eugenia uniflora* ethanol extract.

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