

Anti-Yeast Activity of Cinnamaldehyde, Eugenol and Linalool

Buket Kunduhoglu

Abstract— It is well known that essential oils and their specific constituents have antimicrobial effects against several pathogenic and saprophytic microorganisms. Therefore, essential oils can be used as alternative or complementary antifungal agents against pathogenic yeasts, especially drug-resistant strains. In addition, essential oils can be used to reduce the effective dose of antifungal drugs. This study evaluates the anti-yeast potential of some essential oil constituents (EOCs), namely, cinnamaldehyde, eugenol and linalool. *Candida albicans*, *Candida glabrata*, *Candida tropicalis* and *Saccharomyces cerevisiae* were used as indicator test strains. EOCs exhibited significant fungicidal activity against indicator strains. The minimum fungicidal concentration (MFC) values of the EOCs ranged from 0.048 to 3.12 µl/ml, while the MFC values of amphotericin B and ketoconazole (positive controls) ranged from 0.78 to 1.56 µg/ml and 6.25–12.25 µg/ml, respectively. These compounds could be further developed into new antifungal agents, either alone or in combination with conventional antifungals.

Index Terms— Anti-yeast activity, Cinnamaldehyde, Eugenol, Linalool.

I. INTRODUCTION

Candidiasis is a fungal infection caused by yeast belonging to the *Candida* genus. More than 20 species of *Candida* can cause infections in humans [1]. Candidiasis ranges from superficial infections to deep invasive infections. Candidiasis is an important health issue not only for immunocompromised patients but also for healthy people [2]. The most common *Candida* species isolated from clinical fungal invasive infections is *Candida albicans*, followed by *C. tropicalis*, *C. parapsilosis* and *C. glabrata* [3,4]. The CDC [5] reported that increasing resistance of *Candida* species to antifungal medications is an emerging public health problem worldwide. Consequently, it is very important to discover alternative antimicrobial compounds. Many studies in the literature claim that plant extracts and essential oils (EOs) obtained from plants are one of the most promising alternative sources of antifungal agents [6-18]. Phenolics, polyphenols, terpenoids, alkaloids, lectins and polypeptides are the major groups of phytochemicals that possess antimicrobial properties [6,7,19].

Because of their natural origin, antimicrobials obtained from plants are also considered to be safer compared to synthetic compounds [19,20].

Additionally, plant-derived antifungals show promise for use against drug-resistant yeasts, because they may have different target sites than traditional antimicrobials and different mechanisms of action [19,21-23]. EO compounds are lipophilic, meaning that they can easily pass through the cell wall and cytoplasmic membrane. They disrupt the structure of the polysaccharide, fatty acid, and phospholipid layers, making the membrane permeable. Consequently, the antimicrobial effects of EOs are linked to their composition and cytotoxic effects, including cell membrane damage [24]. Thus, this study was designed to determine the antifungal activity of the essential oil constituents (EOCs) cinnamaldehyde, eugenol and linalool.

II. EXPERIMENTAL

A. Materials

Stock solutions of chemicals

Cinnamaldehyde (C₆H₅CH=CHCHO, 93+%, natural), eugenol (C₆H₁₂O₂, 98+%, natural) and linalool (C₁₀H₁₈O, 95+%, natural) were purchased from the Sigma-Aldrich Chemical Co. (Germany).

Essential oil compounds and ketoconazole were prepared by dissolving in 20% DMSO (Merck, Germany), while amphotericin B was dissolved in sterile distilled water. All stock solutions were then filter sterilized, and serial two-fold dilutions of the compounds were prepared.

B. Methods

LD₅₀ values of the EOCs

Artemia salina (Brine shrimp) acute toxicity assays were used to determine the cytotoxicity levels of the EOCs [25]. The LD₅₀ was defined as the concentration of the EOCs needed to cause half of the tested brine shrimp to die within 24 h.

Indicator test strains

Candida glabrata (NRRL Y-1418), *Candida tropicalis* (NRRL Y-12968) and *Saccharomyces cerevisiae* (NRRL Y-11878) were obtained from the United States Department of Agriculture Agricultural Research Service (NRRL, Peoria, Illinois, USA). *Candida albicans* (ATCC 60193) was purchased from the American Type Culture Collection (LGC Standards GmbH Mercatorstr. 51 46485 Wesel Germany).

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC value was determined using the broth microdilution susceptibility assay according to the NCCLS [26]. First the minimum inhibitory concentration (MIC) and then the minimum lethal/fungicidal concentrations (MFC) of the EOCs (cinnamaldehyde, eugenol and linalool) and the antifungal drugs were determined. The MIC was defined as the lowest concentration of chemical that prevented growth of

the test yeasts. The MFC was defined as the lowest concentration yielding negative subcultures. All tests were performed in duplicate and in parallel.

III. RESULTS AND DISCUSSIONS

A. Acute toxicity values of the EOCs

In this study, an *A. salina* test was used to determine the LD₅₀ values of the EOCs. This *in vivo* acute toxicity test can be used to screen the toxicity of natural and synthetic organic compounds [27] because *A. salina* is highly sensitive to a variety of chemical substances [28]. Additionally, it has been shown that the results of brine shrimp lethality tests correlate with rodent and human acute oral toxicity data [29,30]. The LD₅₀ values of the EOCs ranged from 16.9 to 70.3 µl/ml (Table 1). The assay results were then used to select the EOC dosages to be used in the anti-yeast activity studies.

Table 1. LD₅₀ values of EOCs.

EOCs	LD ₅₀ (µl/ml) values
Cinnamaldehyde	25.6
Eugenol	16.9
Linalool	70.3

B. Anti-yeast potential of the EOCs

Cinnamaldehyde, eugenol and linalool displayed promising fungistatic activity against the yeast strains used in this study (data not shown). In addition, the EOCs had fungicidal activities against all indicator strains tested. The MFCs ranged from 0.048 to 3.12 µl/ml (Figure 1). Fungicidal doses of the EOCs were significantly lower than their LD₅₀ concentrations (between 1/8 to 1/22 of the LD₅₀ concentrations). Eugenol and cinnamaldehyde displayed fungicidal activity at relatively lower doses than linalool. Supporting our results, several studies have reported that cinnamaldehyde [31], eugenol [32] and linalool [33] had significant antifungal activity. Therefore, the EOCs tested in this study have the potential for use in antifungal chemotherapy, either alone or in combination with conventional antifungals. As the MLC values of these EOCs are very low compared to the LD₅₀ values, toxicity may not be a concern; however, more detailed toxicity studies are needed.

C. Anti-yeast activity of antibiotics

Antifungals such as fluconazole, miconazole, itraconazole, nystatin, ketoconazole and amphotericin B are used for the treatment of systemic and superficial fungal infections. In this study, ketoconazole and amphotericin B were used as conventional antibiotics against the test yeasts. All the test yeasts were sensitive to the antifungals; the MFCs of ketoconazole ranged from 6.25 to 12.5 µg/ml, while the MFCs of amphotericin B ranged from 0.78 to 1.56 µg/ml (Figure 2). Additionally, fungicidal concentrations of the antibiotics were significantly higher than those of the EOCs.

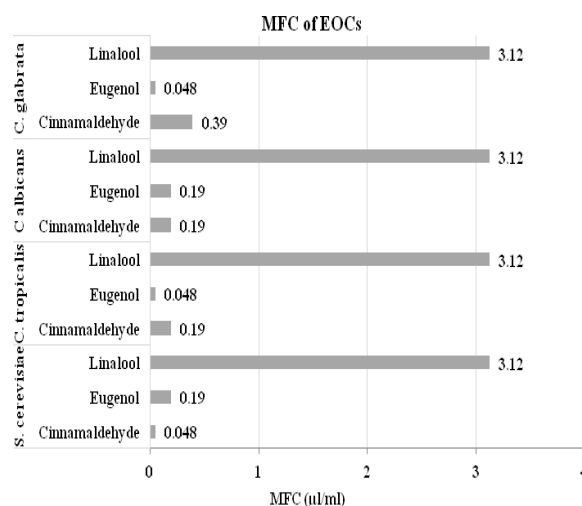


Fig. 1. Minimum fungicidal concentrations (MFCs) of EOCs.

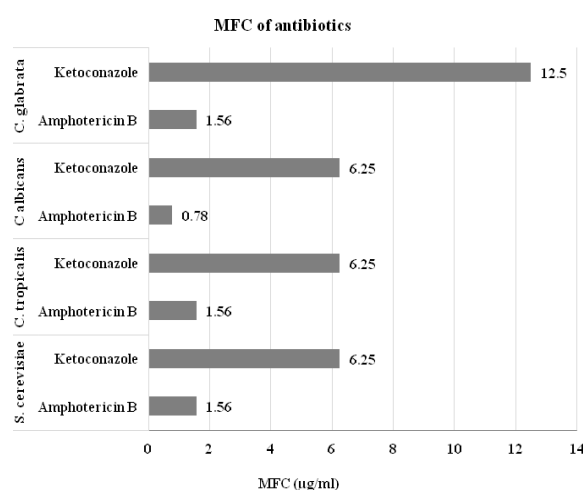


Fig. 2. Minimum fungicidal concentrations (MFCs) of conventional antibiotics.

IV. CONCLUSIONS

The increasing resistance of human pathogens to current antimicrobial agents is a significant problem in modern healthcare. Therefore, there is a real need for the development of new types of antimicrobial substances to treat individuals infected with multidrug-resistant bacteria and fungi. In this study, cinnamaldehyde, eugenol and linalool were evaluated for their antifungal activity. The results showed that cinnamaldehyde, eugenol and linalool exhibited significant fungicidal activity against all indicator test yeasts. Furthermore, these compounds were not toxic at their effective antimicrobial concentrations. In conclusion, these compounds could be used for future development of anti-candidal agents, either alone or combination with conventional antifungal therapeutics. However, the *in vivo* effects of these compounds must be determined to safely use these compounds.

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Dr. Buket Kunduhoglu; Department of Biology, Faculty of Science and Arts, University of Eskişehir Osmangazi, Eskişehir – TURKEY. Bachelor of Department of Biology, Faculty of Science and Arts, University of Anadolu Eskişehir – TURKEY. Lecturer of the general microbiology, food microbiology, microbiology of fermented foods and industrial biotechnology courses.

Publications: Co autor of 30 research papers.

Research interests: antimicrobial agents, biological activity tests, antimicrobial peptides, lactic acid bacteria, probiotics.