Antifungal Properties of Some Commercial Extracts Against *Candida albicans*

Bachir Raho Ghalem^{*}, Boudali Talia, Seddiki Asmaa

Laboratory of Process Engineering and Chemistry of Solutions, Biology Department, University of Mascara, Mascara, Algeria

Email address:

bachir_raho@yahoo.fr (B. R. Ghalem)

^{*}Corresponding author

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Abstract: To evaluate the antifungal activity of five commercial extracts against *Candida albicans in vitro*, five commercial extracts obtained from *Allium sativum*, *Chamaemelum nobile*, *Thymus vulgaris*, *Zingiber officinale* and *Ricinus communis* were tested at three different concentrations (pure, 1/2 and 1/4) for their antimicrobial activity against *C. albicans* using agar disc diffusion method. *C. albicans* was least susceptible to the commercial extracts. The diameter of zone inhibition ranged between 6 and 13 mm. *Z. officinale* and *T. vulgaris* extracts appeared to be the most active, while *A. sativum*, *C. nobile* and *R. communis* extracts exhibited most weak antifungal activity against *C. albicans*. These findings increase the possibility of exploiting these commercial extracts as a safe alternative natural preservative.

Keywords: Commercial Extracts, Antifungal Activity, *C. albicans*

1. Introduction

C. albicans is a harmless commensal dimorphic yeast-like fungus in healthy humans, which can cause superficial as well as life threatening systemic infections like oral candidiasis, vulvovaginal candidiasis and so on in humans [1, 2]. *C. albicans* can infect virtually all body sites because of its high adaptability to different host niches -by the activation of appropriate sets of genes in response to complex environmental signals-and forms microbial biofilms on catheters, dentures and mucosal cell surfaces that makes *C. albicans* resistance to antimicrobial agents and host immune factors [3-5]. An increase in antifungal resistant *Candida* strains has been reported in recent years [6]. The emergence of antibiotic resistant *C. albicans* along with the essential role of *C. albicans* in high incidence of infections make it a pressing mission to discover and identify new hits and leads from synthesized chemicals or natural products. Compared to synthesized chemicals, natural products have many advantages such as structural diversity and relatively low toxicity [7-11]. Plants are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, saponins, alkaloids, flavonoids, and other

compounds, reported to have *in vitro* antifungal properties [12, 13]. In this context, our aim was to evaluate the possible therapeutic potential of some commercial extracts against this human dimorphic commensal organism, which can become a facultative pathogen under altered physiological situations.

2. Materials and Methods

2.1. Commercial Extracts

In this study, five plant extracts were used: *Allium sativum*, *Chamaemelum nobile*, *Thymus vulgaris*, *Zingiber officinale* and *Ricinus communis*. These extracts are imported by the import-export company Enour of Algeria from Eloued city and produced by the Egyptian company El Capitaine (CAPPHARM) for the extraction of oils from natural and cosmetic plants.

2.2. Test Yeast

In our examinations, the yeast *Candida albicans* was used to determine anti-yeast activity of five extracts. For this purpose, clinical isolates from woman patients at the polyclinic of Sidi M'hamed of Mascara. Isolates were

maintained on Sabouraud dextrose agar at 4°C in refrigerator until used in the study.

2.3. Antifungal Assays

Micro-broth dilution and disc diffusion assays were used for antifungal screenings.

a) Disc diffusion assay

Disc diffusion assay of extracts was performed as described by Ghannadi *et al.* (2012) [14], with modifications. In order to test antimicrobial activity, the extracts were dissolved in dimethylsulfoxide to ½ (50%) and ¼ (25%). Sterile discs (6 mm in diameter) were impregnated with 7 µL undiluted extract (100%) and diluted (25% and 50% in DMSO) with approximately 10⁸ yeast and placed in Petri dishes, over agar and dispersed. Inhibition zones were determined after incubation at 30°C for 48 h and measured in mm. Negative controls were prepared on discs impregnated with dimethylsulfoxide (solvent control). Positive control (cultured along with the bacterium without the extract).

b) Minimum Inhibitory Concentration and Minimal fungicidal Concentration

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of five commercial extracts were determined by micro-broth dilution assay in 96 multi-well microtiter plates according to Chebaibi *et al.* (2016) [15], with modifications. 20 µL of DMSO was added to each of the 96 wells of a microtitre plate, followed by 20 µL of each extract to the first row. This was serially diluted descending down the microtitre plate columns.

The wells of a 96-well which content DMSO and extract were filled with 160 µL of Sabouraud broth (SB) inoculated with 20 µL of exponentially growing culture (about 10⁸ colony-forming units/ml). The wells which content SB and growing culture serve as positive controls and this which content DMSO and SB non inoculated were used as negative controls. The microplate was incubated at 30°C for 48 h. MICs were defined as the lowest concentration of essential dilution that inhibits yeast growth after 48 h. MFC values were the first well showing no growth on Sabouraud dextrose agar [16].

3. Results and Discussion

The present study was designated to evaluate anti-yeast activity of five commercial extract plants, which showed different antimicrobial activity against tested microorganisms, depending on the concentration of used extract. Based on data obtained from the examination of the anti-yeast activity of extracts against *Candida albicans*, we obtained following results that are shown in Table 1. According to these results, the diameters of the zones ranged between 6 and 13 mm. *Z. officinale* extract had the largest inhibition zone (13 mm) on *C. albicans* followed by *T. vulgaris* extract (12.5 mm). *A. sativum*, *C. nobile* and *R. communis* extracts were inactive against this yeast with smallest inhibition zone (6 mm). This yeast manifested certain sensitivity to extracts (*Z. officinale* and *T. vulgaris*),

with inhibition zones which increased with extract concentration increasing.

Table 1. Antifungal activities of the extracts against *C. albicans* by disk diffusion method.

Extracts	Pure extract	1/2	1/4	Negative control	Positive control
<i>A. sativum</i>	6	6	6	6	6
<i>C. nobile</i>	6	6	6	6	6
<i>T. vulgaris</i>	12.5	9	6	6	6
<i>Z. officinale</i>	13	8.5	6	6	6
<i>R. communis</i>	6	6	6	6	6

The MIC and MFC values of *Z. officinale* extract against *C. albicans* were 53.75 mg/ml and 215 mg/ml, respectively. The obtained result is agreement with those reported by many researchers who shown the antifungal of ginger extract against this yeast. Mohammadi and Moatar (2007) [17] evaluated the antifungal properties of ginger essential oil on 25 clinical isolates of fluconazole-resistant *C. albicans* and reported that is effective against all isolates of *C. albicans*. Atai *et al.* (2009) [18], Supreetha *et al.* (2011) [19] and Aghazadeh *et al.* (2016) [20] showed that Ethanolic ginger extract was effective against *C. albicans*. Atai *et al.* (2009) [18] said that there are several components in the ginger which have anti-fungal effects and among them the gingerol and shagelol were identified as more active agents. In case of the effect of *T. vulgaris* extract on *C. albicans*, the obtained results are in agreement with those obtained by other investigators. The effectiveness of *Thymus vulgaris* EO on *C. albicans* was reported by Fani and Kohanteb. (2017) [21]. A high anticandidal activity of thyme oil (*T. vulgaris*) was confirmed by Al-Shahrani *et al.* (2017) [22] (MIC50 1 mg/ml). In another study on the essential oil of *T. vulgaris* the growth of *C. albicans* was inhibited with MIC varying between 0.12 and 0.17 µg/mL [23]. Anticandidal activity of *T. vulgaris* and five plant (*Citrus limonum*, *Pelargonium graveolens*, *Cinnamomum cassia*, *Ocimum basilicum*, and *Eugenia caryophyllus*) essential oils distributed by Pollena Aroma (Nowy Dwór Mazowiecki, Poland) was reported by Gucwa *et al.* (2018) [24]. The antimicrobial activities of *T. vulgaris* oil appear to be associated with the phenolic compounds thymol and carvacrol [21].

Many investigators have demonstrated the antifungal potential of thymol against species of yeasts and filamentous fungi. Thymol inhibits the growth of *Candida* species sensitive and resistant (clinical isolates) to azoles and amphotericin B [25-27]. Jamali tested the antifungal activity of seven *Thymus* species: *T. broussonetii*, *T. ciliates*, *T. leptobotrys*, *T. maroccanus*, *T. pallidus*, *T. satureioides*, and *T. serpyllum* collected from different natural regions in southern and southwestern Morocco. The results show high activity on the four tested *Candida* species (*C. albicans*, *C. krusei*, *C. glabrata*, and *C. parapsilosis*) for EOs rich in thymol or carvacrol (MIC 0.43–0.9 mg/mL). *T. serpyllum* EO, in which the predominant compound detected was linalyl acetate (52.2%), had the lowest anticandidal activity (MIC in the range 3.52–7.05 mg/mL) [28].

4. Conclusion

Z. officinale and *T. vulgaris* extracts showed fungistatic activity against strains of *C. albicans*. The extract from *Z. officinale* exhibited the greatest inhibitory activity against strains of *C. albicans*, followed by *T. vulgaris*. The results from this study revealed that *Z. officinale* and *T. vulgaris* extracts could be used as an alternative agent for treatment of infections caused by *Candida albicans*.

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