Plant Archives Vol. 20, Supplement 1, 2020 pp. 172-174 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ANTIFUNGAL ACTIVITY OF MIXTURE *EUGENIA AROMATICUM* AND *THYMUS VULGARIS* ESSENTIAL OILS AGAINST *CANDIDA ALBICANS* CLINICAL STRAINS IN AL-MUTHANNA PROVINCE, IRAQ

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Abstract

The aim of the study was to investigate the antifungal properties of plant essential oils (EOs extracted from Mixture of *Eugenia aromaticum* and *Thymus vulgaris* -M-ET) against *Candida albicans* clinical strains. The antifungal activity of the plant EOs were determined by broth microdilution and microtiter assays. The results indicated a good antifungal activity of M-ET EOs against *C. albicans* strains. The overall antifungal results demonstrated that the investigated plant EOs represent promising approaches to cope with the emerging antifungal resistance.

Keywords **:** EOs, antifungal activity, *Candida albicans*.

Introduction

The novel antibiotic resistance and recurrence of fungal infections linked the attention of scientists to conduct intensive studies about this problem. It has been indicated that immunodeficiency disease (AIDs), diabetes and leukemia are associated with fungal re-infections (Granger 1992).

Candidiasis is a common opportunistic diseases of skin and oral cavity caused by Candida species. The most common and worldwide distributed one is *C. albicans* (Sanata *et al.,* 2010). This pathogenic yeast owns several virulence factors involving; adherence, germ tube formation and enzymes production, such as phospholipase (Taweechaisupapong *et al.,* 2005). Candidaisis is a common recurrent infection due to the presence of the causative agent, *C. albicans* mainly, as a commensal fungi in oral cavity, vaginal and digestive system (Hannula 2000), Getting infected with human immunodeficiency virus (HIV), diabetes, Leukemia or administration of broad spectrum antibiotics may promotes converting the normal flora to opportunistic microbes (Santos 2001, Brooks 2001).

Currently, several plant pharmaceuticals have been used as natural anti-fungal formulations instead of synthetic anti-fungi which are used as skin ointment due to their toxicity on liver and pancreases when used at high concentrations (KwonChung & Bennett 1992) So, urgent approaches are needed to fight against extensively drug-resistant fungal infections. Prevention and control of the emergence and spread of antifungal resistance require global efforts contributing to the solution. New prophylactic and therapeutic strategies must be developed as the existing antifungals become ineffective. Plant essential oils (EOs) constitutes a group of phytochemicals with potential application in development of novel antifungal agents that could contribute to the reduction in drugs use and thus to the maintaining of drugs effectiveness. Additionally, several reports demonstrated that they present a synergistic effect in combination with drugs thus they could be employed in the development of safe drug associations for fighting multi-drug resistance (Saviuc 2013 & Nikolic 2014), also there are a new methods and preservatives techniques, in capsules and dry tablets, have been innovated to facilitate using of these plants and maintain their antimicrobial activity (Al-Ali, 2007). The aim of the present study was the investigation of antifungal properties of plant essential oils (EOs extracted from *Eugenia aromaticum* and *Thymus vulgaris*-M-ET) against *Candida albicans* clinical strains (Al-qertani & Mohamme, 2018).

Materials and Methods

Samples Collection and Isolation of *C. albicans*

A total of 16 clinical isolates were collected using sterile swabs from patients showed a clinical sings of Candidiasis. A samples were collected from different sites, i.e. respiratory tract, wound and urinary tract infections, *Candida albicans* was included in the present study. Samples were collected at admitted at the Al-Hussein teaching Hospital and inoculated directly in a test tubes containing phosphate buffer saline (PBS). All samples were kept at refrigerator (4°C) until the time of inoculation and identification**.**

Direct Examination and culture

Each sample was directly examined microscopically and stained using Gram staining method to identify the Gram-positive yeast (Collee 1996, KwonChung and Bennett 1992). For direct examination and identification of yeast cells and pseudomycilium, light microscope was used at (40X, 100X). Microbial strains were identified using the automatic system Vitek II.

Plant EOs

Essential oils used in this study were extracted in laboratories Al Muthanna university- faculty of science, department of Biology, from the mixture of *Thymus vulgaris* and *Eugenia aromaticum*. These were separately grounded and powdered in domestic blender and hydro distilled in a Clevenger's apparatus by the technique of (Guenther, 1948) to obtained essential oils. Dilution was made after a known volume of each oil was diluted by adding fresh solvent (DMSO) and stored at 4°C till used.

Assessment of the influence of plant EOs on microbial isolates growth

Binary serial microdilution method performed in 96-well microtiter plates was used to determine the minimum inhibitory concentration (MIC) for each of the plant EO against the above mentioned fungal strains. Briefly, microbial suspensions prepared from 24 hours microbial cultures were adjusted to a density of 0.5 Mc Farland, corresponding to 1.5 x 108 CFU/ml and added in wells containing a range of plant EOs prepared by binary serial dilution using DMSO. After inoculation, microtiter plates were incubated for 24 h at 37°C. Wells containing broth inoculated with microbial strains and wells with uninoculated broth were used as positive growth controls and as negative controls, respectively. Solvent control tests were also performed to determine the effect of DMSO on microbial growth. The experiments were performed in duplicate. MICs were determined as the lowest concentration inhibiting microbial growth, indicated by a visible decrease in the growth medium turbidity.

Results and Discussion

We determined the effects of plant essential oils extracted from *Eugenia aromaticum* and *Thymus vulgaris* - *M-ET)*, at various concentrations, on growth of fungal strains isolates using turbidity measurements. Our study revealed that the investigated EOs displayed good antifungal growth effects. The results of antifungal tests are presented in the Table 1. We found that the mixture of M-ET was very efficient. The *C. albicans* strains were susceptible to the tested EOs, with MIC values ranging from 0.7 - 5.56 mg/ml.

Table 1 : Plant EOs MIC values (mg/ml).

Plant EOs **Microbial strains**	***M-ET mixture*** **min – max**
C.albicans(n=16)	0.7 - 5.56

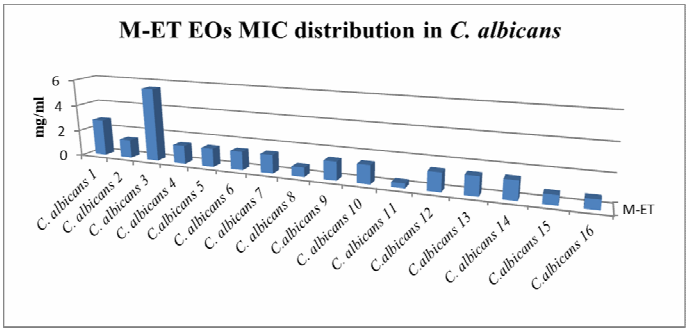

Fig. 1 : minimum inhibitory concentrations for M-ET Eos

Antimicrobial resistance is a global health problem. The emergence and spread of pathogens resistant to current drugs require the development of new strategies (Al-Ali, 2007 and Al-qertani, 2018). Since ancient times, plant EOs are known to possess antimicrobial proprieties, however in order to develop adequate alternatives to the existing antimicrobials, we need first to better understand the mechanisms of the antifungal activity. In this study, we assessed the growth inhibitory activity of mixture *E. aromaticum* and *T. vulgaris* (M-ET) against fungal clinical isolates (*C. albicans*). Entire plant extracts and many of their individual components were demonstrated to possess antimicrobial activity against pathogens *in vitro*. In our study, we found that the tested plant EOs were able to inhibit the fungal growth with different intensities. The clinical isolates exhibited high variations in plant EOs susceptibility that could be explained by the various drug resistance patterns. The M-ET mixture demonstrated the strongest antifungal activity against all clinical isolates that probably correlated with the highest percentage of phenolic compounds, these results are in agreement with previous studies (Hammer 1999, Burt 2004 & Borugă 2015). According to Marchese (2017) p-cymene has enhanced the activity of other antimicrobial substances through synergism, antagonism and additive effects (Marchese, 2017).

The antimicrobial activity of the tested plant EOs could be attributed to high amounts of phenolic compounds: eugenol about 75-85% of *E. aromaticum* EO (Nikolic 2014), thymol, 57.7% and p-cymene 24.79% of *T. vulgaris* EO (Borugă 2014, Zeghad and Merghem, 2013), the major antimicrobial phenolic components are, principally, acting as envelope permeabilizers (Burt, 2004).

Conclusions

With the increasing emergence of antifungal drug resistance, phytochemicals could constitute an alternative therapeutic strategy for fighting infectious diseases. Oily aromatic liquids extracted from plants represent a reservoir of bioactive compounds in nature. In this context, we analyzed the influence of: The mixture of *E. aromaticum* and *T. vulgaris* (M-ET) on drug resistant fungi. The overall results indicated that selected plant EOs displayed a good antifungal activity, that demonstrating their potential as bioactive alternative to conventional synthetic antifungal for controlling drug resistant fungi. However, for drug development additional tests are needed to determine their toxicity and pharmacokinetic and pharmacodynamic properties.

Abbreviations

Eos	Plant essential oils
M-ET	*Eugenia aromaticum* and *Thymus vulgaris* mixture
MIC	Minimum inhibitory concentration

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