



Anti-candidal activities of some *Myrtus Communis* L. extracts obtained using accelerated solvent extraction (ASE)

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ABSTRACT

Candida albicans is a very important organism in production of acute and chronic infections. It can be reason of serious infections in human immune compromised patients as nosocomial infections. In this study, We researched new anti-candida agents from *Myrtus communis* extracts by ASE (Accelerated Solvent Extraction). We were also determined Gallic, Ferulic, Quercetin phenolic compounds known charged with antimicrobial activity by HPLC (High-performance Liquid Chromatography). Gallic acid (2,424 mg/g) and Quercetin (2,289 mg/g) were detected in high amounts in Acetone extract and Methanol extract while Ferulic (0,018 mg/g; 0,038 mg/g; 0,006mg/g) was recorded in low amounts in Methanol, Ethanol and Ethyl acetate extracts, respectively. Methanol, Acetone, Ethanol and Ethyl acetate extracts showed good anti-candida activity against *C. albicans*, with Minimum Inhibitory Concentration (MIC) ranging from 0.187 to 1,5 mg/mL; Minimum Fungicidal Concentration (MFC) ranging from 0,375 to 3 mg/mL.

1. INTRODUCTION

Candidemia is known as the fourth most common nosocomial bloodstream infection. Hence, control of the spread of *Candida albicans* strains and treatment of infections caused by them is very important problem worldwide [1]. In the past decade, the prevalence of resistant to anticandida agents significantly increased. It makes necessary to discover new agents of anti-candidal compounds to treat infections. In particularly, Herbal agents are important in controlling spread of *Candida* [2,3]. In traditional medicine, the leaves of *Myrtus communis* have used mostly in lung and digestive disorders and in the treatment of many types of infectious including candidacies as antiseptic, anti-inflammatory agent, a mouthwash [4]. In recent research, the antifungal effects of extracts of leaves of this plant has been proven. Numerous studies have been devoted to the extraction of *Myrtus communis* leaves [5-8]. In particularly, Phenols in leaf is considered to be main biologically active antimicrobial chemicals produced. *In vitro* experiments have proved its inhibiting effect on *Candida albicans* which known the most frequent human infections [9,10]. The aims of the present study were to determined the anticandidal activities of four extracts of *Myrtus communis* obtained with ASE and researched phenolic component of extracts.

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2. MATERIALS AND METHODS

2.1. Plant material

Aerial parts of *Myrtus communis* were collected in May from Mersin, Turkey and botanical identification of the plant was done by Prof. Ayse Everest at Mersin University Biology Department.

2.2. Preparation of Extracts

The extractions of soluble compounds from *Myrtus communis* by Accelerated Solvent Extraction were performed by using methanol, ethanol, acetone, ethyl acetate as a solvent. The below method was modified by ASE [11].

Aliquots of *Myrtus communis* leaves (2,4 g) were grounded in cold-blender, passed the through a 20 mesh sieve to acquire particles size of <2 mm, mixed and dispersed with diatomaceous earth (1:1) to remove moisture. Then, the samples were placed in a 66 mL stainless steel extraction cell, to perform extractions with solvents on a ASE 150 (Dionex) system.

The dry plant sample (2,4 g) was extracted by one extraction cycles with all solvent (%99 pure) at 100°C, 1500 psi during 5 min.

Then, the cell was rinsed with fresh extraction solvent and purged with a flow of nitrogen (150 psi during 90 s). The final of the experiment, the extraction solution was collected in 66 mL glass vials. This procedure was performed for all of the solvent, separately. The extracts were filtered (with a 0.45 µm membrane) and stored at -20°C in darkness until HPLC and microbial analysis.

2.3. Analysis of Extracts using HPLC

The main compounds of extracts researched on Agilent 1200 HPLC equipment with a autosampler, a DAD, column under the optimum condition modified by Mradu's method [12]. The Column was phenomenex C-18, 3 μ m particle size (50x4.6 mm I.D) type. The condition of HPLC; total run time was 45 minutes, Gradient elution - Solvent A (Acetonitrile) and Solvent B (0.1% Phosphoric acid in water) , program was begun with 85 % B and was held at this concentration for the first 12 minutes, followed by 75 % eluent B for the next 10 minutes after which its concentration was again increased to 85 % for the next 8 minutes. The flow rate was 20 ml/min, the injection volume was 20 μ l, monitored and quantified at 280 nm.

The phenolic compounds of extracts were calculated by the formula:

$$C_x (\mu\text{g/g}) = A_x \times C_s (\mu\text{g/ml}) \times V (\text{ml}) : A_s \times \text{Sample weight (g)}$$

Where C_x is the concentration of the phenolic X, A_x is the peak area of the phenolic X, C_s is the concentration of the Standard and A_s is the peak area of the Standard [13].

2.4. Anti-candida activity of Extracts

The extracts of *Myrtus communis* were tested against *Candida albicans* (clinic strain) to MIC (Minimal Inhibitory Concentration) and MFC (Minimum Fungicidal Concentration). MIC, lowest concentration causing complete visible growth inhibition, and MFC, lowest concentration killing 99.9% cells, were determined by broth microdilution method modified by Dabur and Zore [14, 15]. The yeast inoculum was prepared in 4 ml tryptic soy broth medium, incubated at 37 °C and in sabouraud dextrose broth medium at 28 °C for 24 h before being used overnight, respectively. The culture, 12 hour-cultures were adjusted to 0.5 McFarland Standard Turbidity (~ 10³ for yeast colony forming unit (CFU) per milliliter [16]. Two-fold serial dilutions of extracts of *Myrtus communis* were added to the wells of a 96-well microtiter plate. 100 μ l of each dilution, ranging from 24 to 0,1 mg/ml were mixed with an equal volume of bacterial suspension (25 μ l). Fluconazole antibiotic was used as positive reference standards for yeast. Then, The plates were incubated for 36 hours at 37°C and observed visually for growth (turbidity) inhibition to determine MIC. 5 μ l culture of all wells was inoculated on YPD agar plates for 36 hours at 37°C for determine MFC.

3. RESULTS AND DISCUSSION

Myrtus communis selected for anti-candida activity have been used in traditional medicine for the treatments of related infections. The phenolic content and antimicrobial effect values of some *M.communis* extracts against *C. albicans* were presented in Table 1. *C. albicans* was found sensitive to positive control (Fluconazole). We reported all solvents were toxic more than >12,5 mg/ml of concentration for *Candida albicans* cultures. Hence, for the antimicrobial activity, *M. communis* concentrations which solvent's was not toxic were noted. We reported the Ethanol, methanol, acetone, ethyl acetate extracts showed good

activity against *C. albicans* tested with MIC values ranging from 0.187 to 1,5 mg/mL; MFC ranging from 0,375 to 3 mg/mL. After 36 hours the ranges of MIC obtained with methanol, ethanol, ethyl acetate, acetone extracts were 1,5 mg/ml; 0,75 mg/ml; 0,375 mg/ml; 0,187 mg/ml; MFC were 3 mg/ml; 1,5 mg/ml; 0,75 mg/ml; 0,375 mg/ml, respectively.

Table. 1: The main components of *Myrtus communis* Extracts using HPLC and Minimal Inhibitory and Fungicidal Concentration of Extracts against *Candida albicans*.

	Comp. mg/g	Ret. time	MIC mg/mL	MFC mg/mL
Methanol Ext.	Gallic 0,01	3.553	1,5	3
	Quercetin 2,289	4.225		
	Ferulic 0,018	16.734		
Ethanol Ext.	Gallic 0,019	3.595	0,75	1,5
	Ferulic 0,038	16.706		
Ethyl acetate Ext.	Quercetin 0,336	3.934	0,375	0,75
	Ferulic 0,006	16.771		
Acetone Ext.	Gallic 2,424	3.551	0,187	0,375

Ext: Extraction, Comp: Component, MIC: Minimal Inhibitory Concentration, MFC: Minimum Fungicidal Concentration, Ret.Time: Retention Time. All experimets were performed in five times and averaged.

We reported that all of the extracts in this study had a favorable anti-candidal activity. Acetone extract exhibited strongest activity against *C. albicans* with MIC value of 0,187 and MFC value of 0,375 mg/ml. Concordantly, Gallic acid was found the main component of acetone extract (2,424 mg/g). The lowest activity (MIC: 1,5 mg/ml and MFC: 3 mg/ml) were recorded in methanol extract and the quercetin (2,289 mg/g) were found the main component. Ferulic acid were found in methanol (0,018 mg/g), ethanol (0,038 mg/g) and ethyl acetate (0,006 mg/g) extracts in low amounts while it was not determined in acetone extract. Other studies have shown mostly that essential oils of plant had strong antimicrobial activity [17-20]. However, It has known that classical extraction methods commonly used for the evaluation of antifungal activities of *Myrtus communis*. In literature, Bajur and his colleagues reported the methanol extract of *Myrtus communis* leaves had antifungal activity with MIC: 0,31 mg/mL [21]. It was also shown that alcoholic extract of *Myrtus communis* had different degrees growth inhibition, depending on the *Candida albicans* strains between 25 mg/ml and 2.5 mg/mL [22]. These studies support our study with regard to strong biological activity. Accelerated Solvent Extraction technology was firstly used for evaluation of anticandidal activity of *Myrtus communis* in this study. In previous works, ASE process have been optimized for the extraction of phenolics from Basque Sider apple, Eggplant and Black Cohosh [23-25]. This method is highly advantageous, fast and reliable for plant tissues [26]. Our results corroborate the importance of Accelerated Solvent Extraction (ASE) in the selection of methods for bioactivity screening.

4. CONCLUSION

In sum, We researched for the first time anticandidal activity of *M. communis* extracts obtained using ASE. Datas showed all of the extracts of *M. Communis* had strong anticandidal

activity. We were determined some phenolics (Gallic, Ferulic, Quercetin) were abundant in these extracts. We assume the inhibitory effects of the extracts increased due to ASE process. Hence, there is need further researches to expose components of extracts.

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