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Research Article

Anti-candidal activity of the *Lavandula stoechas* L. against pathogenic *Candida* species isolated from the hospital[‡]

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ABSTRACT

The purpose of this study is to determine antimicrobial activity of extracts of *Lavandula stoechas* L. against five different pathogenic *Candida* species. As test microorganisms, species of *Candida* (*Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida guilliermondii* and *Candida glabrata*) which were isolated from the patients applying to Medical Faculty Hospital of Duzce University were used. Ethyl alcohol, chloroform and ethyl acetate extractions were acquired from the plant via Soxhlet. 25 µl, 50 µl and 75 µl of these extractions were impregnated into sterile discs and the anti-candidal activity spectrums were determined by using the disc diffusion method against test microorganisms. As the control group standard antifungal antibiotics (Griseofulvin, Fluconazole, Amphotericin B, Miconazole, Nystatin, Flucytosine, Clotrimazole, Ketoconazole, Itraconazole) were used and the outcomes were compared. As a result, it is found out that the extractions acquired from *L. stoechas* plant have a very effective anti-candidal activity as compared with standard antibiotics. On the basis of our results, anti-candidal activity *L. stoechas* extracts more effective than standard antifungal antibiotics. So, this situation supports the ethnobotany usage of the plant. In addition to this, the potential extracts of the plant commonly used for alternative medicine may be also used for Candidemia treatment in future.

Keywords: Anti-candidal activity, *Lavandula stoechas* L.

Lavandula stoechas L.' nin hastaneden izole edilen patojenik *Candida* türlerine karşı anti-candidal aktivitesi

ÖZET

Bu çalışmanın amacı, beş patojenik *Candida* türü üzerindeki *Lavandula stoechas* L. ekstraktlarının antimikrobiyal aktivitesini belirlemektir. Test mikroorganizmaları olarak, Düzce Üniversitesi Tıp

Fakültesine başvuran hastalardan izole edilmiş olan *Candida* (*Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida guilliermondii* ve *Candida glabrata*) türleri kullanıldı. Etil alkol, kloroform ve etil asetat ekstraktları, Soxhlet aracılığıyla bitkiden elde edildi. Bu ekstraktlardan 25 ul, 50 ul ve 75 ul si steril disklerle emdirildi ve anti-candidal aktivite spektrumları test mikroorganizmaları üzerinde disk difüzyon metodu kullanılarak belirlendi. Kontrol grubu olarak, standart antifungal antibiyotikler (Griseofulvin, Fluconazole, Amphotericin B, Miconazole, Nystatin, Flucytosine, Clotrimazole, Ketoconazole, Itraconazole) kullanıldı ve sonuçlar karşılaştırıldı. Sonuç itibarıyla, *L. stoechas* L. bitkisinden elde edilen ekstraktların standart antibiyotiklerle karşılaştırıldığında oldukça etkili anti-candidal bir aktiviteye sahip olduğu ortaya çıktı. Sonuçlarımıza göre, *L. stoechas* L. ekstraktlarından elde edilen anti-candidal aktivite standart antifungal antibiyotiklerden çok daha fazladır. Bu yüzden, bu durum bitkinin etnobotanik kullanımını desteklemektedir. Buna ilaveten, alternatif tıpta yaygın olarak kullanılan bitkinin potansiyel ekstraktları gelecekte Candidemia tedavisi için de kullanılabilecektir.

Anahtar Kelimeler: Anti-candidal activity, *Lavandula stoechas* L.

I. INTRODUCTION

THROUGHOUT history people have benefited from herbal medicine in the treatment of many infectious diseases. Today, medicinal plants continue to play an important role as medical drugs with primary health care services in many developing countries [1]. Medicinal aromatic plants are widely used in the treatment of diseases in Turkey.

Lavandula genus is an important member of *Labiatae* (Lamiaceae) family and widely cultivated in the Mediterranean region. In Turkey mainly two species, *Lavandula angustifolia* Mill and *Lavandula stoechas* and their subspecies and hybrid forms grow wildy or cultivated [2].

L. stoechas as been reported by folk healers for various diseases of central nervous system, as migraine, epilepsy, treatment of wounds reduce to blood sugar levels [3]. Not only the plant material and its essential oil has mainly demanded in perfumery cosmetic and food industries. The medicinal importance of the plants is properly documented and the drugs made of this plant are registered in many pharmacopeia [2]. This plant has been used as an antispasmodic in colic pain, carminative, expectorant, urinary infections, cardiac diseases and eczema [4]. In addition, *L. stoechas* has been studied to evaluate the antifungal activity against pathogenic fungi causes of diseases in human [5].

The aim of this research was to indicate an anti-candidal activity of *L. stoechas* extracts in hospital isolates of *Candida* species. The determination of *L. stoechas*'s activity level about *Candida* species which have gained resistance against many antibiotics and have caused patients various infections will contribute to natural antibiotic search done by the research.

II. EXPERIMENT

A. PLANT MATERIAL

The plant were purchased from a public market in Canakkale, Turkey in June, 2014. Specimen was deposited at the Department of Biology, Faculty of Science and Arts, Duzce University, Konuralp-Duzce, Turkey.

B. PREPERATION OF EXTRACT

The leaves of the plant were dried in an oven at 40°C (12h) and powdered. Each dry powdered plant material (15g) was extracted with 150 ml of 95% ethanol (Mecrk, Darmstadt, Germany), 99.5% ethyl acetate (Mecrk, Darmstadt, Germany) and 99.8% chloroform (Mecrk, Darmstadt, Germany), for 24h by using Soxhlet equipment.

C. HOSPITAL ISOLATES

Pathogenic microorganisms were isolated from patients in the Medical Faculty of Duzce University, Konuralp-Duzce, Turkey. *Candida albicans*, *Candida krusei*, *Candida glabrata*, *Candida tropicalis* and *Candida guilliermondii* were used as a test microorganisms. Phoneix system was used for identification. The microorganisms identified were inoculated instead of Nutrient Agar medium. After this process, the *Candida* species were put to incubation at 27-30°C for 24h. At the end of incubation process, microorganism cultures were preserved at +4°C in the refrigerator.

D. DISC DIFFUSION METHOD

Disc diffusion method was used in order to determine the antifungal activity levels of plant extracts [6]. To determine antifungal activity levels Mueller Hinton Agar (OXOID) were used as a medium. The counts of yeast culture were adjusted to yield 10^5 - 10^6 CFU/ml, respectively, using the standard McFarland counting method. The test microorganisms (0.1 ml) were inoculated with a sterile swab on the surface of appropriate solid medium in plates. Three different extracts consisting of 25 µl, 50 µl and 75 µl in concentration were impregnated into 6 mm sterile discs. The yeast cultures were inoculated on Mueller Hinton Agar and incubated for 48 h at 25-27 °C. At the end of the process, the inhibitions of the cultures ejected from the incubator were measured. The standard 6 mm antibiotic discs (Bioanalyse) were used on the object of comparison. For this purpose, GRS10, Griseofulvin 10µg; FLU25, Fluconazole 25µg; AMB100, Amphotericin B 100 µg; MCZ10, Miconazole 10µg; NY100, Nystatin 100 µg; 5FC1, Flucytosine 1µg; CLT10, Clotrimazole 10µg; KTC10, Ketoconazole 10µg; ITR10, Itraconazole 10µg disc were used as positive controls. The study of antifungal activity against all hospital isolates were implemented as three repetitive.

III. RESULTS and DISCUSSION

The *in vitro* results of anti-candidal activity of the *L. stoechas* extracts by the disc diffusion method against five yeast culture of remarkable importance are shown in (Table 1). At different levels of *Candida* species was observed that the inhibition zone. The plant extracts inhibited the growth of *Candida* strains, producing a zone diameter of inhibition from 8.0 mm to 17.0 mm based on the

sensitivity of the *Candida* species. As the highest anti-candidal activity of the plant extracts were examined, it was observed in order of resulting the inhibitions zones of 17.0 mm for *C. glabrata*, 13.0 mm for *C. tropicalis*, 12.0 mm for *C. krusei* and 10.0 mm for *C. albicans*.

Fungi used in this study were chosen primary on the basis of their importance as an opportunistic pathogen of humans. According to findings from the National Nosocomial Infection Surveillance System (NNIS), 61% of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp. and *Cryptococcus* spp. [7]. Some studies, done in hospitals from different geographic regions, report that the most frequent species that cause bloodstream infections in pediatric patients are *C. albicans* and *C. parapsilosis*. In adults, the most frequent isolated species are *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* [8]. *C. glabrata*, *C. krusei*, and *C. parapsilosis*, however, show more resistance to antifungal drugs, especially to the first line treatments [9]. Because of reason, this study was carried out to determine antifungal activity of the *L. stoechas* extracts against the hospital isolates such as *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. guilliermondii*. There are many investigations on antimicrobial activity of *L. stoechas* but the findings on antifungal effects are inadequate. In previous study, the essential oils obtained from the plant were determined to equal effect against *C. albicans* ATCC 10239 compared with standart antibiotic Nystatine by disc diffusion method [10]. In another study, the essential oils *L. stoechas* spp. *stoechas* were evaluated for their anti-candidal activities by broth microdillution method. The modarete anti-candidal activity was observed and the flower essential oil was found to be relatively more active than the leaf oil towards the tested pathogenic microorganisms [11]. In addition, antimicrobial activity of the essential oils from wild populations of Algerian *L. stoechas* was evaluated with minimum inhibitory concentrations (MICs) method. The eleven essential oils exhibited good antimicrobial activities against filamentous fungi and yeast cultures with MICs values ranging from 0.16 to 11.90 mg/ml [12]. Notably, the extracts of *L. stoechas* demonstrated anti-candidal activity in different levels in this study. The differences between our results and those of the mentioned studies may be due to several factors for example, the intraspecific variability in the production of secondary metabolites. In addition, there may be differences in the extraction protocols used to recover the active metabolites as well as differences in the assay methods.

The scientists have studied the medicinal plants having antibiotic feature owing to multi-antibiotic resistance which has recently prevalent. In previous research of certain medicinal plants are known to have antifungal activity *L. stoechas* extract exhibited three high percent components: camphor 31.7%, fenchone 20.7% and 1,8-cineole 10.9% in accordance with compositions previously reported for the oils from other studies. Other minor, but relevant, compound were bornyl acetate 5.0% and myrtenyl acetate 2.1% [13,14]. This plant essential oils contain a high proportion of camphor α -Pinene contained this plant has been shown antibacterial activity and 1,8-cineole, p-cymene was reported to show antifungal effect [15]. Our results indicated that in comparison with the control group standard antifungal antibiotics, all concentrations of the tested plant extracts could substantially inhibited *Candida* species growth and exhibited a moderate to high antifungal activity. *C. glabrata* is more susceptible to the ethanol extract 50 μ l of plant as compared to all standard antifungal antibiotics such as Fluconazole, Amphotericin B and Itraconazole. This activity may be indicative of the presence of metabolic toxins or the mentioned plant compounds.

The ethanol possessed higher antifungal activity than the other solvents (chloroform and ethyl acetate) in this study. Similar studies concerning the effectiveness of extraction methods that ethanol extraction yields higher antimicrobial activity than the other solvents [16].

Table 1. *In vitro* anti-candidal activity of the *L. stoechas* L. extracts

		Mean Diameter of Inhibitory Zones (mm)				
<i>L.stoechas</i> L. Extract		Hospital Isolates				
		<i>C.krusei</i>	<i>C.albicans</i>	<i>C.tropicalis</i>	<i>C.glabrata</i>	<i>C.guilliermondii</i>
Ethanol	25µl	10.0	7.0	10.0	8.0	9.0
	50µl	12.0	6.0	11.0	17.0	13.0
	75µl	10.0	7.0	9.0	9.0	10.0
Chloroform	25µl	9.0	8.0	9.0	11.0	11.0
	50µl	8.0	10.0	12.0	8.0	10.0
	75µl	9.0	6.0	11.0	13.0	8.0
EthylAcetate	25µl	8.0	8.0	10.0	9.0	8.0
	50µl	8.0	10.0	9.0	12.0	10.0
	75µl	11.0	9.0	14.0	11.0	9.0
Antibiotics						
GRS10		8.0	9.0	9.0	9.0	7.0
FLU25		6.0	8.0	6.0	6.0	6.0
AMB100		9.0	9.0	6.0	6.0	8.0
MCZ10		7.0	11.0	10.0	11.0	6.0
NY100		6.0	8.0	6.0	10.0	6.0
5FC1		7.0	11.0	6.0	9.0	8.0
CLT10		7.0	15.0	10.0	15.0	6.0
KTC10		6.0	15.0	6.0	15.0	6.0
ITR10		8.0	10.0	10.0	6.0	7.0

GRS10, Griseofulvin 10 µg; FLU25, Fluconazole 25 µg; AMB100, Amphotericin B 100 µg; MCZ10, Miconazole 10 µg; NY100, Nystatin 100 µg; 5FC1, Flucytosine 1 µg; CLT10, Clotrimazole 10 µg; KTC10, Ketoconazole 10 µg; ITR10, Itraconazole 10 µg.

IV. CONCLUSION

Candidemia is a substantial anxiety in the clinal medicine relevant to the public helath 20%, mostly because of the high mortability rates in the children and adults, 30% and 51% respectively [8]. This study, provides data about the anti-candidal properties of the extracts obtained from *L. stoechas* against the hospital isoletes of *C. albicans*, *C. kruseii*, *C. tropicalis*, *C. guilliermondii*, *C. glabrata*. By isolating and identifying the bioactive compounds from these extracts can be formulated treat for Candidemia. Further phytochemical and pharmacological studies in future on *L. stoechas* in wild-growing in Turkey are necessary to utilize these ethnomedicinallly importants plants successfully. In addition, the findings explain the use of *L. stoechas* in folk medicine.

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