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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Antifungal effect of *Trachyspermum ammi* against susceptible and fluconazole-resistant strains of *Candida albicans*

Effet antifongique de Trachyspermum ammi *contre les souches de* Candida albicans *sensibles ou résistantes au fluconazole*

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Trachyspermum ammi;
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OPC;
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Thymol

Summary
Objective. — *Trachyspermum ammi* (*T. ammi*) has been known as having many therapeutic properties and its antimicrobial activity has currently received a renewed interest. This study aimed to verify the effectiveness of *T. ammi* essential oil to inhibit the growth of *Candida albicans* (*C. albicans*) strains isolated from HIV$^+$ patients with oropharyngeal candidiasis (OPC).
Materials and methods. — The essential oil was obtained by hydrodistillation in a Clevenger apparatus and analyzed by gas chromatography. Susceptibility tests were expressed as inhibition zone by the disk diffusion method and minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) by the broth microdilution method.
Results. — Thymol (63.4%), *p*-cymene (19%) and γ-terpinen (16.9%) were found as the most abundant constituents. The disk diffusion results revealed that 67% of oral *C. albicans* isolates were susceptible, 9% susceptible-dose dependent and 24% resistant to fluconazole. In the broth microdilution method, 68% of isolates were susceptible, 5% susceptible-dose dependent and 27% resistant to fluconazole. The increase in concentration led to a significant reduction in yeasts that were growing in exponential phase. In addition, with increasing in *T. ammi* oil concentration, the time of remaining cells in lag phase was significantly increased.
Conclusion. — This study showed that all clinical *C. albicans* isolates were susceptible to *T. ammi* essential oil, indicating a significant reduction in the yeast growth in exponential phase.

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MOTS CLÉS
Trachyspermum ammi ;
VIH ;
Candidose oro-pharyngée ;
Fluconazole ;
Candida ;
Thymol

Résumé
Objectif. — *Trachyspermum ammi* (*T. ammi*) est connu pour ses nombreuses propriétés thérapeutiques et il y a actuellement un intérêt renouvelé pour son activité antimicrobienne. Cette étude a pour but de vérifier l'efficacité de l'huile essentielle de *T. ammi* sur l'inhibition de croissance de *Candida albicans* (*C. albicans*) isolés de patients positifs pour le VIH avec une candidose oro-pharyngée (OPC).
Matériel et méthodes. — L'huile essentielle a été obtenue par l'hydrodistillation dans un appareil Clevenger et a été analysée par chromatographie gazeuse. Les épreuves de sensibilité ont été exprimées comme la zone d'inhibition par la méthode de diffusion des disques et la concentration inhibitrice minimale (MIC) et la concentration fongicide minimale (MFC) par la méthode de microdilution en milieu liquide.
Résultats. — Le thymol (63,4 %), le *p*-cymène (19 %) et le γ-terpinène (16,9 %) ont été les constituants les plus abondants. Les résultats de diffusion des disques ont révélé que 67 % des souches de *C. albicans* étaient sensibles, 9 % avaient une sensibilité dose-dépendante et 24 % étaient résistants au fluconazole. Avec la méthode de microdilution, 68 % des isolats étaient sensibles, 5 % avaient une sensibilité dose-dépendante, et 27 % étaient résistants au fluconazole. L'augmentation de la concentration a causé une réduction significative des levures en phase exponentielle de croissance. En plus, avec l'augmentation de la concentration d'huile de *T. ammi*, le temps de recroissance des cellules en phase stationnaire a été de façon significative augmenté.
Conclusion. — Cette étude a montré que tous les isolats cliniques de *C. albicans* étaient sensibles à l'huile essentielle de *T. ammi*, avec une réduction significative de la croissance des levures dans leur phase exponentielle.

Introduction

Oropharyngeal candidiasis (OPC) remains the most frequent opportunistic fungal infection among HIV$^+$ patients and is frequently the initial manifestation of HIV infection [24]. Currently, it is estimated that approximately 80—90% of HIV-infected patients develop OPC at some time during the progression of their disease from HIV infection to AIDS [6]. *Candida albicans* (*C. albicans*) is the species responsible for the majority of cases of OPC [9]. The ability of *C. albicans* to adhere to buccal epithelial cells is critical in establishing oral colonization. After colonization, the organisms may persist for months or years in low numbers in the absence of inflammation. In HIV$^+$ patients, antifungals are frequently less efficacious than in patients with other immunodeficiencies such as cancer. Similarly, the time to clinical response also tends to be delayed in this population [2,11]. Moreover, the relapse rate is higher in patients with HIV than in any other patient population [5,24]. For unknown reasons, a subgroup of HIV$^+$ patients experience recurrent episodes of OPC and thus receive numerous courses of antifungals during their lifetime. As their HIV infection progresses, they tend to experience shorter disease-free intervals between episodes of mucosal candidiasis and thus have a greater antifungal exposure, which may ultimately lead to the development of clinical and in vitro antifungal resistance and its associated morbidity and mortality.

In recent years, interest has grown in natural medicinal products, essential oils and other botanicals, in response to the ever-increasing incidence of adverse side effects associated with conventional drugs, and the emergence of resistance to antifungal drugs. There has been particular resurgence of interest in Iranian *Trachyspermum ammi* (*T. ammi*), which has been employed for its antimicrobial activity since ancient times. *T. ammi* L., belonging to family Apiaceae, is a highly valued medicinally important seed spice. The roots possess diuretic in nature and the seeds excellent aphrodisiac properties. The seeds contain up to 5% brown colored oil known as aiwain oil. The main component of this oil is thymol, which is used in the treatment of gastrointestinal ailments, lack of appetite and bronchial problems. The oil exhibits fungicidal [17], antimicrobial [19] and anti-aggregatory effects [20] on humans.

This work evaluates the chemical analysis of *T. ammi* essential oil and its antifungal activity on susceptible, susceptible-dose dependent and resistant strains of *C. albicans* isolated from HIV$^+$ patients with OPC.

Materials and methods

C. albicans isolates

Specimens were obtained from the OPC lesions of the tongue or the buccal mucosa using sterile cotton swabs. These swabs were incubated in sabouraud dextrose agar with chloramphenicol (Merck Co., Darmstadt, Germany) at 32 °C for 48 h (under aerobic conditions) and in CHROMagar^{TM} *Candida* (CHROMagar, France) at 35 °C for 48 h (in the dark). We used sugar fermentation and assimilation tests with RapID^{TM} Yeast Plus System (Remel, USA) according to the manufacturer's instructions for the presumptive identification of *C. albicans* isolates.

Plant material

Fresh seeds of *T. ammi* were purchased from the Pakanbazr Pharmaceutical Company, Isfahan, Iran. Plants were