Fatimazahrae. MOUSSAID et al /J. Pharm. Sci. & Res. Vol. 11(3), 2019, 718-720

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

In vitro antifungal activity of Cinnamum Burmannii and Cuminum Cyminum Essential Oils against two nosocomial strains of Aspergillus Fumigatus

Fatimazahrae. MOUSSAID[1*], Yassine. El ATKI[2], Azzedine. EL BARNOSSI[1],Abdelfattah. ABDELLAOUI[2,] Abdelilah. IRAQI HOUSSEINI[1].

1: Laboratory of Biotechnology. Team of Microbial Biotechnology. Faculty of Sciences Dhar El Mahraz. Sidi Mohammed Ben Abdellah University., B.P. 1796, Atlas, Fez, Morocco.
2: Laboratory of Physiology, Pharmacology and Environmental Health. Faculty of Sciences Dhar El Mahraz. Sidi Mohammed Ben Abdellah University. , B.P. 1796, Atlas, Fez. Morocco.

Abstract:

Molds of the genus *Aspergillus* are among the ubiquitous fungi environment. *Aspergillus fumigatus* is one of the most dangerous nosocomials fungal pathogens especially in immunocompromised. In order to verify the medicinal virtues granted to some essential oils (EOs) which have a broad spectrum of applications (pharmaceutical and medicinal: bactericides, fungicides, antiparasitics), the objective of this study was to evaluate the antifungal activity of the EOs of two aromatic plants: *Cuminum cyminum* and *Cinnamomum verum*, on the mycelial growth of *Aspergillus fumigatus*. In this study, we isolated two strains of *Aspergillus fumigatus* (ASP37 and ASP42) from a pulmonary aspergillosis. The in vitro antifungal effects of the EOs tested were determined using the disk diffusion technique in the agar medium, and the microdilution method. The inhibitory effects of these oils indicated that the isolates of *Aspergillus fumigatus* tested were sensitive to both essential oils. The ASP37 was completely inhibited by both oils, while the ASP42 was inhibited by *Cuminum cyminum* and *Cinnamomum verum* with percentage inhibitions of 53% and 82%respectively. The essential oils of *Cuminum cyminum* and *Cinnamomum verum* can be used as a bio-antifungal against *Aspergillus fumigatus* responsible of chronic pulmonary aspergillosis.

Keywords: Antifungal activity, *Aspergillus fumigatus*, Cinnamomum verum, Cuminum cyminum

INTRODUCTION

Antimicrobial resistance was widely known as a serious threat to global health in the 21st century. because the past decades have seen a dramatic increase in human-pathogenic microorganisms that are resistant to one or multiple antimicrobial agents [1]. The world health organization (who) published in 2017 a report listing the most dangerous multidrug-resistant microorganism to which new antimicrobials should be urgently discovered [2]. The discovery of new antimicrobial agents is mainly based on natural products that can be obtained from different sources. including plants, bacteria, algae, fungi and animals but there has been an increased interest in bioactive compounds provided by the plant as an alternative to the common antimicrobial [3]. Essential oils account for a source of very promising natural compounds for producing new antimicrobial drugs. Numerous studies have reported a strong antibacterial effect for some essential oils essential oils [4–6]. Among these essential oils, the potential antimicrobial of Cinnamon and Cumin has been documented frequently [7][8] [9].

On the other hand, *Aspergillus fumigatus* is an opportunistic pathogen and ubiquitous in the environment. Is classified as one of 'hard to treat' organisms and is one of the most fungicide resistant fungi this century because of its adaptability to different conditions [8]. In humans, *A. fumigatus* can cause severe infections that are generally called aspergillosis diseases, including, Allergic Bronchopulmonary Aspergillosis, Allergic Aspergillus Sinusitis, Aspergilloma, Chronic Pulmonary Aspergillosis and Invasive Aspergillosis [10].

However, to the best of our knowledge, there are no available data about the antifungal activity of Cinnamon and Cumin against multidrug resistant *Aspergillus fumigatus* isolated from a pulmonary Aspergilloma provided by University hospital Complex Fes, Morocco. Therefore, the aim of this study was to evaluate the efficacy of *Cuminum cyminum* and *Cinnamomum verum* EOs on anti-multidrug resistance *Aspergillus fumigatus*, in order to develop an alternative to chemical fungicides.

MATERIALS AND METHODS

Essential Oils Extraction

A portion (300 g) of the Cinnamon bark and the Cumin seeds submitted for 3 hours to water distillation, using a Clevenger-type apparatus, according to the method recommended by the European Pharmacopoeia (1975) [11]. The obtained essential oils were dried with anhydrous "sodium sulphate" and stored in a refrigerator at 4–5°C prior to analysis.

Fungal species

The present study describes the antifungal activity of EOs of two aromatic plants: *Cuminum cyminum* and *Cinnamomum verum*, on the mycelial growth of *Aspergillus fumigatus*. This strain was isolated from a pulmonary Aspergilloma provided by University Hospital Complex (CHU) from Fez. It's was cultivated in sabouraud, malt extract (EMA) and czapec medium, incubated at 37 °C and 42 °C under aerobic conditions for 21 days. Identification of fungi was done according to macro and microscopic morphological characteristics described by Barnett and Hunter key [12-13-14].

Antifungal activity assay

The agar disc diffusion method were employed for the determination of *in vitro* antifungal activity of the essential oils on radial growth of *A. fumigatus* [15]. The medium used was EMA, discs with fungal mycelia (6mm in diameter) were placed on Petri dishes, and 10µl of essential oil (EO) were added to filter paper discs (6 mm in diameter, Whatman No. I) [16] in Petri dish at 1cm. Similarly, a growth control without EO treatment was used. During the 7 days of incubation at 37°C, the diameters of the inhibition zones were measured in millimeters. The percentage of mycelia growth inhibition (**P**) was estimated using as reference the control (**C)** without EO, and colony diameter under the essential oil (**T)** as follows: $P = (C-T)/C\times100$. [17] In this study, all tests were performed in three replicates.

Minimal inhibitory concentration of conidial germination

The minimumin inhibitory concentrations (MICs) of the EOs against *A. fumigatus* were determined by microdilution method in 96-well plates, according to NCCLS standards with some modifications [18-19-20]. From a stock solution of EO diluted in 0.2% agar [21], different concentrations of EO is prepared by the successive dilution 1/2 ranging from 3.33% to 0.0125%. In other to study the effect of these oils on conidial germination, we adjusted the concentration of strains at 1×10^6 conidia/ml. The MIC was the lowest concentration of EO that completely inhibit *A. fumigatus* in the microdilution wells, as can be detected by the unaided eye after 7 days.

The minimal fungicidal concentration

After determining the MICs, the minimum fungicidal concentrations (MFCs) were determined by sub-culturing negative wells on EMA. The MFC was defined as the lowest concentration resulting no growth on the subculture. [19]. MFC was the lowest concentration of oil that resulted in either no growth or less than three colonies after control growth.

Statistical analysis

The antifungal activity of essential oils on mycelial growth was analyzed by ANOVA test using the Graphpad Prism5 software. Statistical significance was considered a P-value less than 0.05.

RESULTS:

Fungal species

In this study, we isolated two isolates from a pulmonary aspergillosis: ASP37 and ASP42. These isolates were identified two different strains of *A. fumigatus*, according to their macro and microscopic appearance, and the barnet and hunter identification key.

Antifungal activity assay:

The Antifungal activity of the essential oils was investigated against *A. fumigatus*, using disc diffusion method in the agar medium. The influence of *Cuminum cyminum* and *Cinnamomum verum* oil on mycelial growth inhibition of tow strains of *A. fumigatus* is illustrated in Figure 1 and Figure 2. The tow EOs (10µl/Disc) significantly inhibited the mycelial growth of tow strains of *A. fumigatus* on EMA ($p < 0.05$). ASP37 strains was completely inhibited (100%) by both oils, while ASP42 strains was inhibited to 82% by *Cuminum cyminum* and 53% by *Cinnamomum verum* compared to the control negative. The inhibitory effects of these oils indicated that strains of *Aspergillus fumigatus* tested were sensitive to both oils.

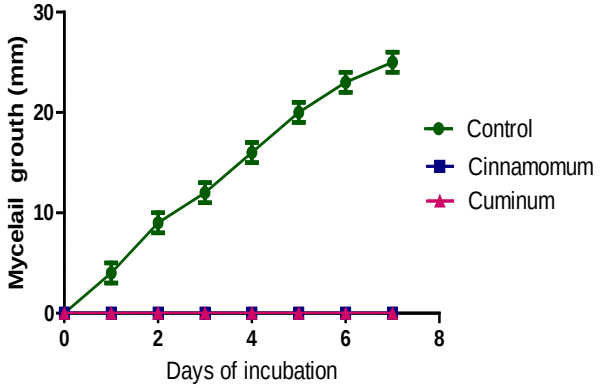

Figure 1: The effect of *Cinnamum Burmannii* and *Cuminum Cyminum* against ASP37

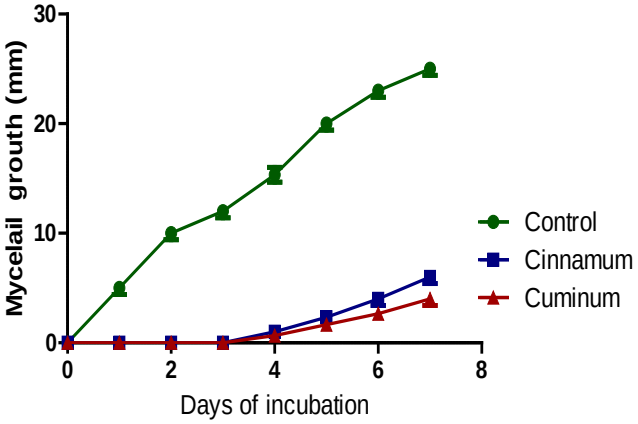

Figure 2: The effect of *Cinnamum Burmannii* and *Cuminum Cyminum against ASP42*

Minimal inhibitory concentration (MIC):

The MIC was determined by the microdilution method, at different concentrations. These results show that the EOs has significant activity ($p < 0.05$) and inhibits conidial germination of two strains. The MICs of *Cinnamomum verum* EO was 0.2% against ASP42 and ASP37. While *Cuminum cyminum* EO had a MIC value of 1.66% and 3.33% for ASP37 and ASP42 respectively. (Table1)

Minimal fungicidal concentrations (MFCs)

Re-seeded negative wells inocula on EMA showed mycelial growth, indicating fungicidal activity of essential oils. The MFC of *Cinnamomum verum* EO produced a fungicidal effect of both strains at 0.2%. While MFC of EO of *Cuminum cyminum* were observed at 3.33% against the two strains of *A. fumigatus.* (Table1)

Table 1: Minimal inhibitory concentration (MICs) and minimal fungicidal concentrations (MFCs) of *Cinnamomum verum* and *Cuminum cyminum* essential oils

Strains	MICs (%)		MFCs (%)	
	C. cyminum	*C. verum*	*C. cyminum*	*C. verum*
ASP37	1.66	0.2	3.33	0.2
ASP42	3.33	0.2	3.33	0.2

DISCUSSION

The growth inhibition of both strains of *A. fumigatus in vitro* by EOs using agar disc diffusion method, showed that the EOs of *C. verum* and *C. cyminum*, represents an important antifungal characteristic against *A. fumigatus*. Moreover, the results showed changes in the hyphae of this fungus. The stability of fungicide properties of tested EOs represented by the inhibition of mycelial growth even after 20 days of culture. These datas generally confirmed those obtained in the microdilution assay. Different studies have shown that *C. cyminum* have a potential antifungal against different fungal strains. Our results are in agreement with the results reported by Khosravi *et al* 2011, the oil concentration of *C. cyminum* at 0.25 mg/ml showed fungistatic activity against *A. fumigatus* [23]**.** In addition, A. Naeini *et al* 2014, demonstrate that the *C. cyminum* oil had a broad-spectrum antifungal activity against different pathogenic *Candida* species with inhibition zone values ranged from 7 to 50 mm [24]. Other Study on three *Malassezia* species showed a similar susceptibility to *C. cyminum* with inhibition value: 48.3 mm. Bushra *et al* 2018 reported that *C. verum* was performed against *Cr. neoformans*, *M. gypseum* and *M. canis*. [25] Lakshmeesha T *et al* 2014 carried out similar results antifungal activity of bark aqueous extracts on the mycelial growth of *A. flavus* [26].

CONCLUSION

To our knowledge, no study was carried on antifungal activity of *Cuminum cyminum* and *Cinnamomum verum* against *A. fumigatus* isolated from a pulmonary Aspergilloma. Our data indicate that EOs of *Cuminum cyminum* and *Cinnamomum* can be considered a potential fungicide for the biocontrol against *A. fumigatus* pathogenic fungi. However, to complete this work, it's necessary more study for determined the compounds of these EOs responsible to anti- *Aspergillus fumigatus* activity.

REFERENCES

[1] Martelli G, Giacomini D. Antibacterial and antioxidant activities for natural and synthetic dual-active compounds. European Journal of Medicinal Chemistry 2018;**158**(5):Pages 91-105.

[2] WorldHealth Organization (WHO). AGlobal HealthGuardian: Climate Change, Air Pollution And Antimicrobial. WorldHealth Organization(*WHO)*. 2017.

[3] Rossiter SE, Fletcher MH, Wuest WM. Natural Products as Platforms To Overcome Antibiotic Resistance. Chemical Reviews 2017;**117**(19):12415–12474.

[5] El-Jalel LFA, Elkady WM, Gonaid MH, El-Gareeb KA. Difference in chemical composition and antimicrobial activity of Thymus capitatus L. essential oil at different altitudes. *Future Journal of Pharmaceutical Sciences* 2018. doi:10.1016/J.FJPS.2017.12.004.

[6] Jalal Z, El Atki Y, Lyoussi B, Abdellaoui A. Phytochemistry of the essential oil of *Melissa officinalis* L. growing wild in Morocco: Preventive approach against nosocomial infections. Asian Pacific Journal of Tropical Biomedicine 2015;**5**(6):458–461.

[7] Unlu M, Ergene E, Unlu GV, Zeytinoglu HS, Vural N. Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (Lauraceae). *Food and Chemical Toxicology* 2010;**7**(48):3274–3280.

[8] Vasconcelos NG, Croda J, Simionatto S. Antibacterial mechanisms of cinnamon and its constituents: A review. *Microbial Pathogenesis* 2018;**120**:198–203.

[9] Petretto GL, Fancello F, Bakhy K, Faiz CA, Sibawayh Z, Chessa M, et al. Chemical composition and antimicrobial activity of essential oils from *Cuminum cyminum* L. collected in different areas of Morocco. *Food Bioscience* 2018;**22**:50–58.

[10] Garcia-rubio R, Alcazar-fuoli L, Iii C. Diseases Caused by *Aspergillus fumigatus*. 2018;:1–9.

[11] Pharmacopoeia E. European Pharmacopoeia. *Maisonneuve, SA, Sainte* 1975;**3**:68–80.

[12] V.S. Pragadheesha, Arvind Sarojc, Anju Yadavb, C.S. Chanotiyaa, b, M. Alamc, A. Samadca. Chemical characterization and antifungal activity of *Cinnamomum camphora* essential oil. Industrial Crops and Products 49 (2013) 628– 633

[13] Barnett. H.L; Barry B. Hunter, 1986; c1998. Illustrated genera of imperfect fungi /H. L. Barnett, Barry B. Hunter. Fourth ed. St. Paul, Minn.: APS Press.

[14] Mambaeva1 , A. Sadanov, O. Shemshura, Ibishev1 , M. Alimzhanova, B. Lozovicka. Prospects of using fungi of genus Trichoderma as agents of biocontrol for fungal diseases of potatoes and cucumbers in kazakhstan A. J. Pharm. Sci. & Res. Vol. 10(11), 2018, 2855-2857.

[15] NCCLS (National Committee for Clinical Laboratory Standards). Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts. Proposed Guideline NCCLS Document M44-P National Committee for Clinical and Laboratory Standards, Wayne, PA, 2003.

[16] A.R. Naeini, M. Nazeri, H. Shokri. Antifungal activity of *Zataria multiflora*, *Pelargonium graveolens* and *Cuminum cyminum* essential oils towards three species of *Malassezia* isolated from patients with pityriasis versicolor. Journal de Mycologie Médicale (2011) 21, 87-91

[17] Francisco Castillo a, Daniel Hernándeza,∗, Gabriel Gallegosa, Martha Mendezb, Raúl Rodríguezb, Alfonso Reyesc, Cristóbal N. Aguilarb. In vitro antifungal activity of plant extracts obtained with alternative organic solvents against *Rhizoctonia solani Kühn*. Industrial Crops and Products 32 (2010) 324–328

[18] NCCLS (National Committee for Clinical Laboratory Standards). Performance Standards for Antimicrobial Susceptibility Testing, ninth International Supplement, M100-S9, Wayne, PA. 1999.

[19] A. Mohammadi, H. Nazari, S. Imani, H. Amrollahi. Antifungal activities and chemical composition of some medicinal plants. Journal de Mycologie Médicale (2014) 24, e1-e8

[20] Mehmet Unlu, Emel Ergene, Gulhan Vardar Unlu, Hulya Sivas Zeytinoglu, Nilufer Vural. Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from *Cinnamomum zeylanicum Blume* (Lauraceae). Food and Chemical Toxicology 48 (2010) 3274–3280.

[21] A Remmal, T Bouchikhia, K Rhayoura, M Ettayebia, et A Tantaoui-Elarakib, Improved method for the determination of microbial activity of essential oil in agar medium. J. Essent. Oil Res., 1993, 5(2), 179-184.

[22] Y. El Ouadi, M. Manssouri, A. Bouyanzer, L. Majidi, H. Bendaif, H. Elmsellem, M.A. Shariati , A. Melhaoui, B. Hammouti. Essential oil composition and antifungal activity of Melissa officinalis originating from north-Est Morocco, Against Postharvest Phytopathogenic Fungi In Apples. Microbial Pathogenesis 107 (2017) 321-326

[23] A.R. Khosravi, Minooeianhaghighi, M.H, Shokri, H. Emami, S.A; Alavi, S.M; Asili, J. The Potential Inhibitory Effect of *Cuminum Cyminum*, *Ziziphora Clinopodioides* and *Nigella Sativa* Essential Oils on the Growth of *Aspergillus Fumigatus* and *Aspergillus Flavus*. Brazilian Journal of Microbiology (2011) 42: 216-224 ISSN 1517-8382

[24] A. Naeini, N. Jalayer Naderi, H. Shokri. Analysis and in vitro anti-*Candida* antifungal activity of *Cuminum cyminum* and *Salvadora persica* herbs extracts against pathogenic *Candida* strains. Journal de Mycologie Médicale (2014) 24, 13-18

[25] Bushra Abdulkarim Moharm, Ibrahim Jantan, Jacinta Santhanam & Jamia Azdina Jamal, Antifungal Activity of the Bark and Leaf Oils of Cinnamomum verum J.S. Presl. Alone and in Combination against Various Fungi. International Journal of Advanced Research, 2018.2(5):1169-1172

[26] Lakshmeesha T. R, Sateesh M. K, Vedashree S. and Mohammad Shafi Sofi. Antifungal activity of Cinnamomum verum on Soybean seed-borne Aspergillus flavus. International Journal of Advanced Research (2014), Volume 2, Issue 5, 1169-1172.