NUSANTARA BIOSCIENCE
Vol. 7, No. 1, pp. 55-59
May 2015

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n070110

Antifungal effect of cumin essential oil alone and in combination with antifungal drugs

SAHADEO PATIL[1,♥], PANKAJ MAKNIKAR[1], SUSHILKUMAR WANKHADE[1], CHANDRAKIRAN UKESH[1], MAHENDRA RAI[2]

[1]Department of Microbiology, Shri Shivaji Science College, Amravati 444603, Maharastra, India. Tel. +91-9372433429;
[♥]email: sdpatil20957@yahoo.co.in
[2]Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati-444602, Maharashtra, India

Manuscript received: 6 March 2015. Revision accepted: 30 April 2015.

Abstract. *Patil S, Maknikar P, Wankhade S, Ukesh C, Rai M. 2015. Antifungal effect of cumin essential oil alone and in combination with antifungal drugs. Nusantara Bioscience 7: 55-59.* We report evaluation of antifungal activity of cumin seed oil and its pharmacological interactions when used in combination with some of the widely used conventional antifungal drugs using CLSI broth microdilution, agar disc diffusion and checkerboard microtitre assay against *Candida*. The essential oil was obtained from cumin seeds using hydrodistillation technique and was later evaluated for the presence of major chemical constituents present in it using gas chromatography and mass spectrometry (GC-MS) assay. The GC-MS assay revealed the abundance of γ-terpinene (35.42%) followed by *p*-cymene (30.72%). The agar disc diffusion assay demonstrated highly potent antifungal effect against *Candida* species. Moreover, the combination of cumin essential oil (CEO) with conventional antifungal drugs was found to reduce the individual MIC of antifungal drug suggesting the occurrence of synergistic interactions. Therefore, the therapy involving combinations of CEO and conventional antifungal drugs can be used for reducing the toxicity induced by antifungal drugs and at the same time enhancing their antifungal efficacy in controlling the infections caused due to *Candida* species.

Keywords: *Cuminum cyminum*, essential oil composition, antifungal activity, combinational study

INTRODUCTION

The sudden rise in the emergence of opportunistic fungal infections is of paramount concern as a cause of morbidity and mortality, which can be the manifestations of any of these immunosuppressive treatments involving use of chemotherapy, immunosuppressants, organ transplantation, HIV infections, consumption of heavy dose of antibiotics and other prolonged immunotherapy (Pappas 2010). The challenges to these infections are further severed by the tolerance shown by these pathogens to the available antimicrobial drugs over a period of time sooner or later. *Candida* species are yeast-like fungi, which are the normal inhabitants of skin and gastrointestinal microflora of human beings and can thrive without imposing any danger under normal conditions. However, under weak immunological barriers, they can manifest into serious health ailments (Bicanic and Harrison 2014).

Brown and Netea (2012) reported that the patients from the Western societies which are severely ill are only prone to the fungal infections including both immuno-compromised and healthy patients whereas patients from developing countries are susceptible to the infections caused by wide range of fungi regardless of their health status. Despite the developments in the antifungal therapy, the mortality due to fungal infection can be traced up to 30-50%. Amphotericin B is generally considered primary line of defense to tackle infections by *Candida* species and is often given in combination with more efficacious and less toxic systemic azole antifungal drugs such as fluconazole, miconazole, ketoconazole, etc. However, amphotericin B overdose can be nephro and hepatotoxic (Safdar et al. 2010) and indiscriminate use of azole drug such as fluconazole can result in its intrinsic tolerance among *C. albicans* and non-*albicans Candida* species (Sanglard et al. 2003). Therefore, resistance to the azole antifungal drugs against *Candida* species has become predictable over a period of time which can be attributed to its fungistatic rather than the fungicidal nature in general (Vandenbosch et al. 2010). Moreover, these problems are further severed by the emergence of cross-resistance to other antifungal drugs of same or different class too (Panackal et al. 2006; Chen et al. 2012). Hence, novel strategies are imperative in controlling the infections caused by *Candida* species. On the backdrop of this, combinational therapy using conventional antifungal drugs and the plant-based products has come up with promising outcomes.

Essential oils are some of the vital secondary metabolites which have been assessed extensively for their antibacterial, antifungal, antitumor and antioxidative efficacies (Begnami et al. 2010; Khan et al. 2010; Prakash et al. 2010, 2012; Al-Ja'fari et al. 2011; Lv et al. 2011; Nikolić et al. 2014). In addition, essential oils or their individual chemical constituents when combined with conventional antibacterial and antifungal drugs have been identified with their capability in enhancing the potency of

these drugs (Mahboubia and Bidgoli 2010; Amber et al. 2010; Silva et al. 2011; Ahmad et al. 2013). *Cuminum cyminum* (cumin) is an annual herb belonging to the Apiaceae family widely cultivated in India, China, and several Middle East Asian countries (Thippeswamy and Naidu 2005; Oroojalian et al. 2010). Cumin seeds possess a characteristic aroma due to its essential oil content, owing to which is widely used in the different cuisines across the globe and ranks only second in the world's most popular spices (Hajlaoui et al. 2010). Cumin essential oil (CEO) has been identified with potent antibacterial, antifungal, insecticidal and antioxidative efficacies (Iacobellis et al. 2005; Gachkar et al. 2007; Packiavathy et al. 2012).

In the current study, antifungal activity of CEO was evaluated alone as well as in combination with four antifungal drugs including fluconazole, miconazole and amphotericin B using disc diffusion, CLSI broth microdilution method and checkerboard assay (Saad et al. 2010).

MATERIALS AND METHODS

Essential oil extraction and chemical composition

The cumin seeds (*Cuminum cyminum*) were purchased from local market and were finely ground before hydrodistillation using Clevenger apparatus for 3 h. The extract was dried over anhydrous sodium sulfate and after filtration was refrigerated in sealed glass vials at 4°C until further use (Palmeira-de-Oliveira et al. 2012). The chemical constituents of essential oil were analyzed by using GC-MS assay in which retention times of chromatographic peaks were identified by comparing with WILEY8.LIB and NIST05s.LIB databases.

Test organisms

Five clinical isolates belonging to *Candida* species including *C. albicans* (*n*=3), *C. glabrata* (*n*=1) and *C. krusei* (*n*=1) were obtained from PDMMC Hospital, Amravati, Maharastra, India.

Disc diffusion assay

The CEO was assessed for its antifungal efficacy using CLSI disc diffusion assay (CLSI 2009). Sterile paper discs (6 mm diameter) procured from HiMedia, India was impregnated with an aliquot of 20 µL of 10, 20 and 30% CEO in DMSO (v/v) and placed on to sterile Sabouraud dextrose agar (SDA) plates prelawned with yeast cultures in log phase. The plates were left undisturbed for 30 min at room temperature in order to diffuse the essential oil and incubated at 37°C for 48 h. The diameters of zone of inhibition were recorded in millimeter. The experiment was performed in triplicate and mean values were recorded.

Determination of MIC and FIC index

The minimum inhibitory concentration (MIC) of four antifungal drugs (fluconazole, miconazole and amphotericin B), as well as CEO, was determined using broth microdilution method according to the guidelines CLSI guidelines (CLSI 2008). The plates were incubated at 37°C for 48 h and MIC endpoints were recorded as the highest dilution resulting in complete inhibition of any visible growth in case of amphotericin B and CEO whereas, in case of fluconazole, miconazole and 80% growth reduction was counted as MIC endpoint (Alexander et al. 2007; Shin and Lim 2004). The fractional inhibitory concentration index (FICI) was defined as summation of fractional inhibitory concentration (FIC) of antifungal drug and FIC of essential oil and was evaluated by using checkerboard assay. The interactions observed were interpreted as synergistic, additive and antagonistic with FICI values of ≤0.5, >0.5 and ≤2 and >2 respectively (Saad et al. 2010).

RESULTS AND DISCUSSION

The results of qualitative and quantitative analysis of CEO using GC-MS assay is as shown in Table 1. The hydrodistillation of cumin seed oil gave a yield of 3.9 to 4.5 % (v/w). GC-MS analysis of the CEO was done with the identification of ten major chemical constituents as shown in Table 1. The CEO deciphered highest content of γ-terpinene and *p*-cymene with 35.42 and 30.72% concentrations respectively. In addition to this, the next eight components were cuminal (5.79%), β-pinene (5.58%), α-pinene (4.65%), carbicol (4.25%), 2-caren-10-al (3.84%), limonene (3.36%), 1,8-cineole (3.35%) and α-terpinene (3.12%). The reports of present study showed α-terpinene (35.42%) and *p*-cymene (30.72%) as major compounds in CEO. According to the report of Gachkar et al. (2007), essential oil of *C. cyminum* grown in Iran contain α-pinene (29.1%), 1,8-cineole (17.9%) and linalool (10.4%) as the major compounds. In another study, Tunisian variety of *Cuminum cyminum* contained

Table 1. Chemical constituents of CEO

Peak no.	RT (min.)	Compound name	Peak area (%)
1	9.63	α-Pinene	4.65
2	9.74	1,8-Cineole	3.25
3	10.12	Limonene	3.36
4	10.33	α-Terpinene	3.12
5	10.58	β-Pinene	5.58
6	10.97	Cuminaldehyde	5.79
7	11.46	γ-Terpinene	35.42
8	12.18	*p*-Cymene	30.72
9	12.57	Carbicol	4.25
10	13.05	2-Caren-10-al	3.84

Table 2. Antifungal activity of CEO against *Candida* species

Test organism	Zone of inhibition (mm) at CEO (%) in DMSO (v/v) µL (Mean±SD)		
	10%	20%	30%
C. albicans (C16)	42.67±2.08	44.00±2.22	49.67±0.58
C. albicans (C27)	31.33±1.52	34.33±2.08	42.33±2.08
C. albicans (C29)	37.00±1.73	43.67±1.52	44.33±2.08
C. glabrata (C39)	36.67±2.08	42.67±1.52	46.67±0.57
C. krusei (C45)	31.00±1.00	33.00±1.73	40.67±1.53

Table 3. Combinational effects of cumin with antifungal drugs against *Candida* species

Isolate	Combination	MICa	MICc	FIC	FICI	Type of interaction
	C. albicans					
C16	Cumin EO-fluconazole					
	Cumin EO	0.08	0.005	0.06	0.31	Synergistic
	Fluconazole	256	64	0.25		
C27	Cumin EO-fluconazole					
	Cumin EO	0.16	0.02	0.125	0.19	Synergistic
	Fluconazole	256	16	0.06		
C27	Cumin EO-amphotericin B					
	Cumin EO	0.16	0.02	0.125	0.19	Synergistic
	Amphotericin B	2	0.125	0.06		
C29	Cumin EO-miconazole					
	Cumin EO	0.31	0.08	0.25	0.5	Synergistic
	Miconazole	8	2	0.25		
	C. glabrata					
C39	Cumin EO-fluconazole					
	Cumin EO	0.08	0.02	0.25	0.375	Synergistic
	Fluconazole	128	16	0.125		
	C. krusei					
C45	Cumin EO-fluconazole					
	Cumin EO	0.08	0.02	0.25	0.375	Synergistic
	Fluconazole	128	16	0.125		

Note: MICa: MIC of EO/antifungal drug alone; MICc: MIC of EO/antifungal drug in combination; FIC: Fractional inhibitory concentration; FICI: Fractional inhibitory concentration index (FIC of EO + FIC of antifungal drug).

cuminaldehyde (39.48%), gamma-terpinene (15.21%), O-cymene (11.82%), and beta-pinene (11.13%) as major components (Hajlaoui et al. 2010).

Table 2 illustrates the results obtained for antifungal activity of CEO using agar disc diffusion assay. The result of CEO antifungal activity was studied at different CEO concentration of 10, 20 and 30%. All the concentrations of CEO were found to be significant antifungal agent against all the tested candidal isolates with the zone of inhibition in between 31.00±1.00 to 49.67±0.58 mm. The zones of inhibition were found to be increasing with the increase in the concentration of CEO which reflected its dose-dependent inhibitory potential. The results of MIC of CEO when tested alone against *Candida* species obtained by broth microdilution technique demonstrated the values in between 0.08 and 0.31 µg/mL. The strong antifungal efficacy of CEO was also evident with its lower MIC values (Table 3). The antimicrobial potential of essential oils is governed by the occurrence of its major and minor chemical constituents. Several investigators have coupled strong inhibitory action of CEO to its phenolic and monoterpenic contents (Naeini et al. 2013). The terpenes in CEO are known to affect respiration in *Candida* probably due to its damaging effects on mitochondria. This reaction renders the morphological alterations in the *Candida* cell leading to cell death (Tepe et al. 2004). *Cuminum cyminum* was of γ-terpinene chemotype and thus, this chemical constituent might have played a key role in killing the candidal population. MIC values (128-256 µg/mL) of selected antifungal drugs against the test organisms demonstrated their strong intrinsic resistance to them when assessed alone (Table 3).

The results of checkerboard microtitre assay show the FICIs values ranging from 0.19 to 0.375. The strategy involving combination of plant essential oils with conventional antifungal drugs has given encouraging results in the recent past (Rosato et al. 2009; Saad et al. 2010; Stringaro et al. 2014). Hence, the antifungal potential of CEO was further analyzed using checkerboard assay for the possible synergistic interactions. The treatment of CEO in combination with antifungal drugs showed significant decrease in the individual MICs of all the antifungal drugs. The combinations involving CEO with fluconazole and amphotericin B demonstrated lowest FICIs (0.19 each) expressing the occurrence of strong synergistic interactions (Saad et al. 2010). Combining the CEO with fluconazole and amphotericin B lowered the individual MICs of both the drugs by sixteen fold against *C. albicans* (C27). CEO-miconazole combination too expressed synergistic interaction by showing the FICI of 0.5. *C. glabrata* and *C. krusei* were both analyzed for possible synergistic interactions combining the CEO with fluconazole which showed identical FICIs of 0.375 each demonstrating the occurrence of synergistic interactions.

The mechanism of action of these combinations was not assessed in the current study. However, the possible synergistic action in enhancing the potency of combinations could be attributed to the individual as well as combined actions of both CEO chemical constituents and antifungal drugs. Azole drugs are known to act by

inhibiting the lanosterol 14α-demethylase, an important enzyme needed for ergosterol synthesis while polyenes antifungals, viz. amphotericin B acts by binding to the fungal membrane sterol rendering the membrane porous that manifests into leakage of cytoplasmic materials and ultimately the cell death (Balkis et al. 2002). The essential oils have been discussed earlier to have involved in the disruption of fungal cell membrane. It has also been reported earlier that, fluconazole is more hydrophilic compound unlike the other antifungal drug (e. g. ketoconazole which is lipophilic) therefore, is poorly absorbed by the cell membrane of *Candida* that is lipophilic in nature (Consiglieri et al. 2010). Thus, the porosity inducing trait of essential oil might have facilitated the entry of fluconazole through cell membrane when treated in combination thereby severing the cell membrane damage. Similarly, the same phenomenon might have assisted in the synergistic interactions produced in presence of other antifungal drugs such as miconazole and amphotericin B. Besides that, the strong antimicrobial potential of essential oils could be correlated to the abundance of different chemical constituents comprised by them. The individual chemical constituents of essential oil have been extensively examined for their biological activities but that can be the reflections of only certain fractions of essential oil rather than expressing the efficacy of essential oil as a whole. The highly abundant chemical constituents may act ferociously against pathogens but chances of other less prevalent chemical components possibly maneuvering their actions also could not be neglected (Bakkali et al. 2008). Therefore, the same principle could also be applied to the efficacy of combinations involving conventional antifungal drugs and CEO when prevalent as well as other scarcely present chemical constituents of essential oil could have facilitated the entry of antifungal drugs thereby increasing its efficacy against the *Candida* species.

From the outcomes of the current study, it was evident that CEO besides being potent antifungal agent when combined with conventional antifungal drugs such as fluconazole, miconazole and amphotericin B is more likely to reduce the individual MICs of these antifungal drugs moreover increasing their efficacy against *Candida* species. However, further research is imperative to assess its practical in vivo therapeutic applications.

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