Effect of Cumin Seeds Extracts on the Sensitivity of Candida Albicans

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ABSTRACT
Background many studies have shown that treatment with antifungal drugs is unsuccessful due to the development of resistant species. Other studies found that ethanol and oil extracts of Cumin seeds have antifungal activity against Candida albicans species. **Objectives** of this study were to evaluate the effect of different concentrations of Cumin seeds (oil and ethanol extracts) on the sensitivity of Candida albicans species. **Materials and Methods** Candida albicans were isolated from patients, sub-cultured, incubated, and tested. Ethanol and oil extracts of Cumin seeds were prepared with soxholate extractor, evaporated by rotary evaporator then kept in a dark place until used. **Results** All the dilutions of oil extracts caused inhibition in the growth of Candida albicans with an inhibition zone in Sabouraud Dextrose Agar Media (SDA) except the (0.75 and 1.5%). There were significant differences between all dilutions ($p \leq 0.05$) except between (25% and 50%) and (50% and Nystatin), also all dilutions of ethanol extracts showed inhibition zone in the SDA media except (25%) and there were significant differences ($p \leq 0.05$) between all dilutions except between (50% and 75%), (75% and 100%) and (100% and Nystatin). **Conclusions** Both extracts affect the sensitivity of Candida albicans. Oil extracts of Cumin seeds were more active than ethanol extracts and control positive Nystatin.

Keywords: Cumin Seeds Oil extracts; Cumin Seeds Ethanol extracts; Candida albicans

Introduction

Candida albicans present in the oral cavity of 3-48 % of healthy peoples, it's an important and command oral fungal pathogen which considered the common micro-organism that caused fungal infection (Ferrari et al, 2005, Sroisiri and Boonyanit, 2010). It has the ability to colonization, proliferation, and adherent on oral tissue leading to form complex biofilm structure that caused Trauma to the oral cavity (Hoshing et al, 2011; Emami et al, 2008; Young et al, 2009). Studies have appeared that treatment with antifungal drugs unsuccessful due to the development of resistant species (Zhang et al, 2020; de Oliveira Mima et al, 2011; Mima et al, 2012). This will lead to the use of systemic antifungal drugs, but systemic antifungal drugs have limitations in terms of toxicity

and drug interactions and more expensive (Farah et al, 2010; Silva et al, 2012). This makes it necessary to seek a new therapeutic approach using herbal extracts that have anti-fungal agents against Candida Albicans. (Petrović et al, 2014; Gleiznys et al, 2015; Rodino et al, 2014; Ismael and Abdul Latif 2019). Medical plants, now play a vital role, because they are considered safer and with few side effects in recommended dosage (Anibal et al, 2010; Al-Nema, 2014). Many studies appeared that the ethanol and oil extracts of Cumin seeds had antifungal activity against Candida albicans species (Kamble, 2015; PATIL et al, 2015). Thymoquinone and carvacrol are considered as anti-candidal agents against Candida albicans in both extracts (İşcan et al, 2016, Al Jabre et al, 2003; Darmawan et al, 2019; Randhawa et al, 2010). The objective of this study was to evaluate the effect of different concentrations of Cumin seeds (oil and ethanol extracts) on the sensitivity of Candida albicans species.

Materials and Methods

Isolation and examination of Candida albicans

Species of Candida were isolated from patients suffering from denture stomatitis in Baghdad Medical City labs, the procedure for taking the species was done by using a sterile cotton swab and gently rub the swab over the intraoral lesions, subcultured on Sabouraud Dextrose Agar Media SDA (OXOID, UK) and incubated at 37°C for 48 h. This is followed by microscopic examination, grams stain, germ tube formation, and API Candida systems were done for identification of Candida albicans (Ponka and Baddar, 2014, Abdulwahhab and Jassim, 2018). Figure: 1(A).

Figure (1): A- Microscopic examination B- MCF densitometer C- (1) effect of DMSO on Candida species. (2) Effect of extracts on Candida species.

Collection and extraction of the seeds

In the present study, Cumin seeds were bought from special local herbals market in Baghdad, washed, and left to dry at room temperature (35°C) without exposure to sunlight, ground electrically with the electrical grind (Brown, Germany). The extraction method of the seeds was done in the Ministry of Science and Technology/ Pollutant Treatment Center, 50g for both oil and ethanol extracts were prepared in soxholate extractor (Cole-Parmer, USA), 500 ml of n-hexane used for oil extract, and (400 ml) of ethanol 98% and (100 ml) distilled water used for ethanol extract for (4h/4 days). Then the plant extracts were evaporated by a rotary evaporator (Cole-Parmer, USA) under low pressure at temperate (50 - 60°C). The residue was put in a sterile Petri dish and placed in an oven at 25°C to dried it completely.

In vitro anti-candidal sensitivity testing

Disc diffusion method (agar diffusion method) was used to test anti-candida sensitivity (Wayne, 2009). The appropriate concentration of Candida Albicans suspension (correct turbidity) was prepared in a tube that equal to 0.5 McFarland using McFarland densitometer Figure: 1 (B). Sterile petri dish (8 cm diameter) with SDA media(20ml), the media surface was stretched, wells with 5mm diameter made on media and filled with different concentrations of the extracts about 50 µl in each well, Nystatin (44µg/mL) (Khoram

et al, 2019)was used as control positive and DMSO (Dimethyl Silfono Oxide) was used as control negative. Four dilutions for ethanol extracts of Cumin seeds (100, 75, 50, 25)% and nine dilutions for oil extract of Cumin seeds (100, 75, 50, 25, 12. 5, 6, 3, 1.5, 0.75) % were prepared using dilution law C1V1=C2V2 (100% prepared by dissolving 10g of crud extract in 100 ml of DMSO, while 75% prepared by dissolving 7.5g, in 100 ml, 50% prepared by dissolving 5g in 100ml and the other percentage calculated on the same manner) (Al-Saraf et al, 2016). After wells filled with dilutions, they left for 10 min before incubation to allow the extracted material to be diffusion into agar media, after that incubated at 37°C for 48h. After 48hrs Figure: 1(C) the inhibition zone was measured in millimeters using a measuring ruler (PATIL et al, 2015). The experiments were accomplished ten replicate and the average was reported.

Statistical Analysis

Data were analyzed using SPSS Ver. 25 for Windows. Descriptive statistics (frequencies, mean ± standard deviation and with tables and graphs) and inferential statistics (Pairwise Post-hoc Bonferroni test and Kendal Wall›s test) were used. P ≤0.05 considered statistically significant

Results

The total sample for oil extracts of Cumin seeds was 70 and each group consists of 10 frequencies (n = 10). According to Table (1), the Mean Inhibition Zone (MIZ) ± Stander Deviation (SD) in oil extracts for 100% (34.5 ± 2.415) and 75% (26.10 ± 1.663) were greater than control positive Nystatin (23.8 ± 0.632) while in ethanol extracts the MIZ ± SD of Nystatin were greater than all dilutions. The minimum mean value of Inhibition Zone in oil extracts was recorded by 3%, while for ethanol extracts the minimum mean value of Inhibition Zone was recorded by 50%

Table (1): Description of the study sample.

Substance and concentrations (g/ml)	Sample size	Mean ± SD	Mini.	Max.
Oil Extract of Cumin seeds	70			
3	10	9.5 ± 0.483	9	10
6	10	13.4 ± 0.843	13	15
12.5	10	15.8 ± 1.229	15	18
25	10	20 ± 1.333	18	22
50	10	22.2 ± 1.476	20	24
75	10	26.10 ± 1.663	24	28
100	10	34.5 ± 2.415	30	37
Ethanol Extract of Cumin seeds	30			
50	10	11.4 ± 0.966	10	12
75	10	17.1 ± 1.449	15	18
100	10	20.6 ± 0.966	20	22
Nystatin	10	23.8 ± 0.632	22	24

Inhibition zone positively correlated with the dilutions of Oil Extract of Cumin seeds which means that inhibition zone increased with increasing the concentration (r 0.834, p 0.001), Figure (2).

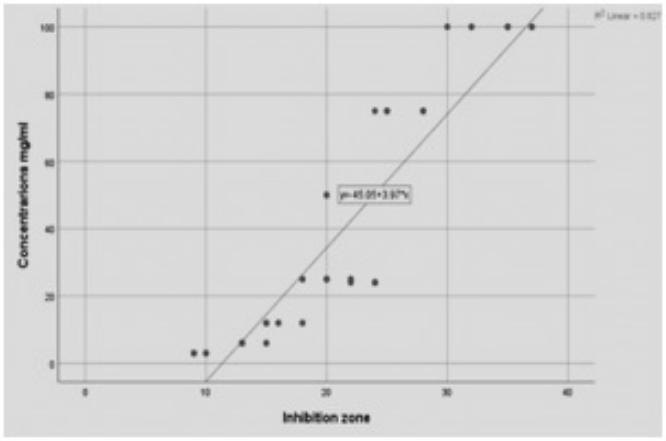

Figure (2): Correlation of inhibition zones with concentrations of oil extract of cumin seeds.

Post-hoc pairwise comparison for oil extracts indicated that there were significant differences (p≤0.05) between all dilutions with Nystatin (control positive) except between (25 and 50) and (50 and Nystatin) there were no significant (p>0.05), as shown in Table 2.

Table (2): Pairwise comparison of oil extract of cumin seeds in different concentrations with nystatin inhibition zone.

Pairs (Concentrations)		Std. Error	Std. Test Statistic	Adj.Sig. *	Sig.
3	6	12.487	4.004	0.001	S
	12	12.737	3.926	0.001	S
	Nystatin	12.301	4.065	0.001	S
	25	12.737	3.926	0.001	S
	50	12.799	3.907	0.001	S
	75	12.773	3.914	0.001	S
	100	12.850	3.891	0.001	S
6	12	12.354	3.561	0.005	S
	Nystatin	12.167	4.110	0.001	S
	25	12.607	3.966	0.001	S
	50	12.670	3.946	0.001	S
	75	12.644	3.954	0.001	S
	100	12.722	3.930	0.001	S
12	Nystatin	12.423	4.025	0.001	S
	25	12.814	3.746	0.003	S
	50	12.917	3.871	0.002	S
	75	12.891	3.879	0.001	S
	100	12.968	3.856	0.002	S
Nystatin	25	12.408	3.949-	0.001	S
	50	11.517	2.692-	1.00	NS
	75	12.221	3.723	0.003	S
	100	12.539	3.987	0.001	S
25	50	12.487	2.803	0.071	NS
	75	12.891	3.879	0.001	S
	100	12.968	3.856	0.002	S
50	75	12.922	3.753	0.002	S
	100	13.028	3.838	0.002	S
75	100	13.003	3.845	0.002	S

* Post-hoc Bonferroni correction for multiple tests.
p>0.05 NS
p≤ 0.05 S

Also in ethanol extracts, all the dilutions had significant differences (p≤0.05) except between (50 and 75%), (75 and 100%), and (100 and Nystatin %) were no significant (p >0.05), as shown in Table 3.

Table (3): Pairwise comparison of ethanol extract of cumin seeds in different concentrations with nystatin inhibition zone.

Pairs (Concentrations)	Std. Error	Std. Test Statistic	Adj. Sig. *	Sig.
50-75	5.153	1.941-	0.314	NS
50-100	5.153	3.911-	0.001	S
50-Nystatin	5.153	5.793	0.0001	S
75-100	5.153	1.970-	0.293	NS
75-Nystatin	5.153	3.892	0.001	S
100-Nystatin	5.153	1.883	0.359	NS

* Post-hoc Bonferroni correction for multiple tests.
P>0.05 NS
P≤ 0.05 S

Discussion

Studies found that antifungal drugs can result in resistance of Candida Albicans species (Mima et al, 2012; de Oliveira Mima et al, 2011; Zhang et al, 2020). This makes it necessary to seek new therapeutic approaches using herbal extracts that have anti-fungal properties against Candida albicans. (Rodino et al, 2014, Petrović et al, 2014; Gleiznys et al, 2015; Ismael and Abdul Latif, 2019). Some studies showed that oil extracts of Cumin seeds have anti-Candida Albicans activity (Kamble, 2015; PATIL et al, 2015). In the present study, the antifungal effect of Cumin seeds was analyzed and observed that Candida Albicans species were reduced when the concentration increased (inhibition zone was increased when the dilution of extracts decrease) because the concentration of the active component in the extracts increased when the concentration increased.

The study demonstrated that oil extract of cumin seeds have a noticeable anti-fungal effect against Candida Albicans especially in (100 and 75%) because they were more effective than Nystatin MIZ. This concurs with the results of (Kamble, 2015), who found that Cumin seed oil strongly inhibited all clinical isolates of C. Albicans and non-albicans Candida with growth inhibition zones ranging from 27 to 72 mm. Wanner et al, (2010) showed that cumin oil had antibacterial activity against gram-positive and gram-negative bacteria, in addition to its anti-Candida Albicans activity.

The activity of Cumin oil against Candida albicans is related to its essential ingredient (Thymoquinone) (Piras et al, 2013; Burits and Bucar, 2000), which has an obvious antimicrobial activity especially against Candida species (İşcan et al, 2016), Thymoquinone, carvacrol, and P-cymene are considered the main components of Cumin oil and they both have the antibacterial effect (Arici et al, 2005; Ali and Blunden, 2003).

This study showed that the inhibition zone in ethanol extract also increases with higher concentrations. This agrees with (Benlafya et al, 2014), who reported that ethanol extract had activity against B. Subtilis and Candida albicans. The antimicrobial activity of ethanol extract has been reported by Bameri et al, (2013), who found that the cumin seeds had exhibited variable antimicrobial activities against the biofilm E. coli. This is related to the active components (Thymoquinone, thymol, and carvacrol) that present in cumin seeds extract which has been reported to be responsible for decreasing the antimicrobial activity of both Candida Albicans and bacteria (Darmawan et al, 2019; Al Jabre et al, 2003; Randhawa et al, 2010).

Conclusion

The present study concluded that both extracts of cumin seeds affected the sensitivity of Candida Albicans. The inhibition zone was increased when the concentration of both extracts increased so that these extracts can be used as anti-fungal agents for these species. The oil extract of cumin seeds was more active than ethanol extract and control positive Nystatin, ethanol extract had less effect than oil extract and control positive Nystatin.

References

ABDULWAHHAB, A. R. & JASSIM, R. K. 2018. The Effect of Aloe vera Extract on Adherence of Candida albicans and Other Properties of Heat Cure Denture Soft Lining Material. International Journal of Medical Research & Health Sciences, 7, 94-103.

AL-NEMA, L. M. 2014. Evaluation of Addition of Plant Fixed Oil Extracts (Ginger, Maramia, Eucalyptus) on Some Properties of Heat Cured Denture Base Material. Al-Rafidain Dental Journal, 14, 132-138.

AL JABRE, S., AL AKLOBY, O. & AL QURASHI, A. 2003. Thymoquinone, an active principle of Nigella sativa, inhibited Aspergillus niger. Pakistan Journal of Medical Research 42:185-191.

ALI, B. & BLUNDEN, G. 2003. Pharmacological and toxicological properties of Nigella sativa. Phytotherapy Research: An international journal devoted to pharmacological and toxicological evaluation of natural product derivatives, 17, 299-305. https:// doi: 10.1002/ptr.1309

ANIBAL, P. C., SARDI, J. D. C. O., PEIXOTO, I. T. A., MORAES, J. J. D. C. & HÖFLING, J. F. 2010. Conventional and alternative antifungal therapies to oral candidiasis. Brazilian Journal of Microbiology, 41, 824-831. https://doi.org/10.1590/S1517-

83822010000400001

Arici M, Sagdic O, Gecgel U. Antibacterial effect of Turkish black cumin (Nigella sativa L.) oils. grasasaceites [Internet]. 2005Dec.31 [cited 2021Mar.22];56(4):259-62. Available from: http://grasasyaceites.revistas.csic.es/index.php/grasasyaceites/article/view/90

BAMERI, Z., AMINI-BOROUJENI, N., BOKAEIAN, M., SAEIDI, S., JALALADDINI, M. & BAZI, S. 2013. Antimicrobial activity of Cyminum cuminum against biofilm E. coli. Intern Res J Appl Basic Sci, 6, 286-288. https://doi: 10.17795/iji-18739.

BENLAFYA, K., KARROUCHI, K., CHARKAOUI, Y., EL KARBANE, M. & RAMLI, Y. 2014. Antimicrobial activity of aqueous, ethanolic, methanolic, cyclohexanic extracts and essential oil of Nigella sativa seeds. Journal of chemical and Pharmaceutical Research, 6, 9-11.

BURITS, M. & BUCAR, F. 2000. Antioxidant activity of Nigella sativa essential oil. Phytotherapy research, 14, 323-328. https://doi.org/10.1002/1099-1573(200008)14:5<323::AID-PTR621>3.0.CO;2-Q

DARMAWAN, W., FARIDA, R. & REDJEKI, S. 2019. The effect of nigella sativa (Black cumin) seed extract on candida albicans viability. International Journal of Applied Pharmaceutics, 11, 88-91. https://doi.org/10.22159/ijap.2019.v11s1.199

DE OLIVEIRA MIMA, E. G., PAVARINA, A. C., SILVA, M. M., RIBEIRO, D. G., VERGANI, C. E., KURACHI, C. & BAGNATO, V. S. 2011. Denture stomatitis treated with photodynamic therapy: five cases. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 112, 602-608. https://doi. 10.1016/j.tripleo.2011.05.019

EMAMI, E., DE GRANDMONT, P., ROMPRÉ, P., BARBEAU, J., PAN, S. & FEINE, J. 2008. Favoring trauma as an etiological factor in denture stomatitis. Journal of dental research, 87, 440-444. https://doi.org/10.1177/154405910808700505

FARAH, C., LYNCH, N. & MCCULLOUGH, M. 2010. Oral fungal infections: an update for the general practitioner. Australian dental journal, 55, 48-54. https://doi: 10.1111/j.1834-7819.2010.01198.x

FERRARI, P., CAI, S. & BOMBANA, A. C. 2005. Effect of endodontic procedures on enterococci, enteric bacteria and yeasts in primary endodontic infections. International Endodontic Journal, 38, 372-380. https://doi.org/10.1111/j.1365-2591.2005.00947.x

GLEIZNYS, A., ZDANAVIČIENĖ, E. & ŽILINSKAS, J. 2015. Candida albicans importance to denture wearers. A literature review. Stomatologija, 17, 54-66.

HOSHING, C., DIXIT, S., MOOTHA, A. & DIWAN, N. 2011. Role of Candida albicans in denture stomatitis. Journal of Indian Academy of Oral Medicine and Radiology, 23, 617. https://doi.10.5005/jp-journals-10011-1234

İşcan G, İşcan A, Demirci F. Anticandidal Effects of Thymoquinone: Mode of Action Determined by Transmission Electron Microscopy (TEM). Nat Prod Commun. 2016 Jul;11(7):977-978.

ISMAEL, T. K. & ABDUL LATIF , A. 2019. Antifungal activity of some plants extract. Journal of oral and Dental Research 6, 13-24.

KAMBLE, V. A. 2015. In vitro anti-fungal

activity of Cuminum cyminum (cumin seed) essential oil against clinical isolates of Candida species. Am J Phytomed Clin Ther, 3, 264-275.

MIMA, E., VERGANI, C. E., MACHADO, A. L., MASSUCATO, E. M. S., COLOMBO, A. L., BAGNATO, V. S. & PAVARINA, A. C. 2012. Comparison of Photodynamic Therapy versus conventional antifungal therapy for the treatment of denture stomatitis: a randomized clinical trial. Clinical Microbiology and Infection, 18, E380-E388. https://doi.org/10.1111/j.1469-0691.2012.03933.x https://doi.org/10.1111/j.1469-0691.2012.03933.x

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. https://doi.org/10.13057/nusbiosci/n070110

PETROVIĆ, M., KOSTIĆ, M., KOSTIĆ, M., KRUNIĆ, N., IGIĆ, M., PEŠIĆ, Z. & OTAŠEVIĆ, S. 2014. Therapeutic alternatives of natural compounds in treatment of Candida-associated denture stomatitis. Acta Medica Medianae, 53, 73-79. https://doi:10.5633/amm.2014.0113

PIRAS, A., ROSA, A., MARONGIU, B., PORCEDDA, S., FALCONIERI, D., DESSÌ, M. A., OZCELIK, B. & KOCA, U. 2013. Chemical composition and in vitro bioactivity of the volatile and fixed oils of Nigella sativa L. extracted by supercritical carbon dioxide. Industrial Crops and Products, 46, 317-323. https://doi: 10.1556/066.2018.47.3.14

PONKA, D. & BADDAR, F. 2014. Microscopic potassium hydroxide preparation. Canadian Family Physician, 60, 57-57.

Al-Ali A, Alkhawajah AA, Randhawa MA, Shaikh NA. Oral and intraperitoneal LD50 of thymoquinone, an active principle of Nigella sativa, in mice and rats. J Ayub Med Coll Abbottabad. 2008 Apr-Jun;20(2):25-7. PMID: 19385451.

RODINO, S., BUTU, M., PETRACHE, P., BUTU, A. & CORNEA, C. P. 2014. Antifungal activity of four plants against Alternaria alternata. Scientific Bulletin. Series F. Biotechnologies, 18, 60-65.

SILVA, M. M., DE OLIVEIRA MIMA, E. G., COLOMBO, A. L., SANITÁ, P. V., JORGE, J. H., MASSUCATO, E. M. S. & VERGANI, C. E. 2012. Comparison of denture microwave disinfection and conventional antifungal therapy in the treatment of denture stomatitis: a randomized clinical study. Oral surgery, Oral medicine, Oral pathology and Oral radiology, 114, 469-479. https://doi.org/10.1016/j.oooo.2012.05.006

SROISIRI, T. & BOONYANIT, T. 2010. Inhibition of candida adhesion to denture acrylic by Boesenbergia pandurata. Asian Pacific Journal of Tropical Medicine, 3, 272-275. https://doi.org/10.1016/S1995-7645(10)60066-1

WANNER, J., BAIL, S., JIROVETZ, L., BUCHBAUER, G., SCHMIDT, E., GOCHEV, V., GIROVA, T., ATANASOVA, T. & STOYANOVA, A. 2010. Chemical composition and antimicrobial activity of cumin oil (Cuminum cyminum, Apiaceae). Natural product communications, 5, 1934578X1000500904.

WAYNE, P. 2009. Zone diameter interpretive standards, corresponding minimal inhibitory concentration (MIC) interpretive breakpoints, and quality control limits for antifungal disk diffusion susceptibility testing of yeasts. Third International Supplement CLSI document-M444-S3, New York, US.

Young B, Jose A, Cameron D, McCord F, Murray C, Bagg J, Ramage G. Attachment of Candida albicans to denture base acrylic resin processed by three different methods. Int J Prosthodont. 2009 Sep-Oct;22(5):488-9.

ZHANG, M.-R., ZHAO, F., WANG, S., LV, S., MOU, Y., YAO, C.-L., ZHOU, Y. & LI, F.-Q. 2020. Molecular mechanism of azoles resistant Candida albicans in a patient with chronic mucocutaneous candidiasis. BMC Infectious Diseases, 20, 1-6.https:// doi: 10.1186/s12879-020-4856-8.