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Research Article

EFFECT OF *NIGELLA SATIVA* SEED EXTRACT ON THE VIABILITY OF *CANDIDA GLABRATA*

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ABSTRACT

Objective: Candidiasis is a common opportunistic infection of the oral cavity caused by a yeast-like fungus called *Candida*. *Candida glabrata* is the second most frequently isolated species from this condition, after *Candida albicans*. This study aimed to evaluate the effect of *Nigella sativa* (black cumin), known to possess antifungal properties, on the viability of *C. glabrata*.

Methods: *C. glabrata* was added to a 96-microwell plate that was coated with artificial saliva and exposed to various concentrations (6.25%, 12.5%, 25%, and 50%) of *N. sativa* seed extract; amphotericin B (250 mg/mL) was used as the positive control and 200 µL of yeast nitrogen base medium as the negative control. The viability percentage of *C. glabrata* was determined by MTT assay.

Results: The results showed that the viability values of *C. glabrata* were lower after exposure to the *N. sativa* seed extract when compared with the negative control.

Conclusion: The viability of *Candida glabrata* was decreased with increasing concentrations of the extract.

Keywords: *Candida glabrata*, *Nigella sativa*, MTT assay, Optical density, Viability.

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INTRODUCTION

Candidiasis is the most common opportunistic infection of the oral cavity and is caused by the yeast-like fungus *Candida albicans* [1]. Oral candidiasis is mostly found in the elderly and in patients with dentures and HIV-AIDS. It has been reported that more than 90% of individuals infected with HIV suffer from oral candidiasis [2].

C. *albicans* is the most commonly isolated pathogenic fungus in the oral cavity, but the number of isolated species other than *C. albicans* has also increased [2]. The incidence of isolated *C. albicans* in the oral cavity was reported to be 95% in individuals with HIV [3]. The other species that plays a role in oral infections in humans include *Candida glabrata*, *Candida tropicalis*, *Candida dubliniensis*, *Candida pseudo tropicalis*, *Candida guilliermondii*, *Candida krusei*, *Candida lusitaniae*, *Candida parapsilosis*, and *Candida stellatoidea* [2-4].

C. glabrata is the second most frequently isolated species in oral candidiasis after *C. albicans* [5]. Increased use of immunosuppressive agents in conjunction with broad-spectrum antimycotic therapy has resulted in a significant increase in mucosal and systemic infections caused by *C. glabrata*. A major constraint in *C. glabrata* infections is the increase in the number of *C. glabrata* due to their innate resistance mechanisms against antimycotic azole therapy to maintain viability [6,7]. Thus, biofilms formed by *C. glabrata* may be highly resistant to some antifungal medications [8]. It has been reported that 6.8% of *C. glabrata* are resistant to fluconazole, 23.7% are resistant to itraconazole, and 2.5% are resistant to amphotericin B [9,10].

The most common side effects of amphotericin B and the azole antifungal group of medications are gastrointestinal symptoms, with hepatotoxicity being one of the most severe side effects [11,12]. Consequently, these antifungal agents are less effective for the treatment of oral candidiasis due to their side effects and also due to the resistance of *Candida* to these drugs, thus warranting the need for the development of better antifungal drugs against *C. glabrata*.

Medicinal plants serve as an alternative therapy, a safe option, and in some cases, the only effective mode of treatment [13]. One type of plant that has been under consideration among herbalists is *Nigella sativa* (black cumin) [14]. *N. sativa* seeds have been traditionally used for centuries in the Middle East, North Africa, and South Asia as a natural remedy for various diseases [15].

N. sativa seed extract has active antifungal properties. Several previous studies have been conducted to investigate the effects of these seeds as antifungals; the extracts of *N. sativa* seeds have been shown to inhibit the growth of several types of *Candida* fungi such as *C. albicans*, *C. parapsilosis*, and *C. tropicalis* [15-17]. However, the active nature of *N. sativa* seeds as an antifungal agent and the effect of the seed extract on the viability of *C. glabrata* is unknown. Hence, the present study aimed to evaluate the effect of *N. sativa* seed extract on the viability of *C. glabrata*.

METHODS

Premade yeast nitrogen base (YNB) medium (5 mL) was diluted with 45 mL sterile Milli-Q in a 50 mL tube, ensuring that the pH was neutral (pH=7). Next, 10 mL of the diluted YNB was poured into a sterile reaction tube. A single colony of *C. glabrata* in Sabouraud Dextrose Agar medium was added to the test tube. The culture of *C. glabrata* in YNB medium was made in Duplo. After incubation of the culture for 18 h on a shaker (150 rpm) at 37°C, the growth of *C. glabrata* in YNB medium was measured by determining the optical density (OD) value using a spectrophotometer. In a microwell plate, 200 µL of sterile single dilution YNB was added as a blank. The cultured *C. glabrata* (200 µL) was then added to the wells. This procedure was performed Duplo. The OD values were read at a wavelength of 490 nm. The *C. glabrata* suspension was subsequently diluted to 10^{-10}.

After obtaining an OD of 0.514 and a colony-forming unit of 13×1011/mL, 200 mL of the *C. glabrata* suspension was poured into a 96-microwell plate coated with saliva and incubated for 120 min at 37°C. The microorganisms that remained unattached to the saliva were

removed following two rinses with 200 µL of PBS. Subsequently, 200 µL of *N. sativa* seed extracts of varying concentrations (6.25%, 12.5%, 25%, and 50%) were added into each well. Amphotericin B (4 µL) and YNB (196 µL) were added to the wells as positive controls, whereas 200 µL of YNB was taken as the negative control. This procedure was performed in 12 wells for each group. The 96-microwell plate was then incubated for 24 h at 37°C. Images of the morphology of *C. glabrata* were obtained. In addition, an MTT test was performed.

The OD values were obtained from the spectrophotometer at a wavelength of 490 nm, and the viability of *C. glabrata* was calculated using the following formula: The viability of *S. sanguinis* was calculated using the formula:

$$\text{Cell viability}\,(\%) = \frac{\text{Absorbance value of treatment group}}{\text{Absorbance value of control group}} \times 100\%$$

Statistical analysis

Data were processed statistically using. The Kolmogorov–Smirnov test was used to assess the distribution of data, and one-way analysis of variance followed by *post hoc* tests was used to determine the level of significance between groups. Pearson's correlation was used to assess linear associations between the concentrations of the extract and the viability of the microorganisms. The level of significance was set at $p<0.05$.

RESULTS

The percentage of viability of *C. glabrata* was 18% in the positive control group (amphotericin B) and 100% in the negative control group (Fig. 1). In the treatment group, *C. glabrata* viability after exposure with 6.25% *N. sativa* seed extract was 50%, whereas exposure with 12.5% seed extract resulted in viability of 47%. Exposure to 25% and 50% *N. sativa* seed extract demonstrated 24% and 35% viability of *C. glabrata*, respectively. Among the various concentrations of the *N. sativa* seed extract, the highest percentage of *C. glabrata* viability was found in the treatment group, which decreased with increasing concentrations of the extract, except for the 50% concentration.

The average OD values obtained from the spectrophotometer are shown in Fig. 2. The mean OD in the negative control group (0.773±0.050) was higher than that in the treatment groups with the various concentrations of the seed extract. The average ODs of the 6.25%, 12.5%, 25%, and 50% *N. sativa* seed extracts were 0.388±0.052, 0.367±0.114, 0.187±0.027, and 0.187±0.027, respectively.

The results of the Kolmogorov–Smirnov normality test revealed that the data had a normal distribution ($p>0.05$). Homogeneity variance was evaluated using the Levene test, which resulted in a value of 8753 with 0.000 significance, thus accepting the null hypothesis of the current study that *N. sativa* seed extracts can decrease the viability of *C. glabrata*.

Statistically significant differences in average OD ($p<0.05$) were observed between the control group with the various treatment groups (6.25%, 12.5%, 25%, and 50%). Conversely, average OD in the 6.25% treatment group (0.388) was not significantly different from that in the 12.5% group (0.367). Furthermore, no significant difference in average OD was noted between the group exposed to amphotericin B (0.143) and that exposed to 25% *N. sativa* seed extract (0.187).

The correlation coefficient between the OD of *C. glabrata* and the acting group was −0.592**, thus indicating a negative relationship between the ODs of *C. glabrata* and the treatment group. The viability of *C. glabrata* was found to decrease with the increase in the concentration of the *N. sativa* seed extract ($p=0.000$).

DISCUSSION

The present study aimed to evaluate the effect of various concentrations of *N. sativa* seed extract (6.25%, 12.5%, 25%, and 50%) on the viability of *C. glabrata*.

The percentage of viability of *C. glabrata* was calculated by comparing the mean OD values between the treatment groups and the negative control group. The 6.25%, 12.5%, 25%, and 50% concentrations of *N. sativa* seed extract decreased the viability of *C. glabrata*; the viability in the group exposed to the 6.25% *N. sativa* seed extract was lower than

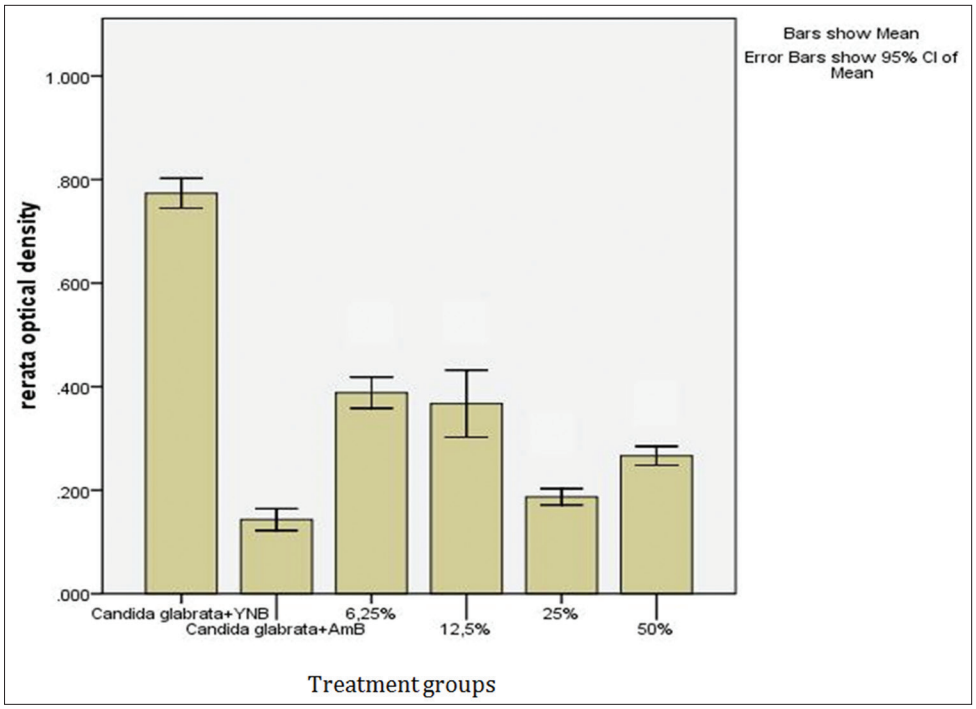

Fig. 1. Percentage of *Candida glabrata* viability after exposure to various concentrations of *Nigella sativa* seed extract for 24 h at 37°C

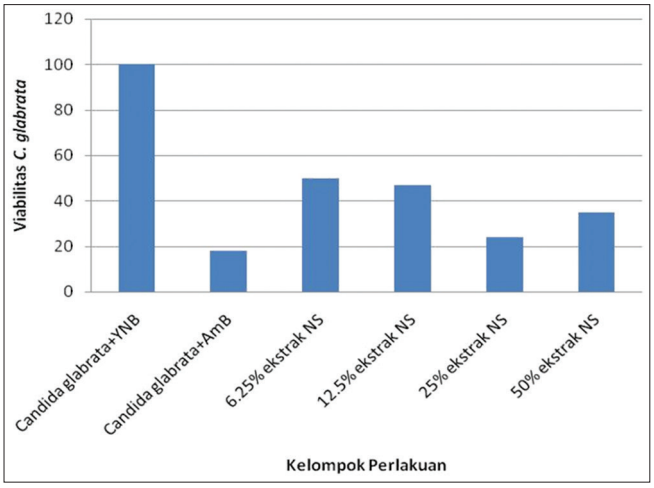

Fig. 2: Bar graph showing the mean optical densities after exposure to *Nigella sativa* seed extract for 24 h at 37°C

that in the control group, indicating that exposure to low concentrations of the extract might decrease the viability of these microorganisms. Exposure to the 12.5% extract resulted in a slight decrease in *C. glabrata* viability when compared with the 6.25% extract, statistical significance notwithstanding.

The present study did not investigate the mechanism by which, *N. sativa* seed extract causes an increase or decrease in *C. glabrata* viability. In a previous study, phytochemical analysis of *N. sativa* seed ethanolic extracts had revealed the presence of two main components: Thymoquinone and nigellone; thymoquinone is thought to be involved in reducing the viability of *C. glabrata* [18].

The thymoquinone-mediated antifungal activity of *N. sativa* seeds was evaluated by Aljabre *et al*. and Akhtar *et al*., and it was found to effectively inhibit the growth of the dermatophytes *Aspergillus niger* (minimum inhibitory concentration, 2 mg/mL) and *Fusarium solani* [19,20]. Dermatophytes are a group of fungi that attack the stratum corneum of the skin, hair, and nails and can cause a type of infection called tinea or ringworm infection. Arthrospora is a morphological form of dermatophytes that play an important role in infection. Subsequently, the effect of thymoquinone on arthrospora dermatophytes was investigated by Aljabre *et al*. in 2009, wherein thymoquinone was reported to inhibit the germination of arthrospores [19]. On the basis of the results of their study, it may be assumed that the decrease in the viability of *C. glabrata* by *N. sativa* seed extract occurs due to the inhibition of the germination of blastoconidia through thymoquinone activity.

In addition to thymoquinone, the other components of the *N. sativa* seed extract suspected to have antifungal effects are thymol and carvacrol. In a previous study, thymol and carvacrol were reported to inhibit ergosterol synthesis in *Candida* [21]. Ergosterol is the main component of sterol found in the yeast cell membrane and is responsible for maintaining cell function. The viability of *C. glabrata* was decreased by *N. sativa* seed extract due to the inhibition of ergosterol synthesis by thymol and carvacrol, which occurs as a result of interactions with demetilase C14A, an enzyme that depends on P-450 cytochrome required for the conversion of lanosterol to ergosterol [21]. Ergosterol deficiency causes membrane fluidity, resulting in increased permeability and growth restriction, and cell multiplication [12]. Inhibition of ergosterol synthesis may also lead to the disruption of nucleic acid synthesis or accumulation of peroxides in fungal cells that can cause damage [11].

The lowest percentage of *C. glabrata* viability was observed at a *N. sativa* seed extract concentration of 25%, which suggests that this concentration is optimum for use during treatment. In 50% concentration group, the viability of *C. glabrata* was increased, probably due to the ability of these microorganisms to tolerate 14α demetilase deficiency during ergosterol biosynthesis; thus, the higher concentrations of the extracts had exceeded the optimum dose [22]. The decrease in effectiveness after reaching the optimum dose has been explained using a hyperbolic curve, wherein the increase in drug concentration will lead to an increase in the effectiveness of the drug until it reaches its maximum point; however, the effectiveness of the drug decreases if the concentration of the drug is increased beyond the maximum point on the curve [23].

In another study where static *N. sativa* seed oil was used as a test material against *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. krusei*, the best antifungal activity was found against *C. glabrata* (inhibition zone, 12 mm) and *C. parapsilosis* (inhibition zone, 13.33 mm) [24].

Ethanol (96%) has been used as a solvent for the extraction of *N. sativa* seed oil and shown no antifungal effects, indicating that it does not affect the viability of *C. glabrata* [25]. In the current study, we did not use 96% ethanol in the negative controls.

The results of the present study indicated that an unsuitable MTT test was used to test the cytotoxic effect of *N. sativa* seed oil, which may be due to the colorimetric nature of the test. The seed oil of *N. sativa* is dark brown in color that turns dark purple after MTT testing. Dense color changes can result in higher absorbance values, which affect the OD value leading to higher values in the treatment group when compared with the controls. In addition to the color factor, volatile oil turbidity may also affect the OD value.

CONCLUSION

N. sativa seed extract can reduce the viability of *C. glabrata*, which was found to decrease with increased concentrations of the extract in the current study.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

REFERENCES

1. Burket LW, Greenberg MS, Glick M. Burket's Oral Medicine: Diagnosis and Treatment. 10th ed. New Jersey: BC Decker Inc.; 2003. p. 94-101.
2. Meurman JH, Siikala E, Richardson M, Rautemaa R. Non-*Candida albicans Candida* yeasts of the oral cavity. Formatex 2007;1:719-31.
3. Akpan A, Morgan R. Oral candidiasis. Postgrad Med J 2002;78:455-9.
4. Brooks G, Butel J, Mourse S, Adelber JM. Medical Microbiologi. 23th ed. New York: Connecticut Appleton and Lange; 2004. p. 645-7.
5. Luo G, Samaranayake LP. *Candida glabrata*, an emerging fungal pathogen, exhibits superior relative cell surface hydrophobicity and adhesion to denture acrylic surfaces compared with *Candida albicans*. APMIS 2002;110:601-10.
6. Fidel PL Jr., Vazquez JA, Sobel JD. *Candida glabrata*: Review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clin Microbiol Rev 1999;12:80-96.
7. Arzumanyan VG, Semenov BF. Drug sensitivity of *Candida* yeast isolated from patients with allergic diseases. Bull Exp Biol Med 2001;131:346.
8. Tscherner M, Schwarzmüller T, Kuchler K. Pathogenesis and antifungal drug resistance of the human fungal pathogen *Candida glabrata*. Pharmaceuticals 2011;4:169-86.
9. Kuriyama T, Williams DW, Bagg J, Coulter WA, Ready D, Lewis MA. *In vitro* susceptibility of oral *Candida* to seven antifungal agents. Oral Microbiol Immunol 2005;20:349-53.
10. Yang YL, Li SY, Cheng HH, Lo HJ. Susceptibilities to amphotericin B and fluconazole of *Candida* species in TSARY 2002. Diagn Microbiol Infect Dis 2005;51:179-83.
11. Ganiswarna SG, editor. Farmakologi dan Terapi. 4th ed. Jakarta: Gaya Baru; 2000.
12. Herman MJ. Antijamur sistemik. Cermin Dunia Kedokteran 1996;108:37-44.
13. Salem ML. Immunomodulatory and therapeutic properties of the

Nigella sativa L. Seed. Int Immunopharmacol 2005;5:1749-70.
14. Junaedi IE, Yulianti IS, Suty DS, Kuncari SS. Kedahsyatan Habbatussauda Mengobati Berbagai Penyakit. Jakarta: Agromedia Pustaka; 2011. p. 9-11.
15. Mashhadian NV, Rakhshandeh H. Antibacterial and antifungal effect of *Nigella sativa* extracts against *S. aureus, P. Aeroginosa* and *C. albicans*. Pak J Med Sci 2005;21:47-52.
16. Raval BP, Shah TG, Suthar MP, Ganure AL. Screening of *Nigella sativa* seeds for antifungal activity. Ann Biol Res 2010;1:164-71.
17. Haloci E, Manfredini S, Toska V, Vertuani S, Topi PZ, Kolani H. Antibacterial and antifungal activity assessment of *Nigella sativa* essential oils. World Acad Sci Eng Technol 2012;66:1198-200.
18. Nehar S, Rani P. HPTLC studies on ethanolic extract of *Nigella sativa* Linn. seeds and its phytochemical standardization. Ecoscan 2011;1:105-8.
19. Aljabre SM, Randhawa MA, Alakloby OM, Alzahrani AJ. Thymoquinone inhibits germination of dermatophyte arthrospores. Saudi Med J 2009;30:443-5.
20. Akhtar N, Alakloby OM, Aljabre SH, Alqurashi AR, Randhawa MA. Comparison of antifungal activity of thymoquinone and amphotericin B against *Fusarium solani in-vitro*. Sci J King Faisal Uni (Basic Appl Sci) 2007;8:137-45.
21. Pinto E, Pina-Vaz C, Salgueiro L, Gonçalves MJ, Costa-de-Oliveira S, Cavaleiro C, *et al.* Antifungal activity of the essential oil of thymus pulegioides on *Candida*, *Aspergillus* and *Dermatophyte* species. J Med Microbiol 2006;55:1367-73.
22. Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, *et al*. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. Eur J Clin Microbiol Infect Dis 2011;30:41-50.
23. Bourne HR, James MP. Drug receptors and pharmacodynamics. In: Basic and Clinica Pharmacology. 3rd ed. Connecticut: Appleton and Lange; 1987. p. 10.
24. Harzallah HJ, Noumi E, Bekir K, Bakhrouf A, Mahjoub T. Chemical composition, antibacterial and antifungal properties of Tunisian *Nigella sativa* fixed oil. Afr J Microbiol Res 2012;6:4675-9.
25. Elvas AR. Effects of Dyes on *Candida* spp. Viability. Ciências da Saúde Effects: Universidade da Beira Interior; 2011.