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Antifungal activity of *Nigella sativa* (Ranunculaceae) extracts against dermatophytes and *Candida* species

Actividad antifúngica de los extractos de *Nigella sativa* (Ranunculaceae) contra dermatófitos y especies de *Candida*

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ABSTRACT

Dermatophytoses and candidiasis are widespread fungal infections in both humans and animals. However, they can be life-threatening in individuals with compromised immune systems. Moreover, emerging drug resistance has led to treatment failure. It is therefore necessary to explore natural therapeutic alternatives capable of counteracting growing antifungal resistance and offering an effective complement to conventional treatments.

Nigella sativa was selected for this study due to its well-known beneficial effects, particularly in dermatology, making it an excellent candidate for skincare applications. The antifungal activity of its vegetable oil, ethanolic, and methanolic extracts was evaluated through both *in vitro* and *in vivo* methods. Their *in vitro* efficacy was tested against five common pathogenic fungi, followed by *in vivo* assessment using mouse models. *Microsporum canis* was the most susceptible species, inhibited at an exceptionally low minimum inhibitory concentration (MIC) of 0.005 µl/mL of the vegetable oil. *In vivo*, only 60 µL of this oil was required to achieve full recovery within three days, highlighting its rapid and effective therapeutic potential. The methanolic extract also showed notable *in vivo* activity against *Candida* species, notably *C. albicans*, showing 0% mortality rate after five days of treatment. In contrast, the ethanolic extract exhibited comparatively lower efficacy against all species. The outcomes of our study highlighted that *M. canis* was the most susceptible and that *C. glabrata* presented greater resistance, requiring higher MICs than other fungi for all the extracts, emphasizing variability in susceptibility among fungal pathogens.

Keywords: Antifungals, black seed, candidiasis, dermatomycoses.

RESUMEN

Las dermatofitosis y la candidiasis son infecciones fúngicas muy extendidas tanto en humanos como en animales. Sin embargo, pueden poner en peligro la vida de las personas con sistemas inmunitarios comprometidos. Además, la resistencia emergente a los medicamentos ha provocado el fracaso de los tratamientos. Por lo tanto, es necesario explorar alternativas terapéuticas naturales capaces de contrarrestar la creciente resistencia a los antifúngicos y ofrecer un complemento eficaz a los tratamientos convencionales. Se seleccionó la *Nigella sativa* para este estudio debido a sus conocidos efectos beneficiosos, especialmente en dermatología, lo que la convierte en una excelente candidata para aplicaciones en el cuidado de la piel. Se evaluó la actividad antifúngica de su aceite vegetal y de sus extractos etanólicos y metanólicos mediante métodos *in vitro* e *in vivo*. Se probó su eficacia *in vitro* frente a cinco hongos patógenos comunes, seguida de una evaluación *in vivo* utilizando modelos de ratón. *Microsporum canis* fue la especie más vulnerable, inhibida a una concentración mínima inhibitoria (CMI) excepcionalmente baja de 0.005 µl/mL del aceite vegetal. *In vivo*, solo se necesitaron 60 µL de este aceite para lograr la recuperación completa en tres días, lo que destaca su rápido y eficaz potencial terapéutico. El extracto metanólico también mostró una notable actividad *in vivo* contra las especies de *Candida*, en particular *C. albicans*, con una tasa de mortalidad del 0% tras cinco días de tratamiento. Por su parte, el extracto etanólico mostró una eficacia comparativamente menor contra todas las especies. Los resultados de nuestro estudio pusieron de relieve que *M. canis* era el más

susceptible y que *C. glabrata* presentaba una mayor resistencia, requiriendo CMI más altas que otros hongos para todos los extractos, lo que pone de relieve la variabilidad en la susceptibilidad entre los hongos patógenos.

Palabras clave: Antifúngicos, candidiasis, dermatomicosis, semilla negra.

INTRODUCTION

Over a billion people are affected by mycoses, leading to a high mortality rate each year (Last *et al.,* 2021). Among these pathologies, dermatophytoses and candidiasis are the most common fungal infections worldwide. Although dermatophytoses are typically not life-threatening for healthy individuals, they can result in severe infections in immunocompromised patients, creating serious health concerns (Hill *et al.,* 2024). *Candida* species can lead to invasive candidiasis, with mortality rates approaching 50%, primarily affecting those with weakened immune systems (Pristov and Ghannoum, 2019). Meanwhile, the rising global resistance in dermatophytoses, particularly among pathogens like *Trichophyton*, *Microsporum*, and *Candida*, complicates clinical management and undermines treatment efficacy (Williams *et al.,* 2020; Hill *et al.,* 2024).

Current antifungal treatments often require prolonged administration and are limited by side effects and poor patient adherence. Patients frequently stop therapy prematurely once symptoms subside, rather than completing the full course, promoting the emergence of resistant, more contagious fungal strains, further complicating clinical control of mycoses (Burstein *et al.,* 2020; Hill *et al.,* 2024). Given these challenges, there is an urgent need to identify natural antifungal agents with strong antimicrobial properties and favorable safety profiles.

Nigella sativa (Black seed), a medicinal plant with a rich history in traditional medicine, has garnered scientific interest due to its essential oil, which is rich in bioactive compounds like thymoquinone, thymol, and carvacrol (Mahmoudvand *et al.*, 2014; Shokri, 2016; Tiji *et al.*, 2021; Shafodino *et al.,* 2022). However, the antifungal activity of vegetable oil and alcoholic extracts from this plant have yet to be thoroughly established. Previous studies have shown that the aforementioned metabolites combat fungal pathogens, including *Candida* species, through multiple complementary mechanisms. These include disruption of cell membrane integrity, inhibition of ergosterol biosynthesis (a process essential for fungal cell viability) and interference with key enzymatic pathways (Mahmoudvand *et al.*, 2014; Khwaza and Aderibigb, 2023; Mączka *et al.,* 2023). Notably, thymoquinone, the predominant active compound, induces oxidative stress within fungal cells, triggering programmed cell death and effectively reducing fungal viability (Mahmoudvand *et al.*, 2014; Almshawit and Macreadie, 2017). Despite these promising findings, the detailed antifungal efficacy and underlying mechanisms of *N. sativa* preparations against dermatophytes remain inadequately defined. Taken together, the evidence underscores the significant potential of *N. sativa* as a safe, natural substitute for managing superficial fungal infections, especially at a time when conventional antifungal therapies face increasing resistance challenges.

To explore whether *N. sativa* contains additional antifungal metabolites, this study aimed to test its vegetable oil, methanolic and ethanolic extracts, both *in vitro* and *in vivo* against five predominant pathogenic agents, including *Microsporum canis*, *M. audouinii*, *Trichophyton rubrum*, *Candida albicans*, and *C. glabrata*.

MATERIALS AND METHODS

Fungal strains, seeds, and vegetable oil origin: Authenticated *N. sativa* seeds and cold-pressed vegetable oil, preserving their bioactive compounds, were sourced from the Department of Environmental and Agricultural Sciences, Faculty of Natural and Life Sciences, University of Jijel (Algeria).

Clinical dermatophyte isolates included three strains of *M. canis* and one strain of *M. audouinii*, recovered from patients with superficial infections, as well as two strains of *T. rubrum* obtained from individuals diagnosed with onychomycosis. These strains were supplied by the Hospital of Jijel and the University Hospital Center of Constantine, and identified through an integrative approach involving detailed macroscopic and microscopic observations, complemented by molecular characterization to ensure precise species-level confirmation. Additionally, yeast isolates consisting of three strains each of *C. albicans* and *C. glabrata* were collected from candidiasis patients at the Laboratory for Medical Analysis in Jijel, where identification was confirmed by both biochemical and genetic methods.

Preparation of the different extracts: The vegetable oil was diluted with methanol to obtain different concentrations ranging from 5 µl/mL to 100 µl/mL. Methanolic and ethanolic extracts were prepared by maceration of 10 g of *N. sativa* seeds in 100 mL of solvent mixtures: methanol and water (50:50, V/V), and ethanol and water (80:20, V/V), respectively. The extraction was conducted over three consecutive days in the dark at room temperature with continuous magnetic stirring to maximize solvent penetration and extract bioactive compounds effectively. The solvent was renewed daily while retaining the same plant material to optimize yield. On the final day, the pooled extracts were carefully concentrated under reduced pressure at 50 °C using a rotary evaporator, ensuring complete solvent removal while protecting the integrity of thermolabile constituents. Evaporation proceeded until the extract was completely dry, as confirmed visually. The resulting crude extract of 1 mg (10%, W/W) was then reconstituted in methanol/ethanol to obtain a concentration of 1 mg/mL.

Subsequently, serial dilutions were meticulously prepared with distilled water, reaching concentrations ranging from 0.005 mg/mL to 0.100 mg/mL (Mahmoudvand *et al.*, 2014; Akroum, 2021).

Preparation of the fungal suspensions: The *in vitro* antifungal activity was assessed on five fungi: *M. canis*, *M. audouinii*, *T. rubrum*, *C. albicans,* and *C. glabrata*. They were cultivated in Petri dishes containing Sabouraud agar medium with cycloheximide. A concentration of 10^7 colony-forming units (CFU/mL) of fungal suspensions was prepared with phosphate-buffered saline solution (PBS). For the *in vivo* assays against yeasts, an inoculum of $1.5x10^5$ CFU/mL was prepared. Antifungal susceptibility testing was performed according to the CLSI M100 standard to ensure reliable and reproducible MIC determination (Mahmoudvand *et al.,* 2014; Akroum, 2018).

***In vitro* antifungal assay:** The inoculation of each fungal suspension was done on Petri plates containing Sabouraud agar medium supplemented with cycloheximide and 1 mL of the different concentrations of the vegetable oil or the extracts tested. Also, terbinafine (0.050 mg/mL terbinafine hydrochloride) and distilled water were used as positive and negative controls, respectively. Moulds were incubated at 25 °C for 7 days, and yeasts at 30 °C for 24 h. MIC values were determined at the end of the incubation period of each fungus (Bita *et al.,* 2012; Akroum, 2018; Egbe *et al.,* 2023).

Treatment of dermatophytosis caused by *M. canis*: The test was performed on white mice that were immunosuppressed by a subcutaneous injection of 2 mg of estradiol valerate. They were then infected by spraying 1 mL of *M. canis* suspension (10^7 CFU/mL) onto an area of scarified and shaved skin. When the mycosis occurred, the treatment was carried out by spreading the vegetable oil and the extracts on the wounds at a rate of 20 µL/mouse per day for five successive days. The skin lesions were graded from one (significant crusting and erythema) to five (hair growth) based on the severity of the lesions. In this experiment, five mice were assigned to each experimental and control group. Two control tests were conducted: positive and negative. The first test (positive) was carried out on immunocompromised, infected, and treated mice using terbinafine. The second test (negative) was performed on immunocompromised, infected, and untreated mice, which had undergone mycosis with erythema and squames. The treatment cured mycosis after five consecutive days of use (Akroum, 2018).

Treatment of candidiasis caused by *Candida* species: A group of mice was immunosuppressed by an intraperitoneal injection of 2 mg of cyclophosphamide. The infection was induced by oral gavage of 1 mL of each suspension (10^5 CFU/mL). Treatment commenced 48 h post-inoculation and was administered daily for five consecutive days, delivering 20 µL of vegetable oil or extracts per 20 g of mouse body weight. Each experimental group consisted of five mice. The mortality was checked daily (Akroum, 2018; Akroum, 2021).

The experiments were carried out in accordance with the guidelines established by the Algerian Association of Experimental Animal Sciences (AAEAS) (http://www.aasea.asso.dz/). Approval to use the animals was granted by the Department of Applied Microbiology and Food Sciences in the Faculty of Nature and Life Sciences at the University of Jijel.

Statistical analysis: The *in vitro* antifungal experiments were carried out in triplicate, and the results were presented as the mean MICs ± standard deviation (SD). In contrast, the *in vivo* assay outcomes were expressed as the percentage mortality of yeasts over a 5-day period. Data processing was conducted using Microsoft Office Excel 2019. Statistical analyses were performed using SPSS software version 20.0. One-way ANOVA was applied at a significance level of 5%, followed by post-hoc comparisons with Tukey's HSD test to identify significant differences among treatments ($p < 0.05$).

RESULTS

***In vitro* antifungal assay:** The findings revealed that all the extracts exhibited antifungal activity against the strains used in this study. Notably, vegetable oil showed the best activity against all fungi ($p < 0.05$), outperforming terbinafine, methanolic and ethanolic extracts, which showed moderate activity (Table 1). In fact, methanolic and ethanolic extracts were often not statistically different from each other ($p > 0.05$).

M. canis was the most sensitive species, expressing the lowest MIC value of 0.005 µl/mL, followed by *M. audouinii*, *C. albicans*, *T. rubrum*, and *C. glabrata*. The latter was the most resistant fungus, requiring high extract concentrations for inhibition as high as 0.075 mg/mL, reflecting its resistance (Fig. 1)

Treatment of dermatophytosis caused by *M. canis*: According to our results, the best outcomes were those obtained with 60 µL of vegetable oil, which resulted in complete recovery of the mice within three days and promoted hair growth by the fourth day, corresponding to stages four and five, respectively, as compared to terbinafine used as a control. So, it was the most appropriate remedy for dermatophytosis. On the other hand, methanolic and ethanolic extracts gave good results; however, complete skin recovery required a higher dose of 100 µL and an extended period of five days. Additionally, these groups exhibited varying degrees of crusting and erythema, indicative of earlier stages of infection (stages 1, 2, and 3), as illustrated in (Fig. 2). These findings were consistently reproduced across three independent experiments.

Treatment of candidiasis caused by *Candida* species: The results obtained were compared with two control groups : the first comprised immunocompromised, infected,

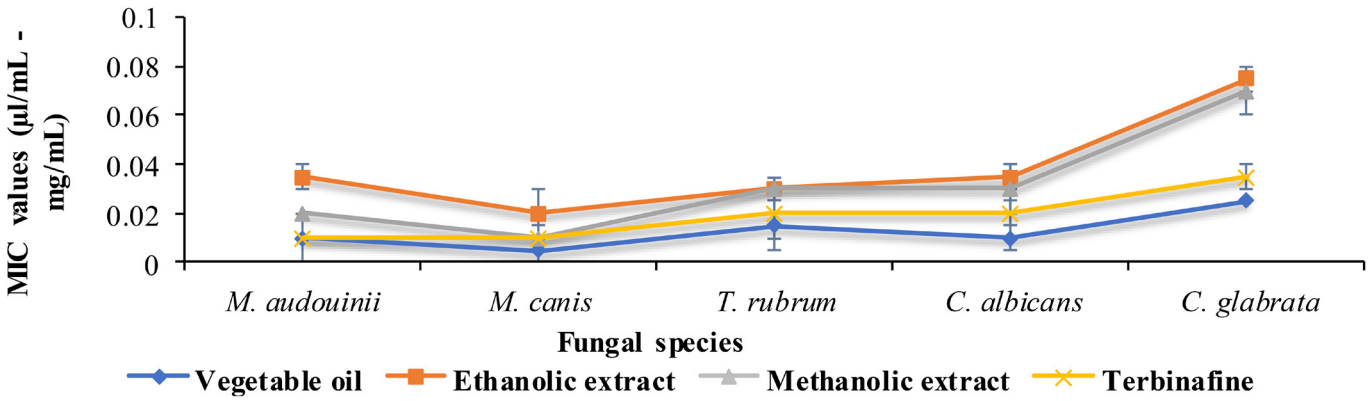

Figure 1. Comparative MIC values of different *N. sativa* extracts and terbinafine against all tested species.

and untreated mice, while the second group consisted of healthy mice that were infected, and treated with 100 µL of the tested extracts. The first group exhibited a mortality rate of 100%, with all five animals in the group deceased, whereas the second group experienced a 0% mortality rate, as all animals survived throughout the duration of the study (Akroum, 2018; Akroum, 2021).

This activity demonstrated that mice infected with *Candida* species responded most effectively to methanolic extract. Indeed, it was the most effective candidiasis treatment, significantly inhibiting growth of both yeasts after five days, followed by ethanolic extract, which showed lower efficacy ($p < 0.001$). Vegetable oil, on the other hand, exhibited significantly lower efficacy (higher mortality rates) than the other extracts ($p < 0.001$) (Fig. 3). This difference is likely attributable to variations in absorption, as the uptake of vegetable oil in mice appears to be less efficient compared to that of polar solvent extracts. The data suggest that bioactive constituents such as phenols and flavonoids, concentrated in methanolic extracts, are more readily absorbed across the intestinal barrier in animals, facilitating greater systemic availability and thus enhancing antifungal activity against *Candida* species. Conversely, the vegetable oil, characterized by a predominance of lipophilic compounds, undergoes slower and less complete absorption, compromising its bioavailability and diminishing its antifungal effectiveness against candidiasis (Sadgrove and Jones, 2019; Shafodino *et al.,* 2022).

DISCUSSION

The present investigation establishes the pronounced antifungal efficacy of *N. sativa* extracts, with its vegetable oil demonstrating promising therapeutic potential against dermatophytic infections in both *in vitro* and *in vivo* experimental paradigms. Notably, *M. canis* showed exceptional susceptibility to treatment, achieving complete pathological resolution within three days in the murine model. This finding corroborates previous studies attributing this antimycotic activity to bioactive monoterpenes, including carvacrol and thymol, which exert their therapeutic effects through disruption of fungal cell membrane integrity and inhibition of spore germination, biofilm formation and ergosterol synthesis (Ali-Shtayeh *et al.,* 2019; Jung *et al.,* 2021; Michalczyk and Ostrowska, 2021; Mączka *et al.,* 2023).

The methanolic extract demonstrated comparable therapeutic efficacy, particularly against candidiasis, substantiating previous investigations (Bita *et al.,* 2012; Mahmoudvand *et al.,* 2014). However, a notable pharmacokinetic disparity emerged, wherein the vegetable oil's activity against *Candida* species proved less pronounced *in vivo* compared to its remarkable effectiveness against dermatophytes, a phenomenon likely attributable to differential pharmacokinetic factors (Sadgrove and Jones, 2019). The methanolic extract's therapeutic mechanism appears to be mediated by its abundant phenolic constituents along with thymoquinone, which orchestrates antifungal properties through multiple pathways, including oxidative stress induction, membrane destabilization, and direct interaction with fungal genetic material (Almshawit and Macreadie, 2017; Lavaee *et al.,* 2018; Shafodino *et al.,* 2022).

While the antifungal properties of various *N. sativa* preparations, including essential oils against pathogenic fungi have been extensively documented (Aljabre *et al.,* 2005; Mahmoudvand *et al.,* 2014; Shokri, 2016; Almshawit and Macreadie, 2017), the molecular mechanisms underlying these medicinal effects remain inadequately elucidated. This knowledge gap becomes particularly pronounced when contrasted with conventional antifungals, which possess well-characterized molecular targets such as sterol biosynthesis enzymes, ribosomal functions, and micro-tubular structures (Fioriti *et al.,* 2022; Vanreppelen *et al.,* 2023). Contemporary research on *N. sativa* predominantly focuses on phenotypic manifestations of antifungal activity, growth inhibition parameters, MIC values, and viability reduction metrics without comprehensively delineating the specific molecular pathways disrupted within fungal pathogens. In contrast, a recent investigation by Egbe *et al.* (2023) represents a significant advancement in understanding *N. sativa's* mode of action against *C. albicans*. This study revealed that *N.*

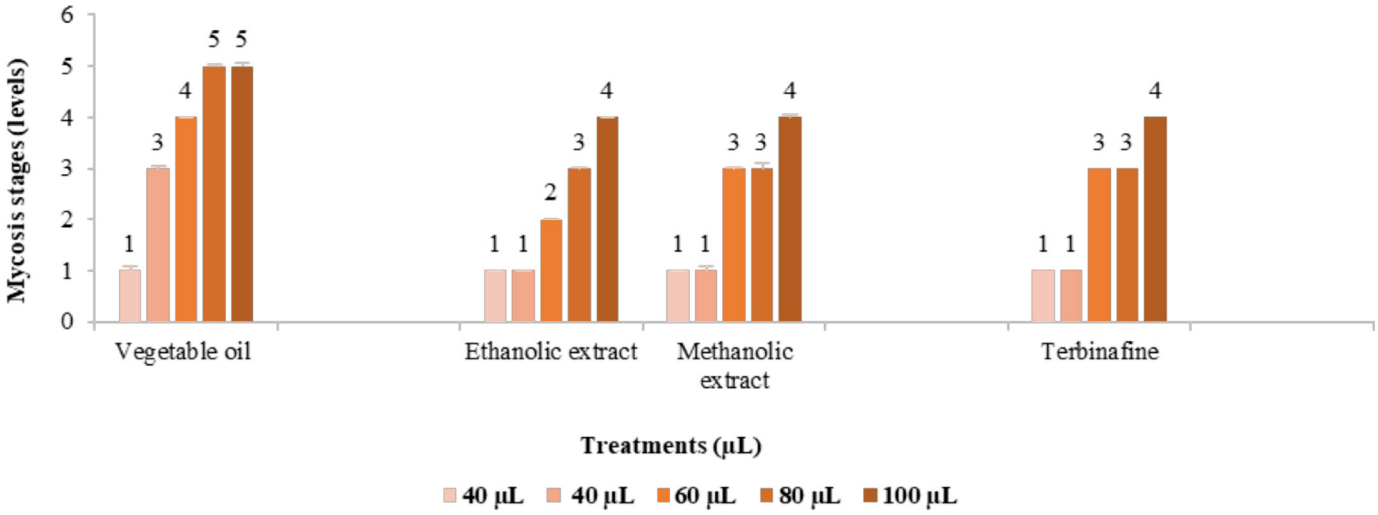

Figure 2. Stages of mycosis development depending on the treatment doses received. Infection levels: 1: Significant crusting and erythema, 2: Medium crusting and erythema, 3: Slight crusting and erythema, 4: Healthy skin, 5: Hair growth.

sativa oil demonstrated broad-spectrum efficacy against both wild-type and *URA3*-deleted *C. albicans* strains, including fluconazole-resistant variants. While aqueous and methanolic extracts exhibited moderate effectiveness against wild-type strains, the seed oil's superior antimycotic activity suggests a mode of action that circumvents or overcomes established resistance mechanisms. Particularly noteworthy is the complex molecular interplay between *N. sativa* bioactive compounds and *C. albicans* drug resistance genes, specifically *MDS3* and *MDR1*, indicating potential therapeutic mechanisms involving gene expression modulation or amplification pathways. These findings warrant further mechanistic studies to fully elucidate the specific molecular basis of *N. sativa's* antifungal properties in dermatophytes.

Our findings confirm that *C. glabrata* exhibits significantly greater resistance compared to *C. albicans*, consistent with prior studies (Lavaee *et al.,* 2018; Yassin *et al.,* 2020; Akroum, 2021). Renowned for its remarkable adaptability, *C. glabrata* has rapidly developed multifaceted resistance mechanisms against major antifungal classes, including azoles, echinocandins, and polyenes. These mechanisms encompass mutations in key drug target genes such as *PDR1* (pleomorphic drug resistance) and *FKS1/2*, overexpression of efflux transporters like *CDR1* and *CDR2*, and robust biofilm formation, collectively contributing to its enhanced antifungal tolerance (Frías-De-León *et al.,* 2021; Hassan *et al.,* 2021; Tortorano *et al.,* 2021). This sophisticated arsenal enables *C. glabrata* to survive and proliferate despite therapeutic interventions (Vanreppelen *et al.,* 2023).

CONCLUSIONS

To conclude, the vegetable oil and methanolic extract of *N. sativa* show great promise as natural, effective treatments for dermatophytoses and candidiases, respectively. In contrast, the ethanolic extract demonstrated limited antifungal activity against the tested species, suggesting it may not be a viable therapeutic option. However, this study faces certain limitations, including the absence of detailed phytochemical profiling of the *N. sativa* extracts, which limits the ability to identify the specific compounds responsible for antifungal effects. Additionally, while the *in vivo* murine model provides valuable insights, it cannot fully replicate the complexity of human fungal infections, which may account for some differences observed between *in vitro* and *in vivo* findings. Moreover, essential pharmacokinetic and pharmacodynamics properties of the extracts and oil remain uncharacterized, which limits the ability to guide optimal dosing and delivery.

Looking forward, future research should prioritize comprehensive chemical characterization of *N. sativa* extracts to identify active constituents with antifungal properties. Exploring synergistic interactions with existing antifungal drugs could unlock powerful combination therapies capable of overcoming resistance. Detailed mechanistic studies using molecular and cellular techniques will be crucial to unravel how these extracts exert their effects and how fungal resistance might be circumvented. Ultimately, translating these findings into clinical practice depends on robust, well-designed human trials focused on safety, efficacy, improved bioavailability, and innovative delivery systems. Such efforts will pave the way for harnessing *N. sativa* as a safe, effective, and accessible natural antifungal solution, addressing a growing global need for alternative therapies.

AUTHOR CONTRIBUTIONS

All persons who meet the authorship criteria are listed as authors and all authors certify that they have contributed sufficiently to this work. Conceptualization, methodology, investigation, writing–review and editing, writing original

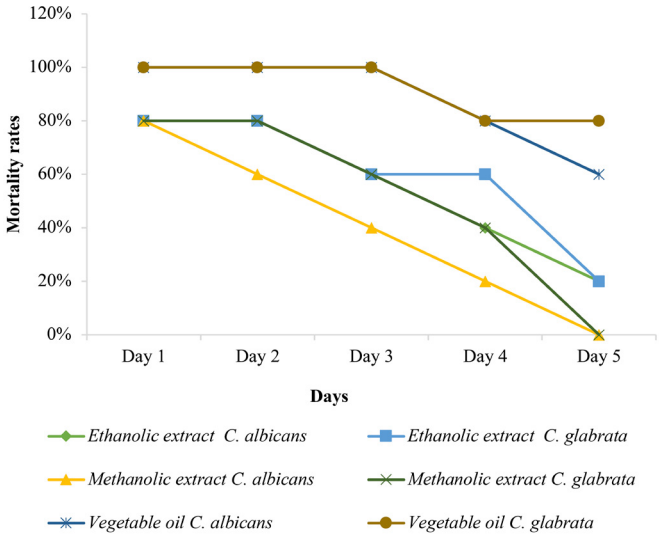

Figure 3. Mortality rate in mice infected with *Candida* species after five days of treatment.

draft were performed by BFZ. Supervision and validation were insured by AS. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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SUPPLEMENTARY MATERIAL

Table 1. Results of the *in vitro* antifungal activity (expressed in µl/mL for vegetable oil and mg/mL for extracts) (Mean ± SD).

	M. audouinii	***M. canis***	***T. rubrum***	***C. albicans***	***C. glabrata***
Vegetable oil	0.010 ± 0.010	0.005 ± 0.005	0.015 ± 0.010	0.010 ± 0.005	0.025 ± 0.000
Ethanolic extract	0.035 ± 0.005	0.020 ± 0.010	0.030 ± 0.005	0.035 ± 0.005	0.075 ± 0.005
Methanolic extract	0.020 ± 0.000	0.010 ± 0.000	0.030 ± 0.005	0.030 ± 0.010	0.070 ± 0.010
Terbinafine	0.010 ± 0.000	0.010 ± 0.005	0.020 ± 0.010	0.020 ± 0.005	0.035 ± 0.005