Evaluation of antimycotic activity of black cumin seed extract: In vitro study

Keerthiga Nagarajan¹, R. V. Geetha ²*,

ABSTRACT

Aim: This study aims to study the antimycotic activity of Nigella sativa extract in vitro. Background: N. sativa Linnaeus (Family Ranunculaceae), a herbal plant commonly referred to as black cumin seed and Karunjeeragam (in Tamil), is immune stimulating, hypotensive, anti-inflammatory, anticancerous, antioxidant, hypoglycemic, and spasmyloytic and also has bronchodilating properties. An antimycotic medication is a pharmaceutical fungicide or fungistatic used to treat and prevent mycoses such as athlete’s foot, ringworm, and candidiasis. Using herbal alternatives against chemical alternatives are thus explored in this research. Materials and Methods: Ethanolic extract of N. sativa was tested for antifungal (antimycotic) activity against Candida albicans. Agar well diffusion method was used to study the antifungal activity by measuring the zone of inhibition compared with control. Results: N. sativa extract displayed antimycotic property against C. albicans. Conclusion: Fungal infections are prevalent throughout South India. Antimycotic effect of herbs such as N. sativa can, thus, be purified and used as an additional ingredient in mouthwashes and toothpaste.

KEY WORDS: Antifungal, Antimycotic, Black cumin seed extract, Candidiasis, Nigella sativa

INTRODUCTION

Nigella sativa L. (Family: Ranunculaceae) is an indigenous herb of Southwest Asia including regions of Iran, India, and Pakistan. Growing to a maximum height of about 40–70 cm, this plant has finely divided foliage and pale blue and white flowers. From the fruit capsules, many small caraway-type black seeds are produced (length: 2.5–3.5 mm and width: 1.5–2 mm).[1] The plant is known by various names in different languages; black cumin, black seed, black caraway (English), Karunjeeragam (Tamil), Kalonji (Hindi), Habbah Al-Sawda; seed of blessing (Arabic), Chernushka (Russian), çörek otu (Turkish), and Cyah daneh in Persian. For a long span of time, the seeds of this plant have been used as a spice and additive in bread, cookies, and other dishes in many Asian and Eastern countries.

Therapeutic benefits of this miraculous spice and its active ingredients are being explored.[2] N. sativa has been extensively studied for its biological activities and therapeutic potential and shown to possess a wide spectrum of activities, namely as diuretic, antihistamine,[3] hypoglycemic,[4] antihypertensive, antidiabetic, anticancer and immunomodulatory,[5] analgesic, antimicrobial, anthelmintic, analgesics and anti-inflammatory, spasmyloytic, bronchodilator, gastroprotective, hepatoprotective, renal protective, and antioxidant properties. The seeds of N. sativa are widely used in the treatment of various diseases such as bronchitis, asthma, diarrhea, rheumatism, and skin disorders. It is also used as liver tonic, digestive, antidiarrheal, appetite stimulant, and emmenagogue, to increase milk production in nursing mothers, to fight parasitic infections, and to support immune system.

The composition of N. sativa seed includes fixed oil, proteins, alkaloid, saponin, and essential oil. The volatile oil (0.4–0.45%) contains saturated fatty acids which includes nigellone that is the only component of the carbonyl fraction of the oil, thymoquinone (TQ), thymohydroquinone, dithymoquinone, thymol, carvacrol, α and β-pinene, d-limonene, d-citronellol, and p-cymene, volatile oil of the seed also contains p-cymene, carvacrol, t-anethole, 4-terpineol, and longifolene. Black cumin seed has two different

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forms of alkaloids: Isoquinoline alkaloid that includes nigelicicmine, nigelicicmine n-oxide, and pyrazole alkaloid that includes nigellidine and nigelicicine. *N. sativa*’s nutritional components are vitamins, carbohydrates, mineral elements, fats, and proteins that include eight or nine essential amino acids. Black cumin seeds also have saponin and alpha-hederin and in trace amount have curcumen, limonene, and citronellol, as well as provide relatively good amounts of different vitamins and minerals such as Fe, Ca, K, Zn, P, and Cu. The fixed oil (32–40%) contains unsaturated fatty acids which include arachidonic, eicosadienoic, linoleic, linolenic, oleic, palmitoleic, palmitic, stearic, and myristic acid as well as beta-sitosterol, cycloartenol, sterol esters, and sterol glucosides.\(^\text{[6]}\) Most of the therapeutic properties of this plant are due to the presence of TQ which is a major active chemical component of the essential oil.\(^\text{[7]}\) The present study was conducted to assess the antimycotic activity of ethanolic extract of *N. sativa* against *Candida albicans*.

*C. albicans* is a commensal fungus commonly inhabiting human mucosal surfaces. *C. albicans* has adapted to the human host and has evolved because of the specific demands of the human host environment for a long time now. Peculiarly, colonizing *C. albicans* strains can become opportunistic pathogens causing persistent mucosal infections and life-threatening disseminated infections with high mortality rates. The oral cavity because of its unique environment is a primary target for opportunistic infections, particularly candidiasis.\(^\text{[8]}\)

The resistance of infectious disease-causing microorganisms to various antibiotics has led to enormous medical complications for the treatment due to the frequent use of commercial antimicrobial drugs to treat infections.\(^\text{[9]}\) Thus, leading to an upsetting increase of fungal infections\(^\text{[10]}\) making it necessary for the preparation of alternative drugs from medicinal plants.\(^\text{[11]}\) Hence, this study aims to study the antimycotic activity of black cumin seed (*N. sativa*) extract *in vitro*.

**MATERIALS AND METHODS**

*C. albicans* was isolated from clinical samples and cultured and maintained on Sabouraud’s dextrose agar medium at 30°C. Ethanolic extract of *N. sativa* was prepared and used for the study.

**Methodology**

The extracts were prepared in the following concentrations in sterile water, 5 mg/ml, 10 mg/ml, and 20 mg/ml. 100 µl of extract of different concentrations were loaded on sterile filter paper discs measuring 6 mm in diameter so that the concentration of the extract on each disc was 500 µg, 1000 µg, and 2000 µg, respectively. The discs were dried and kept aseptically.\(^\text{[12]}\)

**Screening of Antifungal Activity (Disc Diffusion Technique)**

The ethanolic extract of *N. sativa* was screened for antifungal activity by disc diffusion method. Activated cultures of *C. albicans* in Sabouraud’s broth were adjusted to 0.5 McFarland standards (108 cfu/ml). 100 µl of the inoculum was introduced to molten Sabouraud’s dextrose agar and poured in the sterile Petri plates and allowed to set. Sterile filter paper discs (6.0 mm diameter) impregnated with 2000 µg/disc, 1000 µg/disc, and 500 µg/disc of the plant extract dissolved in sterile water were placed on fungal seeded plates and incubated at 28°C for 48 h. As a positive control, fluconazole (10 mcg/disc) and amphotericin B (100 units/disc) were used. Following an incubation period of 48 h, plates were removed from the incubator and antifungal activity was evaluated by measuring zones of inhibition of fungal growth. Clear zones within which fungal growth was absent were measured and recorded as the diameter (mm) of complete growth inhibition. The whole experiment was performed 3 times to minimize test error.\(^\text{[13]}\)

**RESULTS**

The antimycotic activity of the extract at different concentrations was screened by agar well diffusion technique and the zone of inhibition was measured in mm diameter. The test solution inhibited the fungal strains with varying degree of sensitivity for different concentrations. The antifungal activity of the extract against *C. albicans* is shown in Table 1 and Figure 1.

**Table 1: Antimycotic activity of ethanolic extract of *Nigella sativa***

<table>
<thead>
<tr>
<th>Concentration of the extract</th>
<th>Zone of inhibition (in mm diameter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 µg/ml</td>
<td>16</td>
</tr>
<tr>
<td>1000 µg/ml</td>
<td>20</td>
</tr>
<tr>
<td>2000 µg/ml</td>
<td>25</td>
</tr>
<tr>
<td>Fluconazole (10 mcg/disc)</td>
<td>24</td>
</tr>
<tr>
<td>Amphotericin B (100 units/disc)</td>
<td>27</td>
</tr>
</tbody>
</table>

**Figure 1: Graph showing antimycotic activity of ethanolic extract of *Nigella sativa***
The extract showed good antymycotic activity at different concentrations with maximum zone of inhibition of 25 mm at concentration of 2000 µg/ml.

DISCUSSION

In Hanafy MS et al., 1991s, study, N. sativa extract showed antibacterial synergism with streptomycin and gentamicin and showed additive antibacterial action with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin, and sulfamethoxazole-trimethoprim combination. It also exterminated a non-fatal subcutaneous staphylococcal infection in mice when injected at the site of infection.[13] Randhawa et al., 2015, did a study where nanoparticulated drugs - amphotericin-B, ketoconazole, and TQ (an active ingredient of N. sativa) were investigated in vitro against C. albicans yeasts and Candida biofilm and were found to be effective in disinfecting both the Candida yeasts and Candida biofilm.[14] According to the study conducted by Shokri et al., 2012, the mean zone of inhibition value for N. sativa was found to be 40.8 mm, showing strong anti-Candida zeylanoides activities, which reinforces the potential use of N. sativa for the treatment of candidiasis.[15] Nadaf et al., 2015, performed a study to analyze the anti-yeast activity of N. sativa extract by agar well diffusion method against C. albicans NCIM 3466. Supplementary exposition of anti-yeast activity was done by scanning electron microscopy. The phytochemicals were identified using gas chromatography–mass spectrometry and the antioxidant properties checked using 2,2-diphenyl-1-picrylhydrazyl assay. The maximum anti-yeast activity was reported at pH 7 and 30°C temperature. The study confirmed the anti-yeast and antioxidant properties of compounds extracted from N. sativa seeds which might be useful for further expertise advancements in drug manufacturing.[16] Khan et al., 2016, did an in vitro study that checked the efficacy of N. sativa extract on the prevention of C. albicans growth on soft denture reliners. According to Khan et al.[17] N. sativa extract used in the study was significantly effective against C. albicans. According to Naeni’s study et al., 2008, Iranian medicinal herb oils including N. sativa and 13 other oils of the 16 oils tested confirmed a significant activity against C. albicans tested with minimal inhibitory concentration values ranging from 150 to 2300 µg/ml using broth macrodilution method and the growth inhibition zone ranging from 16 mm to 55 mm using disc diffusion method.[18]

In the present study, N. sativa was found to show good antifungal activity at different concentrations with a maximum zone of inhibition of 25 mm at a concentration of 2000 µg/ml. Despite all the significant advancements in modern medicine, traditional herbs have given rise to many important drugs. Medicinal plants are being studied for their potential mechanism of action and therapeutic properties.[21] Recent studies suggest good correlation between medicinal use and the in vitro antymycotic activity of medicinal herbs.[22]

The results of the present study evidently indicate the antifungal activity of N. sativa extract and may be used as a source for the isolation of active compounds that may serve as lead compounds in antifungal drug development. Further studies on their cytotoxicity or toxicity will be advantageous to know the possible harmful effects of this extract for common used by the local communities. Black cumin seed extract can thus undeniably be commercially modified to be used as an antifungal drug to drastically reduce the incidence of candidiasis. The antymycotic property can further be investigated to treat fungal infections of other origins as well and in the synthesis of more advanced, strain-specific antifungal drugs.

CONCLUSION

Reconnoitering avenues in pharmacological benefits of herbs and spices can pave the way for safer, easier, and cost-effective developments in synthesis of drugs for oral candidiasis and other fungal infections. Further, research may, however, be needed to explore the exact mechanism of action of N. sativa extract on C. albicans. The antymycotic effect of herbs such as N. sativa can, thus, be purified and used as an additional ingredient in mouthwashes and toothpastes to prevent fungal infections.

REFERENCES


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