



Assessment of Antifungal Effectiveness of Aqueous *Nigella sativa* Extract and Aqueous Neem Leaf Extract Against *Candida albicans*: An *in vitro* Study

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ABSTRACT

Background: Due to increasing interest to develop antimicrobial aids from alternative sources such as medicinal plants. *Nigella sativa* and *Neem* are known to have antimicrobial properties. *Aim:* The study aimed at detecting the anti-fungal activity aqueous *Nigella sativa* extract and aqueous *Neem* leaf extract as Against *Candida albicans*. *Materials and Methods:* Strains of *C. albicans* and selective media for growing micro-organisms were procured. Antimicrobial activity was assessed by determining the minimum inhibitory concentration (MIC) using the microbroth dilution method. *Results:* This study shows MIC against *C. albicans* of *N. sativa* was 8.6% and minimal inhibitory concentration of aqueous *neem* leaf extract is 4.8% against *C. albicans*. *Conclusion:* The result of the study established that both plant extracts possess anti-fungal activity against *C. albicans* present in the oral cavity.

Keywords: *C. albicans*, *N. sativa*, anti-fungal activity

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INTRODUCTION

Microorganisms are crucial in initiating and sustaining pulp and periapical pathosis. Fungi are a significant component of the root canal microbiota and contribute significantly to root canal therapy failure. In the oral cavity, fungi are a common opportunistic pathogen. One-third of healthy persons appear to have fungi as part of their normal flora. *Candida albicans* (*C. albicans*) is the most significant genus.[1] Numerous identification techniques (culturing, molecular genetics, and in situ electron microscopy) have demonstrated that fungi are more prevalent in secondary endodontic infections than primary endodontic infections.[2, 3] Even calcium hydroxide, the most often used intracanal medication, is ineffective against *Candida* species.

Sodium hypochlorite (NaOCl) is the most frequently used root canal irrigant because of its antimicrobial and tissue dissolving properties. 0.5 % NaOCl has been shown to destroy *Candida albicans* after a 10 - second contact period.[4] However, it has some drawbacks, including high toxicity, an unpleasant taste, corrosive to instruments, inability to remove the inorganic portion of the smear layer, and a decrease in dentin's elastic modulus and flexural strength.[5] As a result of the drawbacks associated with these extensively used traditional irrigation solutions and rising antimicrobial resistance to pharmaceutical treatments, the usage of natural alternatives has become the definitive. As a result, a better alternative is required, which has resulted in the hunt for a herbal substitute.

Black seed (*Nigella sativa*) has significant medicinal potential and mainly untapped for the endodontic purpose. *Nigella sativa* has various active components, such as thymoquinone (TQ), alkaloids, saponins (Alpha hederin) flavonoids, proteins, fatty acids, and many other substances, with substantial advantages in the treatment of patients with different conditions. Neem (*A. indica*) has proven anti-bacterial properties. Studies have shown that active components like alkaloids, terpenoids, tannins, nimbidin, nimbin, azadirachtin, etc are effective against microbial infections.[6] The present research was conducted with an intention to investigate anti-fungal properties of aqueous black seed extract and neem leaf extract. This study aimed to determine the anti-fungal activity of these two herbal extracts against planktonic forms of selected endodontic pathogen *C. albicans* (ATCC 10231);

MATERIAL AND METHODS

The present study was conducted after obtaining ethical clearance from the institution's ethical committee.

Aqueous herbal extract preparation:

The plants' sample (*Nigella sativa* seed powder and Neem leaf powder) was obtained from IMPCOPS Chennai, India *Nigella sativa* were mixed with 100 ml of double-distilled water and boiled for 15 min at 600C using a water bath. After cooling, the extracts were filtered using Whatman No. 1 filter paper and stored at 40C. Aqueous neem leaf extract were prepared similarly for this study.

Obtaining microbes and resurrecting microorganisms:

Strains of *C. albicans* for this investigation were received from a facility in Mumbai and grown fresh on Selective media. In the Laminar Air Flow chamber, the vial containing *C.albicans* was broken and introduced to the flask holding autoclaved enhanced nutritional broth. The flask was then incubated at 37°C for 48 hours.

MIC determination by standard microbroth dilution method:

The MIC values of black seed and neem leaf extraction *C.albicans* were determined by standard microbroth dilution method described by the Clinical and Laboratory Standards Institute (CLSI, 2015), with minor modifications. A known concentration of black seed and neem leaf extract (50 µg/ml) was added into 96-well microtitre plates containing 100 µl of MH broth. Dilutions were performed by the two-fold serial dilution method. Later, 100 µl of tested pathogens were inoculated to all wells and the microtitre plates were incubated at 37°C for 24 hours. After the incubation period, the optical densities of cultures are measured at 600 nm using a microplate reader. The medium containing microorganism with the medium containing microorganism (*C.albicans*) alone (Control) were considered controls. The MIC value was determined as the concentration, which the measured absorbance was equal or lower than that of the control.[7] All assays were done in triplicate

RESULTS

TABLE 1: Two-fold serial dilution of aqueous *Nigella sativa* extract against *C. albicans*

Concentration (mg/ml)	I	II	III	Average	Inhibition (%)	MIC Concentration
Control	1.102	1.125	1.139	1.122	0	
50	0.689	0.729	0.708	0.708667	36.83897802	86.37
25	0.821	0.839	0.855	0.838333	25.28223411	
12.5	0.954	0.921	0.944	0.939667	16.25074272	
6.25	1.054	1.069	1.095	1.072667	4.396910279	

TABLE 2: Two-fold serial dilution of aqueous neem leaf extract against *C. albicans*

Concentration (mg/ml)	I	II	III	Average	Inhibition (%)	MIC Concentration
Control (0)	1.102	1.125	1.139	1.122	0	
100	0.344	0.359	0.326	0.343	69.42959002	
50	0.554	0.578	0.599	0.577	48.57397504	48.73
25	0.754	0.768	0.727	0.749667	33.18478907	
12.5	0.9	0.889	0.87	0.886333	21.00415924	
6.25	1.004	0.989	1.012	1.001667	10.72489602	

TABLE 3: Shows Minimal inhibitory concentration (MIC) of test solution

S. No	Groups	Minimum inhibitory concentration
1	Aqueous <i>N. sativa</i> extract	8.6%
2	Aqueous Neem leaf extract	4.8%

Minimal inhibitory concentration represents the lowest concentration at which an irrigant inhibited growth by the absence of turbidity. This study shows MIC against *C albicans* of *N. sativa* was 8.6%, and minimal inhibitory concentration of aqueous neem leaf extract is 4.8% against *C albicans*. This indicates that both herbal irrigants have anti-fungal activity against *C albicans*.

DISCUSSION

This study aims to evaluate the anti-fungal activity of two aqueous herbal extracts, *N. sativa* and neem leaf extract, against *C.albicans*, which are commonly isolated from infected root canals. In the present study, aqueous *N.sativa* and neem leaf extract exhibited anti-fungal activity against *C. albicans*. Herbs extracts used in this study had several antibacterial, anti-inflammatory, antiviral, and anti-fungal effects. In dentistry, past research has assessed the irrigating capacity of herbs like curcumin, Triphala, propolis, aloe vera, and Neem.[8]

An *in vitro* evaluation of 5 different herbal extracts as endodontic irrigants against *E. faecalis* and *C. albicans* using quantitative polymerase chain reaction revealed that Neem was highly efficient to 5.25% NaOCl in reducing the counts of these microorganisms within the root canals when compared with other extracts.[9] In an experimental study by Khan et al. (2003), the aqueous extract of *N. sativa* seed exhibited an inhibitory effect against candidiasis in mice.[10]

Another study done by agar diffusion method to evaluate anti-fungal activity using aqueous *Nigella sativa* extract and neem leaf extract as root canal irrigant shows, aqueous Neem leaf extract showed good anti-fungal activity (mean zone of inhibition - 23mm) followed by control, aqueous *Nigella sativa* extract(19mm), and 3% NaOCl. At the same time, 2 % chlorhexidine shows less activity compared to other test groups. Which supports that *Nigella sativa* extract and neem leaf extract have anti-fungal properties.[8] And concluded that Herbal extracts (Aqueous *Nigella sativa* and Neem leaf extract) showed better antimicrobial activity.

In a study comparing cytotoxicity of herbal and conventional root canal irrigants, 25% aqueous *Nigella sativa* extract had the least cytotoxic effects, which also supports our study that herbal irrigants can be alternative to non-herbal endodontic irrigants.[11]

CONCLUSION

The results of this study presented that both herbal irrigants tested in this study can be an excellent alternative to irrigation solution in endodontic treatment when used against *C.albicans*. Laboratory tests are only the first steps in studying the antimicrobial effects of antiseptics. The fungal efficiency of irrigation solutions in microbroth dilution method studies may not demonstrate clinical conditions. This study should be followed by a clinical study for a final evaluation of the antimicrobial capability of aqueous *Nigella sativa* and aqueous neem leaf extract as a root canal irrigant.

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