

A Study of Antibacterial Effect of *Nigella Sativa* Seed Extract on Clinical Isolates of Methicillin-Resistant *Staphylococcus Aureus* (MRSA)

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Abstract

Objective: A study of antibacterial effect of *Nigella sativa* seed extract on clinical isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA).

Methods: Samples received at Microbiology laboratory attached to a tertiary care hospital in a period of three months which yield either heavy growth of *Staphylococcus aureus* or pure growth of *S.aureus* from patients with wound infection were included in the study. We did punch well diffusion method to determine the antibacterial effect of *Nigella sativa* seed extract against *S.aureus* isolates.

Results: For MRSA strains, the average zone size for concentrated solution of the seed extract was 25.45± 1.05 mm.; that for 1 in 5 dilution of the extract (166 mg/ml) was 17.75± 1.07 mm and that of 1in 10 dilution (90.9 mg/ml) was 12.30± 0.865 mm. The zone size for 1 in 20 dilutions (47.62 mg/ ml) was not consistent. Out of the 20 MSSA strains tested, 16 (80%) did not show any zone. Out of the 20 MRSA tested, 17 (85%) did not show any zone of inhibition, so the average zone size for 1 in 20 dilution of the extract, was 1.45±2.98 mm for MRSA and 1.10± 2.69 mm for MSSA. For MSSA strains the average zone size for concentrated solution of the seed extract was 25.55± 1.05 mm.; that for 1 in 5 dilution of the extract (166 mg/ml) was 17.55± 1.05 mm and that of 1in 10 dilution (90.9 mg/ml) was 12.30± 0.923 mm.

Conclusion: The present study shows the antibacterial effect of the methanolic extract of *N.sativa* against our clinical isolates of *S.aureus*.

Keywords: Biopharmaceuticals, Methanol, MRSA, *Nigella sativa*, Seeds, *Staphylococcal* infection,

Introduction

Microbial resistance to majority of antibiotics has led to increase in alternative use of antimicrobial agents that can help in the treatment of diseases.¹ Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most frequent pathogenic bacteria isolated in clinical and laboratory practices. New antimicrobials are required to combat this problem.¹

Plant extracts have been known to have certain antimicrobial effects. One such example is the seeds of *Nigella sativa* belonging to family Ranunculaceae, commonly known as black seed or black cumin. The biological activity of the seeds is said to be due to thymoquinone which has anti-inflammatory, analgesic, antipyretic, antimicrobial and antineoplastic activity.²⁻⁵ The antimicrobial activity of *N. sativa* seed extract has been extensively studied for its action against a wide range of bacterial, fungal and parasitic organisms. However, its efficacy against MRSA has limited data as far as Indian studies are concerned.²⁻⁵ So, we studied the antibacterial effect of methanolic extract of *N. sativa* seed on MRSA and Methicillin-sensitive *Staphylococcus aureus* MSSA isolated from the cases of

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wound infection.

Materials and Methods

It was a prospective study for the duration of 3 months from 1st January 2017 – 31st March 2017. Samples received at Microbiology laboratory attached to a tertiary care hospital in this period which yielded either heavy growth of *Staphylococcus aureus* or pure growth of *S.aureus* from patients with wound infection were included in the study. Samples received on dates other than the three months period or which do not yield *S.aureus* or yield scanty or mixed growth of *S.aureus* with other organisms were excluded from the study. Non random sampling was done. Institutional ethics committee clearance was obtained for the present study.

Hundred grams of dried and powdered seeds (Fig. 1) was subjected to Soxhlet apparatus using methanol as a solvent for 4h. The extract obtained was filtered using whatman filter paper no.1. The filtrate was concentrated using mantle and stored at 4 °C for further use.⁶

Antimicrobial susceptibility testing: The extract was diluted by double dilution (1:5, 1:10 and 1:20) in 10% dimethyl sulfoxide (DMSO). An overnight broth culture of various clinical isolates of MRSA and MSSA in Muller Hinton broth was standardized to 0.5 McFarland and a lawn culture of each isolate was made on

Muller Hinton agar plates. The wells of 6 mm diameter were made and 50 µl of extract of different dilutions and also pure DMSO were inoculated into the wells and incubated overnight at 37° C. The zones of inhibition around the wells were measured.⁷

Results

Table 1 shows the results of antibacterial activity of the *N. sativa* seed extract on 20 strains each of MRSA and MSSA expressed as zone size in mm. For MRSA strains, the average zone size for concentrated solution of the seed extract was 25.45± 1.05 mm.; that for 1 in 5 dilution of the extract (166 mg/ml) was 17.75± 1.07 mm and that of 1 in 10 dilution (90.9 mg/ml) was 12.30± 0.865 mm. The zone size for 1 in 20 dilutions (47.62 mg/ml) was not consistent. Out of the 20 MSSA strains tested, 16 (80%) did not show any zone. Out of the 20 MRSA tested, 17 (85%) did not show any zone of inhibition, so the average zone size for 1 in 20 dilution of the extract, was 1.45±2.98 mm for MRSA and 1.10± 2.69 mm for MSSA. For MSSA strains the average zone size for concentrated solution of the seed extract was 25.55± 1.05 mm.; that for 1 in 5 dilution of the extract (166 mg/ml) was 17.55± 1.05 mm and that of 1 in 10 dilution (90.9 mg/ml) was 12.30± 0.923 mm. (Fig. 2)

Table 1: The results of antibacterial activity of the *Nigella sativa* seed extract on 20 strains each of MRSA and MSSA expressed as zone size in mm.

S.aureus Isolates*	Dilutions of the extract				10% DMSO
	Neat (m.m.)	1 in 5 (m.m.)	1 in 10 (m.m.)	1 in 20 (m.m.)	
MRSA n = 20	25.45± 1.05	17.75± 1.07	12.30± 0.865	1.45± 2.98	0
MSSA n =20	25.55± 1.05	17.55± 1.05	12.30± 0.923	1.10± 2.69	0



Figure 1: Seeds of *N.sativa*

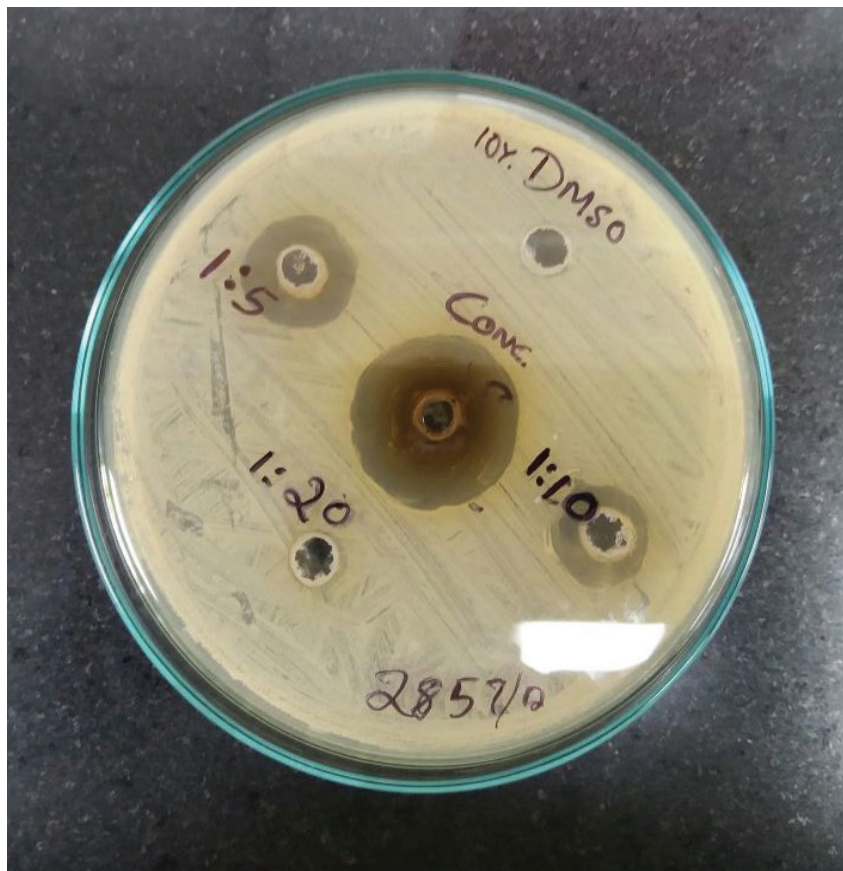


Figure 2: showing the zone of inhibition by *N.sativa* seed extract on *S.aureus*

Discussion

In a study methanolic extract of the seed was investigated for the presence of novel bioactive compounds using Gas Chromatography and Mass Spectrometry analysis method. The results showed that methanolic seed extract contains 13 novel bioactive compounds and has remarkable antifungal activity.⁶ The biological activity of the *Nigella sativa* seed extract is said to be due to thymoquinone which has anti-inflammatory, analgesic, antipyretic, antimicrobial and antineoplastic activity.²⁻⁵

In a study using ground seeds suspended in sterile distilled water was used, clear inhibition of the growth of *Staphylococcus aureus* was observed at a concentration of 300 mg/ml. No inhibition was found in the growth of *E.coli* and *Enterobacter* spp.³ In a study methanol extract at the concentration of 100 mg/mL had a remarkable sensitivity towards all tested bacteria in this study. But aqueous extract of the seed was not effective against gram negative bacteria.⁴ In a study on effect of *Nigella sativa* seed oil extract on MRSA isolated from diabetic wound infection, out of 19 isolates, 8 (42%) were sensitive to undiluted oil sample; 4(21%) of these showed sensitivity at 200 mg/ml, 400 mg/ml and 800 mg/ml respectively. Eleven (58%) of the isolates were completely resistant to all the oil concentrations.⁵

In a study the isopropanol extracts showed the best antimicrobial potential. It significantly inhibited the growth of almost all the pathogenic bacteria tested. The methanol and ethanol extracts showed the highest activity on 5th and 9th day of germination. Some of the results showed that the extracts were better antibacterial agents when compared with commercially available broad-spectrum antibiotics.⁷

In a study the methanolic extract and oil of *Nigella sativa* were found active against 38 and 35 multi-drug resistant strains respectively. Both the oil and methanolic extract showed remarkable dose dependant antibacterial activity against the tested strains up to a dilution of 1:50 as evident from the zones of inhibition.⁸

In a study distilled methanolic extracts of *N. sativa* showed significant antimicrobial activity against tested clinical strains of Gram-positive and Gram-negative bacteria. Results showed day-dependent and dose-

dependent activity and a significant antimicrobial effect was observed as germination proceeded.⁹

N. sativa ground seeds in a dose of 2 g/d when given along with 40 mg/d omeprazole possess clinically useful anti-*Helicobacter pylori* activity, comparable to that of the standard triple therapy.¹⁰

A statistically significant inhibitory effect on biofilm formation by *S.aureus* ATCC 25923 and *S. epidermidis* CIP 106510 was noted after TQ supplementation.¹¹ The study showed thymoquinone (TQ) had a good antibacterial effect with MICs values in the range of 8 to 32 µg/ml especially against Gram-positive cocci (*S.aureus* ATCC 25923 and *S. epidermidis* CIP 106510).¹¹ A study showed antibacterial activity against *S.constellatus* with a MIC of 4 µg/ml. The essential oil showed the strongest activity against *S. mitis*, *Streptococcus mutans*, *S. constellatus* and *Gemella haemolysans* with an MIC of 2.13 mg/ml, but against *Enterococcus faecalis* and *E. faecium* less effective with an MIC of more than 8.5 mg/ml.¹²

It also showed TQ (150 µg/disc) was very effective against *S. mutans* and *S. mitis* (zone of inhibition were: 24.5 ± 0.71 and 22 ± 1.41 mm, respectively) also weak antibacterial activity against *E. faecalis*, *E. faecium* and *S. salivarius* (9 ± 0.00 , 9.5 ± 0.71 and 9.5 ± 0.71 mm, respectively).¹² The essential oil (2.43 mg/disc) also had good activity against *S. mitis*, *S. oralis*, *S. mutans*, *S. constellatus* and *G. haemolysans* with a zone of inhibition ranging from 13.5 to 15.5 mm, but not effective against *E. faecalis*, *E. faecium* and *S. salivarius*.¹²

In a study the seed extract has been loaded into the polymeric micelle and its effectiveness has been evaluated against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* by in vitro disc diffusion method *Nigella sativa* loaded polymeric micelles was found to be more efficient in comparison to plain extract.¹³ A study showed that *N.sativa* oil had good antibacterial action against *S. aureus* in comparison to *P. aeruginosa*.¹⁴ A clinical trial showed that the crude extract was as nearly effective as the standard drug, mupirocin on neonates with pustules, no side effect was observed.¹⁵

Our study results are consistent with the studies conducted in the past. We too found significant antibacterial activity of *N. sativa* extract against *S.aureus*

both MRSA strains and MSSA strains isolated from cases of wound infection. As *N. sativa* is a nontoxic edible substance easily larger doses of this extract may be considered for the treatment of wound infection.

Conclusions

We found significant antibacterial activity of methanolic extract of *N. sativa* by punch well agar diffusion method against 20 strains each of MRSA and MSSA isolated from cases of wound infection

Ethical Clearance- has been taken from the Institutional Ethical Committee

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Conflict of Interest – The authors have no conflict of interest

Conference presentation: The article has been presented as POSTER in the annual parasitology conference, TROPACON 2019 in KMC, Manipal.

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