



# INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)

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## IN VITRO BACTERICIDAL ACTIVITY OF SEEDS EXTRACT OF *NIGELLA SATIVA* AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM TERTIARY CARE HOSPITAL DELHI NCR REGION INDIA.

Mohd Aslam<sup>1\*</sup>,  
Moinuddin Khan<sup>1</sup>, Sayeed Ahmad<sup>2</sup>

<sup>2</sup> Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Jamia Hamdard Delhi.

<sup>1</sup>School of Life Sciences Simghania University Rajasthan.

### ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) continues to be one of the commonest pathogens encountered in clinical as well as laboratory practice. It has become a major health problem worldwide. Newer antimicrobials/agents are urgently needed to combat this problem. MRSA resist to various anti-staphylococcal agents. In the back-drop of this difficult situation *Nigella Sativa* commonly known as black seed extract was aimed to evaluate if it had any anti-staphylococcal activity. The extract was prepared by Maceration/solvent extraction method by absolute ethanol. Disc diffusion methods was performed to assess the antibacterial activity. *S. aureus* ATCC 25923 strain was used as the standard reference. 90.3 % of MRSA strains were sensitive to *N. sativa* extract at a concentration of 5 mg/disc. The results indicated that *N. sativa* has inhibitory effect on MRSA. This finding warrants necessity of further investigation of this product of folk medicine.

### Correspondence to Author



**Mohd Aslam**

School of Life Sciences Simghania  
University Rajasthan.

### Email

aslam\_sadaf@yahoo.co.in

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*S.aureus*, MRSA, resistance, *N. sativa*, extract.

## INTRODUCTION

*Staphylococcus aureus* is a gram positive bacterium responsible for severe morbidity and mortality worldwide. It is one of the leading causes of human infections in the skin, soft tissues, bones joints and abscesses. The organism flourishes in the hospital setting producing bloodstream and surgical wound infections [1,2]. Methicillin was introduced 1959 to treat staphylococcal infections not responding to penicillin therapy. However only within a year some strains of *S. aureus* were reported to be resistant to it. These strains were named as 'Methicillin Resistant *Staphylococcus aureus*' (MRSA). During the past four decades MRSA has spread throughout the world and has become highly endemic in many geographic areas [3]. MRSA infections are difficult to treat because of their resistance to the commonly used anti staphylococcal antibiotics viz macrolides, tetracyclines, aminoglycosides etc. Some of these MRSA strains are resistant to even the most powerful antibiotics such as vancomycin [4]. WHO has been suggesting there is need to find some new antibiotics or new approaches to overcome this problem.

*Nigella Sativa* is an herbaceous plant found in the Middle East, Europe and Western and Middle Asia. Its seeds have a great medicinal importance and have been reported to exhibit many pharmacological effects that include anti-parasitic, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory activities [5]. The seeds have also been used to treat bacterial, fungal and parasitic infections. Its oil is used as condiment, carminative, food preservative, analgesic and to treat many ailments in different parts of world [6]. In the light of the above pharmacological properties exhibited by *N. sativa* seeds, there is a need to investigate its efficacy against MRSA.

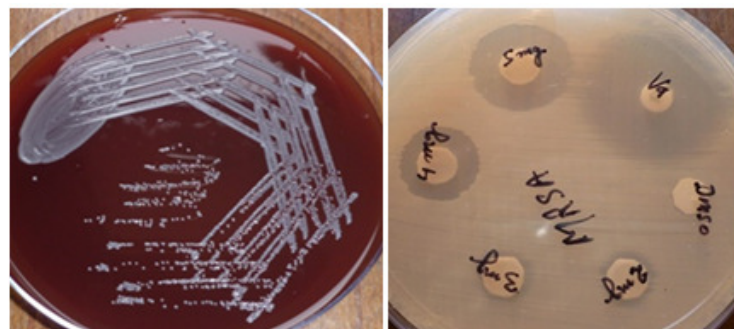
## MATERIALS AND METHODS

A total of 72 clinical isolates of MRSA were obtained from Artemis Hospital of Delhi NCR. Identification was done on the basis of morphology, cultural characteristics, biochemical reactions and resistant to Oxacillin discs (1 µg) using Mueller-Hinton Agar. *Nigella Sativa* seeds were procured from Rex (U&A) Remedies

Pvt.Ltd. Delhi. They were freed of dust and crushed in a domestic grinder and then soaked in absolute ethanol for 5 days at room temperature at the working bench. The amount of absolute ethanol was just enough to adequately cover the crushed seeds. After 5 days it was filtered by Sintered Bronze Filter. The filtrate was put into rotary evaporator to evaporate ethanol and lyophilized to remove excess of solvent from extract. Prepared extract was stored in refrigerator till use.

Whatman Filter paper No. 1 was used to prepare discs (6 mm). The discs were then sterilized by autoclaving. The extract was diluted with 10 % DMSO and its different concentrations were prepared. Discs with final concentration 2.0, 3.0, 4.0 and 5.0 mg per discs were prepared. Prepared discs were stored at 4 °C in the refrigerator till use. To avoid any condensation the discs were kept at room temperature for one hour to before use.

MH agar was prepared,autoclave and poured 20 ml into 100 mm petriplate. All the strains of MRSA were tested against extract impregnated discs by disk diffusion method. Broth cultures equal to 0.5McFarland standards were made from each strain. A sterile cotton swab was dipped into the broth and a lawn was made on the MH agar plate. The discs were placed and the plates were incubated at 35 °C overnight. Vancomycin 30 µg and 10 % DMSO discs were used as positive and negative controls respectively as shown in fig.1. The diameter of the clear zones around the discs was measured in mm with the help of digital calipers.



**Fig.1:** Growth of MRSA and bactericidal activity of *N.sativa* seeds extract.

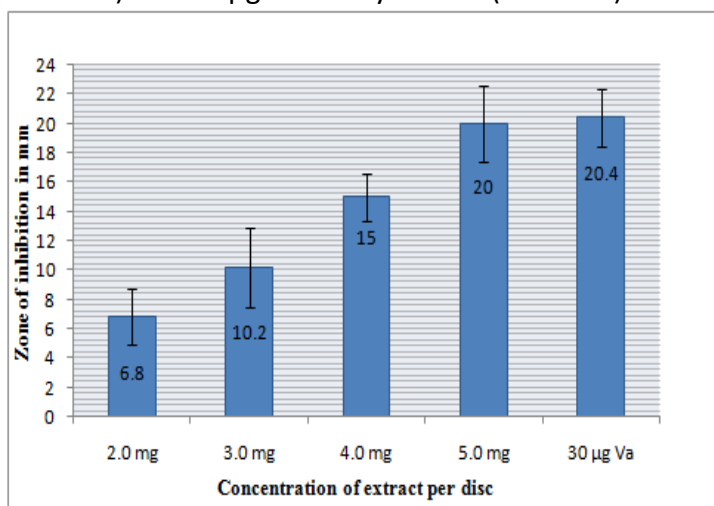
**Results:**

The average zone of inhibition of the extract at its different concentrations is shown in Table-1. Disc containing 10 % DMSO (diluent of the extract)

produced no zone of inhibition whereas vancomycin disc used as positive control produced significant zone of inhibition ( $\geq 15$  mm according to CLSI standards).

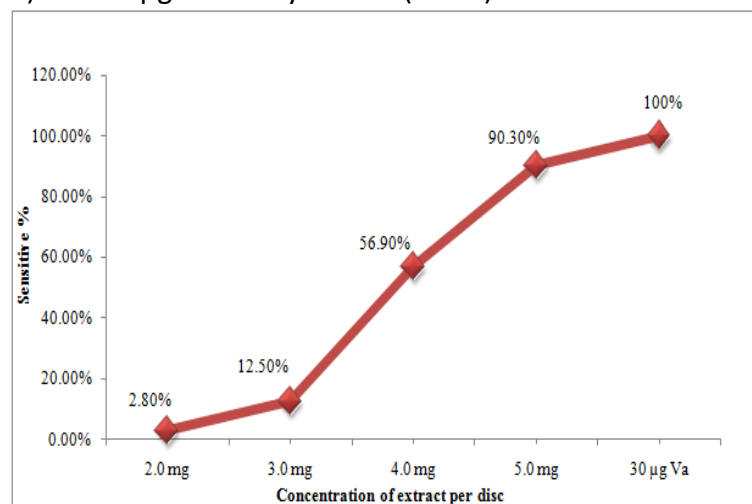
MRSA N-72	<i>N.Sativa</i> extract (zones in mm)				Vancomycin disc
	2.0 mg	3.0 mg	4.0 mg	5.0 mg	30 $\mu$ g
ATCC-25923	6.0	8.0	15.0	19.0	22.0
Mean of zone size	6.8	10.2	15.0	20.0	20.4
Mean $\pm$ SD	6.8 $\pm$ 1.9	10.2 $\pm$ 2.7	15.0 $\pm$ 1.6	20.0 $\pm$ 2.6	20.4 $\pm$ 2.0
Number of sensitive	02	09	41	65	72
Percentage Sensitive	2.8 %	12.5 %	56.9 %	90.3 %	100 %

In vitro Bactericidal activity of seeds extract of *N.sativa* against methicillin resistant *S. aureus* isolated (N-72) were tested by disc diffusion method. Total four different concentrations were used to test all 72 strain of MRSA. The average zone diameter of extract against MRSA as shown in fig.2; 2.0 mg per disc (6.8), 3.0 mg per disc (10.2), 4.0 mg per disc (15), 5.0 mg per disc (20) and 30  $\mu$ g Vancomycin disc(20.4) ( $\geq 15$  mm according to CLSI standards).The overall standard deviation in zone size against impregnated discs were minimal and acceptable as follow; 2.0 mg per disc (6.8 $\pm$ 1.9), 3.0 mg per disc (10.2 $\pm$ 2.7), 4.0 mg per disc (15.0 $\pm$ 1.6), 5.0 mg per disc (20.0 $\pm$ 2.6) and 30  $\mu$ g Vancomycin disc (20.4 $\pm$ 2.0).



**Fig.2:** Bar chart showing correlation between zone size and dose (mg/disc)

The number of sensitive strains by disc diffusion method against discs impregnated by *N.sativa* extract as shown in table 1; 2.0 mg per disc (02), 3.0 mg per disc (09), 4.0 mg per disc (41), and 5.0 mg per disc (65) and 30  $\mu$ g Vancomycin disc (72). The percentage of sensitivity as shown in fig.3; 2.0 mg per disc (2.8 %), 3.0 mg per disc (12.5 %), 4.0 mg per disc (56.9 %), 5.0 mg per disc (90.3 %) and 30  $\mu$ g Vancomycin disc (100%).



**DISCUSSION**

*N. sativa* seed extract has been extensively studied for its antimicrobial activity against a wide range of bacterial, fungal and parasitic organisms. However only a limited data is available so far regarding its efficacy against MRSA. The present study was therefore designed to evaluate this aspect of *N. sativa*. The results of disc diffusion assay demonstrated that MRSA strains were almost completely inhibited at 5 mg/disc (zone size  $\geq 15$  mm was considered to be significant). However a concentration of 2.0 mg per disc almost failed to inhibit these strains.

Zone of inhibition was more than 20 mm at 15 mg per disc and 8 mg per disc for chloroform and methanolic extract respectively whereas our study proves that a concentration of 5 mg/disc is enough to give a significant zone [9]. It is very important to develop guidelines for all procedures adopted in evaluating bactericidal activity of *N. sativa* seed and analyze extracts of *N. sativa* seed of different regions for the actual ingredient which is responsible for their bactericidal activity. There is also an urgent need that a standard method may be devised for extract preparation.

**CONCLUSION**

It is concluded from this study that *N. sativa* seed extract has antimicrobial activity against MRSA. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

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