



## ANTIBACTERIAL ACTIVITY OF *Nigella sativa* SEED EXTRACTS

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### ABSTRACT

*The development and alarming increase of bacterial resistance to existing antimicrobial agents has become a real challenge and a serious problem facing patients suffering from various infections worldwide. This study was designed to evaluate the antibacterial activity of black seed extracts against bacterial isolates (Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa). The powdered seeds were extracted using percolation technique with methanol and petroleum ether. Agar well diffusion method was used to test the antibacterial activity. Methanolic extracts at 100mg/ml had a remarkable activity against S. aureus (19mm) and P. aeruginosa (12mm). Also the petroleum ether extract had the same activity against S. aureus and P. aeruginosa (10mm). This implies that methanolic and petroleum ether extracts of N. sativa were found to be active against S. aureus and P. aeruginosa. While E. coli showed resistance to the extracts at all concentrations. Our study shows that species, strains and concentrations of N. sativa extracts are some of the factors that may influence the sensitivity of tested organisms.*

**Keywords:** Antibacterial activity, *Nigella sativa*, Black seed extract, Agar well diffusion, Bacterial isolates.

### INTRODUCTION

The alarming increase in bacterial resistance to existing antibiotics as well as public interest in herbal medicine demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria (Sultana *et al.*, 2015). *Nigella sativa* linn. (family - Ranunculaceae) commonly known as black seeds or black cumin (English) or “*Habbatu saudda*” (Hausa) are native to the Mediterranean region but has been cultivated in other parts of the world including Saudi Arabia, northern Africa and parts of Asia (Sultana *et al.*, 2015). Black seeds have been used for thousands of years as spice and food preservative, as well as a protective and curative remedy for several disorders (Sultana *et al.*, 2015). Traditionally, there is a common Islamic belief that black seed is a universal remedy for all ailments, but cannot prevent aging or death (Sultana *et al.*, 2015). More so, black seeds have a great medicinal importance and have been reported to exhibit many pharmacological importance that include antioxidant, anti-inflammatory, antiparasitic, antifungal and antibacterial activities (Hasan *et al.*, 2013). Crude extracts and essential oil of black seeds possess antibacterial activity against several bacterial (Zuridah *et al.*, 2008).

However, researches on the effects of extracts of *N. sativa* against multi drug resistant and pathogenic bacteria are very few in Kano, Nigeria. Hence the present study aimed at evaluating the antibacterial activity of *N. sativa* extracts against some bacterial isolates.

### Material and Methods

#### Collection of Plant Materials

*Nigella sativa* seeds were procured from Islamic medicine store at Kurmi market Kano, Nigeria and authenticated by a botanist in the Department of Plant Biology, Bayero University Kano. The seeds were pulverized into a fine powder using a domestic blender.

**Extraction:** Methanolic and petroleum ether extracts were prepared using HPLC grade methanol and petroleum ether by percolation as previously described by Salman *et al.*, (2009). Briefly 350grams of *N. sativa* powder were soaked in 500ml of methanol and petroleum ether respectively for 7 days at ambient temperature, followed by filtration using Whatman No.1 filter paper and evaporated using rotary evaporation apparatus. The extracts were further dried in an hot air oven 50°C for 24h and finally kept at 4°C until further testing.

**Concentrations of Extracts:** One gram (1g) of each extract was dissolved separately in 1ml of 10% dimethyl sulfoxide (DMSO) to give a stock solution of 1000mg/ml. Four different concentrations, 100, 50, 25 and 12.5mg/ml were prepared from the stock solution by serial dilutions from 10<sup>-1</sup> to 10<sup>-4</sup>.

**Bacterial Strains:** The bacterial strains used (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) were collected from Department of Microbiology, Bayero University, Kano.

Inocula were prepared in 5ml nutrient broth with 3 to 5 colonies of each bacterial strain and were incubated at 35°C for 2-3hrs and adjusted to match 0.5 McFarland Standard for susceptibility testing (NCCLS, 1993).

**Antibacterial Activity**

The agar well diffusion method was used to test the antibacterial activity of *N. Sativa* extracts against the bacterial isolates (Al-Mahmood, 2009). Sterilized nutrient agar was poured into sterile petri dishes and allowed to solidify. A sterile swab stick was dipped into standardized inocula and spread on the solidified agar aseptically and labeled. The inoculated plates were allowed to stay for 30minutes to enable the organisms adhere properly to the surface of the agar. Six wells were bored aseptically using sterile cork borer of 6mm diameter. The wells were then filled with 0.1ml of *N. sativa* extracts (100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml). A 0.1 of 30mg/ml ciprofloxacin solution and 0.1ml DMSO were used as positive and negative controls respectively. The plates were allowed to dry and subsequently incubated at 37°C for 24hrs, after which zones of inhibition were observed, measured and recorded in millimeter.

**Minimum Inhibitory Concentration (MIC)**

The broth dilution assay was adopted as described by Al - Mahmood (2009). Extracts were diluted to 10 folds in nutrient broth. To each dilution of extracts 0.1ml of standardized bacterial inoculum was added. Negative control tubes with no bacterial inoculation were simultaneously maintained. Tubes were incubated aerobically at 37°C for 24hrs. The lowest concentration of extract that produced no visible growth (turbidity) was recorded as MIC.

**RESULTS AND DISCUSSION**

This study reports the antibacterial activity of four (4) concentrations of *Nigella sativa* against *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa*. The results of the antibacterial activity of the investigated extracts are shown

in (Table 1 and 2). At a concentration of 100mg/ml, the highest antibacterial activity of 19mm was recorded against *S. aureus*, for methanol extract and 10mm for petroleum ether extract. This findings is in agreement with the result reported by Zuridah *et al.*, (2008) using the same genus of plant tested. Also *N. sativa* had a remarkable activity towards *Psuedomonas aeruginosa* with inhibition zones of 12mm and 10mm at 100mg/ml both extracts respectively (Table 1). Both methanol and petroleum ether extracts of *N. sativa* showed no inhibition against *E. coli* at all concentrations. Generally, the methanol extract exhibited higher antibacterial effect compared with petroleum ether extracts. The extraction of the biologically active compounds from the plant material depends on the type of solvents used for extraction (Hasan, *et al.*, 2013).

The minimum inhibitory concentrations of *N. sativa* extracts were moderate (12.5mg/ml) for *S. aureus* (PET extract) whereas it was maximum for *P. aeruginosa* (25mg/ml) and least for *S. aureus* (6.25mg/ml) with the methanol extract. From this data, it could be said that the methanol extracts of *N. sativa* was more effective in inhibiting *S. aureus* at a very low concentration of 6.25mg/ml (Table 2).

Both extracts were found to be more effective on Gram positive than Gram negative bacteria (Tables 1 and 2). Gram negative bacteria have effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphiphatic compounds and multi drug resistance pumps that extrude toxins across this barrier (Nagi *et al.*, 2008). It is possible that the apparent ineffectiveness of the plant antibacterial activity against *E. coli* is largely due to this permeability barrier. This is in conformity with a number of earlier studies where compounds derived from plants often show considerable activity against gram positive bacteria but not against gram negative species (Nagi *et al.*, 2008).

**Table 1: Antibacterial Activities of *N. sativa* extracts against Bacterial Isolates**

Test bacteria	Diameter zone of inhibition (mm)/extract concentration (mg/ml)							
	MET				PET			
	12.5	25	50	100	12.5	25	50	100
<i>S. aureus</i>	10	11	13	19	08	08	09	10
<i>E. coli</i>	06	06	06	06	06	06	06	06
<i>P. aeruginosa</i>	06	07	10	12	06	07	08	10

Key: MET = Methanol, PET = Petroleum ether

**Table 2: MIC of *N. sativa* extract on Bacterial Isolates**

Test bacteria	MIC/mg/ml)	
	MET	PET
<i>S. aureus</i>	6.25	12.50
<i>E. coli</i>	*	*
<i>P. aeruginosa</i>	25.00	0.00

Key: MET = Methanol, PET = Petroleum ether and MIC = Minimum Inhibitory Concentration

\* = MIC greater than the highest concentration used

## CONCLUSION

It may be concluded from this study that *Nigella sativa* seed extract exhibits some degree of antibacterial activity towards *S. aureus* and *Pseudomonas aeruginosa*. Thus, it shows that *N. Sativa* has a great potential as an effective antibacterial agent for medicinal purposes.

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## Recommendations

In future studies, isolated phytoconstituents from *N. sativa* seeds needs to be evaluated scientifically using specific experimental animal models so as to understand the molecular mechanisms of action.