

Antimicrobial activity of *Nigella sativa* L. seed oil against multi-drug resistant *Staphylococcus aureus* isolated from diabetic wounds

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Abstract: Microbial resistance to existing antibiotics has led to an increase in the use of medicinal plants that show beneficial effects for various infectious diseases. The study evaluates the susceptibility of multidrug resistant *Staphylococcus aureus* to *Nigella sativa* oil. *Staphylococcus aureus* was isolated from 34 diabetic patient's wounds attending the Renaissance hospital, Nsukka, Southeast Nigeria. The isolates were characterized and identified using standard microbiological techniques. Isolates were cultured and a comparative *In vitro* antibiotic susceptibility test was carried out using the disk diffusion method. Of the 34 samples collected, 19(56%) showed multidrug resistance to the commonly used antibiotics. *Nigella sativa* oil was then studied for antibacterial activity against these multidrug resistant isolates of *Staphylococcus aureus* in varying concentration by well diffusion method. The oil showed pronounced dose dependent antibacterial activity against the isolates. 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. The present study, reports the isolation of multi-drug resistant *S. aureus* from diabetic wounds and that more than half of isolates were susceptible to different concentrations *N. sativa* oil.

Keywords: Susceptibility, concentrations, antibacterial activity, patients, bacterial infections.

INTRODUCTION

Staphylococcus aureus and coagulase negative staphylococci represent the major constituent of the human cutaneous microflora and responsible for variety of infections ranging from superficial to deep wound and septicemia, (Komolade and Adegoke, 2008). Reports have shown that bacterial isolates have developed resistance to antimicrobial chemotherapy and their prevalence in both hospital and community acquired infections is of potential threat (Daum and Seal, 2001; Kaplan *et al.*, 2005). Adegoke and Komolafe (2009) reported the isolation of multidrug resistant *S. aureus* from patients in southwest Nigeria. The infection rate for *S. aureus* is high. Therefore, it is obvious that the beneficial retrospective studies on multi-drug resistance must put the available conventional antibiotics in the area, into consideration. Antimicrobial resistance in *Staphylococcus aureus* is a major public health threat, compounded by the emergence of strains resistant to Vancomycin and Daptomycin, both of which are last line antimicrobials Menichetti 2005; Van *et al.*, 20011; Sass *et al.*, 2012).

Resistance by bacteria to existing antibiotics has led to an increase in the search for medicinal plants that are important aid to various ailments. Nature has naturally been a source of medicinal plants for thousands of years and the use of medicinal plants in traditional medicine,

has been well established (Kafaru 1994). Herbal drugs are less toxic and the active ingredients have the advantage of being combined with other substances (Manna and Abalaka 2000; Sheriff 2001). In folklore traditions, various medicinal plants are used for the treatment of infections that are resistant to modern medicines. One of such medicinal plants is *Nigella sativa*. A herbaceous plant, *N. sativa* Linn. (Black cumin) has been used for centuries for the treatment of various ailments, including infectious diseases. The seeds are reported to possess several medicinal properties, which are commonly used in Asian and Mediterranean recipes (Ali and Blunden 2003; Randhawa and Al-Ghami 2002). They have also been used in folk traditional medicine as remedy for asthma, hypertension, diabetes and cough. The crude extracts and essential oils have been reported to possess antibacterial activity (Ali and Blunden 2003; Mouhajir and Pedersen 1999). Many active principles have been isolated from *Nigella sativa* seed/oil (El-Fataty 1975) including thymoquinone (TQ). TQ (2-isopropyl-5-methyl-1,4-benzoquinone) is the main bioactive constituent of *Nigella Sativa* Oil (El-Dakhakhany 1963) showing antibacterial (Lui *et al.*, 1996; Halawani 2009) and antifungal activity^[14]. These previous studies have used seed as a whole or organic extracts to evaluate its antimicrobial potentials (El-Dakhakhany 1963, Lui *et al.*, 1996; Halawani 2009; Al-Jabre *et al.*, 2003; Emeka *et al.*, 2014). The antibacterial and antifungal activity of total extracts and essential oils of *N. sativa* seed in mice has been studied (Hosseinzadeh *et al.*, 2007; Entela *et al.*,

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2012). As well as the effect of the oil extract in the treatment of experimentally induced Rhinosinusitis (Yoruk *et al.*, 2010). The activity of the *N sativa* seed oil against multidrug resistant bacteria from clinical isolates and multidrug resistant coagulase negative *Staphylococci* was also investigated (Salman *et al.*, 2008a; Salman *et al.*, 2008b). However, so far none of such investigations was to elucidate the activity against multidrug resistant *S. aureus* isolated from diabetic wounds. Thus, the present study was undertaken to evaluate antibacterial activity of *Nigella sativa* oil against the multi-drug resistance strains isolated from diabetic wound infections.

MATERIALS AND METHODS

An experimental *in vitro* study design was adopted to evaluate the effect of *Nigella sativa* oil and its dilution against resistant strains of *S. aureus* isolates.

Sample collection

Samples were collected from diabetic patients presenting with wounds at Renaissance hospital in Nsukka area, Enugu State of Nigeria. Approximately 500 patients attend the hospital monthly and a number of them with varying cases of wounds. Swabs were collected from patients who have had these wound for up to three months.

All wounds were judged as infected by the presence of purulent material. The exudates from each wound site were carefully swabbed, plated out on Mannitol salt agar (MSA), (Oxoid England) and incubated at 37°C for 24 hours. Characterization of the bacterial isolates was based on standard microbiological methods [24]. Each distinctive morph type of mannitol-fermenting colony was selected from the MSA plate and sub-cultured on blood agar (Zayo Sigma, Germany) at 37°C for 24 h. Incubated cultures on blood agar were screened using method described by Cowan and Steel (2004; Bauer *et al.*, 1966).

Ethical consideration

Ethical approval was taken from the Renaissance hospital in Nsukka area, Enugu State of Nigeria for sample collection from the patients. Patient with diabetes mellitus and wounds infections, who were willing to participate were given written consents. Thereafter sample was taken for purpose of research. Any information that may disclose the patient identity was not kept in consideration.

Microorganism used

The bacterial isolates used were *Staphylococcus aureus* that had been tested against different antibiotics using disk diffusion method and had shown multidrug resistance. All the resistant bacteria isolates were labeled serially as DW1 DW19 (Diabetic wound 1 to Diabetic wound 19).

Nigella sativa (Black cumin) oil

The black seed oil was bought from a pharmacy at Hofuf, Al Ahsa, Saudi Arabia. It is a product of Al-Hussan food products Factory, Riyadh. According to manufacturer guideline, it is cold press extracted 100% pure organic oil.

Antimicrobial susceptibility testing

The disk diffusion method for *in vitro* antibiotic susceptibility tests described by Bauer was used for the study (Bauer *et al.*, 1966). The concentrations of antimicrobial sensitivity and interpretation of sizes of zones of inhibition were in accordance to Performance Standards for Antimicrobial Disk Susceptibility Tests, NCCL, (2002). All the antibiotics were from Abtek biological Ltd, Liverpool, UK. The antibiotics tested and the concentrations used were Cotrimoxazol (25ug), Cloxacillin (5ug), Erythromycin (5ug), Gentamicin (10ug), Augmentin (30ug), Streptomycin (10ug), Tetracycline (10ug) and (25ug), Chloramphenicol (10ug), Ofloxacin (5ug), Nalidixic acid (30ug), Nitrofurantoin (20ug), Amoxycillin (25ug), Cotrimoxazole (25ug).

Well diffusion susceptibility method

The method of Rios (1998) was used in this study to determine the antimicrobial activity of *N. Sativa* oil. Nutrient agar plates seeded with multidrug resistant strains of isolated *S. aureus* were used for this study. A well was cut in the middle of each of the agar media with a sterilized (0.8mm) cork borer. The oil was introduced into each of the wells, and inoculated plates with wells were then incubated at 37°C for 24 hrs. Zones of inhibition were measured in mm. Three replicates were prepared for each resistant specimen.

RESULTS

A total of 34 specimens were collected from outpatients department of Renaissance hospital, Nsukka. *Staphylococcus aureus* was isolated from all the specimens. Nineteen (56%) of them exhibited multidrug resistance to commonly used antibiotics. Thirty-three (97%) of the isolates were each resistant to the beta lactam antibiotics, Cloxacillin and Amoxycillin. While 30(88%) were resistant to Augmentin. The result presented in the fig. 1 also shows that resistance remained high for the Quinolones at 25(74%) and 20(59%) for Nalidixic acid and Ofloxacin, respectively. Among the aminoglycosides, 17(50%) and 22(65%) of the isolates showed resistance to Gentamicin and Streptomycin respectively (fig. 1). For other antibiotics tested in the study, the results presented in fig. 1, also shows that 23(68%) and 18(53%) of the isolates were resistant to Chloramphenicol, and Nitrofurantoin respectively, while 26(76%) of the isolates showed resistance to Cotrimoxazole. Overall, aminoglycosides and tetracycline revealed a better antibacterial activity than the beta-lactams and Quinolones.

Table 1: Zones of inhibition produced by different concentrations of oil extract of *Nigella sativa*.

S/N Of Organism	Concentrations of Oil extract of <i>Nigella Sativa</i> (mg/ml)			
	200	400	800	1000
DWI. 2	-	-	-	1.8mm
DWI. 9	0.3 mm	0.5 mm	0.8 mm	2.5 mm
DWI. 10	0.1 mm	0.2 mm	0.5 mm	1.8 mm
DWI. 11	-	-	-	1.2 mm
DWI. 14	0.5 mm	0.4 mm	0.7 mm	2.5 mm
DWI. 15	0.2 mm	0.6 mm	1.0 mm	3.0 mm
DWI. 17	-	-	-	2.6 mm
DWI. 18	-	-	-	0.1 mm

DWI = Diabetic wound isolate Represents no zone of inhibition.

Table 2: Comparison of bacterial susceptibility to antibiotics and oil extract of *Nigella sativa*

Sample No.	Zones of inhibition (mm)											
	Oil extract (mg/ml)				Antibiotics							
	200	400	800	1000	AUG	NAL	NIT	GEN	OFL	TET	STR	AMX
DWI. 1	-	-	-	-	-	-	-	-	-	-	-	-
DWI.2	-	-	-	1.8	-	-	-	-	-	-	-	-
DWI.3	-	-	-	-	-	-	-	-	-	-	-	-
DWI.4	-	-	-	-	-	0.4	-	-	-	-	-	-
DWI.5	-	-	-	-	-	0.5	0.3	-	0.8	-	-	-
DWI.6	-	-	-	-	-	-	-	-	-	-	-	-
DWI.7	-	-	-	-	-	-	-	-	-	-	-	-
DWI.8	-	-	-	-	-	0.4	-	-	-	-	-	-
DWI.9	0.3	0.5	0.8	2.5	-	0.3	-	-	-	-	-	-
DWI.10	0.1	0.2	0.5	1.8	-	-	-	-	-	-	-	-
DWI.11	-	-	-	1.2	-	-	-	-	-	0.15	-	-
DWI.12	-	-	-	-	-	-	-	-	-	0.4	-	-
DWI.13	-	-	-	-	0.4	-	-	-	-	-	-	0.5
DWI.14	0.5	0.4	0.7	2.5	-	-	-	-	-	-	-	-
DWI.15	0.2	0.6	1.0	3.0	-	-	-	-	-	-	-	-
DWI.16	-	-	-	-	-	0.5	-	0.4	0.5	-	0.2	-
DWI.17	-	-	-	2.6	-	-	0.2	-	-	-	-	-
DWI.18	-	-	-	0.1	-	-	0.3	0.5	-	-	-	-
DWI.19	-	-	-	-	-	-	-	-	-	-	-	-

DWI= Diabetic wound isolates, - no activity

Table 3: Percentage Resistance and sensitivity of *Staphylococcus aureus* to various concentrations of *Nigella sativa* oil

Concentration of oil extract mg/ml	No. resistant	No. sensitive	Mean diameter zone of inhibition	% difference in zone of inhibition in different oil dilution
200	15 (78.94%)	4 (21.05%)	0.28	14.00%
400	15 (78.94%)	4 (21.05%)	0.43	22.00%
800	15 (78.94%)	4 (21.05%)	0.75	39.00%
1000	11 (57.89%)	8 (42.10%)	1.94	100.00 %

Table 4: Comparison of zones of inhibition between *Nigella sativa* and other antibiotics

Oil extract (mg/ml)	Antibiotics								
1000 NG oil	AUG	NAL	NIT	GEN	OFL	TET	STR	AMX	
Z score	-2.433	-1.963	-2.314	-2.316	-2.296	-2.433	-2.433	-2.433	-2.433
p-values	0.015	0.050	0.021	0.021	0.022	0.015	0.015	0.015	0.015

Two related Willcoxon test, p value less 0.

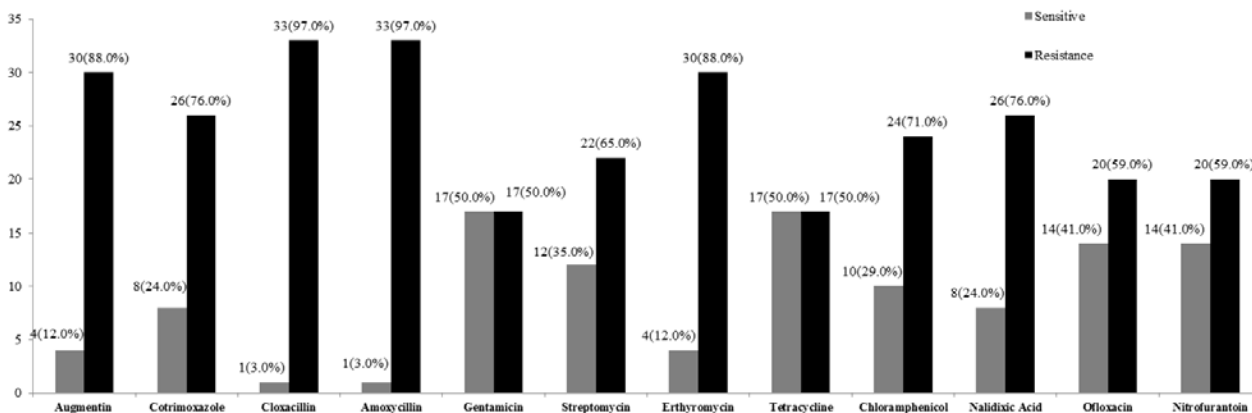


Fig. 1 : Resistance and Sesity pattern of Isolates to commonly used antibiotics

The mean zone of inhibition to the various test drugs is shown in fig. 2. Nitrofurantoin and Streptomycin produce the highest inhibitory zones. Zones of inhibition were lowest with Nalidixic acid and Augumentin at 0.45mm and 0.4mm, respectively.

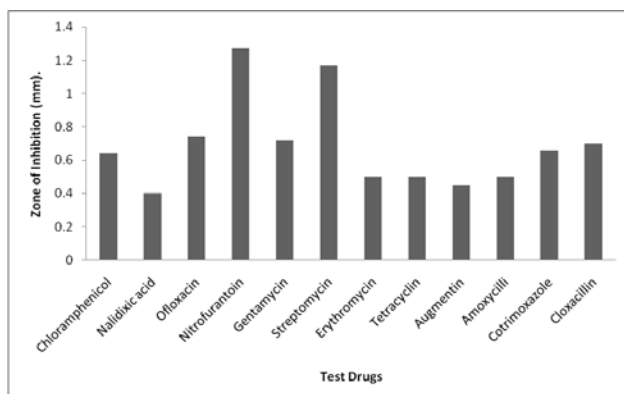


Fig. 2: Mean zone of inhibition by the Isolates to the Various Antibiotics.

Activity of *Nigella sativa* (Black cumin) oil extracts/dilutions against the resistant isolates

Eight (24%) of the 34 isolated *S. aureus* were seen to be resistant to all the antibiotics while eleven (32.4%) exhibited multidrug resistance, sensitive to a maximum of three of the test drugs. These isolates were tested against *Nigella sativa* (Black cumin) oil extracts. The results obtained showed that some of the multidrug resistant *S. aureus* isolated were sensitivity to varying concentrations of *Nigella sativa* oil as presented in table 1 with MIC for the different concentrations of the oil at 50% each for 1000 mg/ml and 200 mg/ml, (fig. 3). table 2 shows the comparison between isolated bacterial susceptibility to antibiotic and oil extract of *N. sativa*. Out of 19 resistant isolates, diabetic wound isolates (DWI) 1, 3, 6, 7 and 19 showed complete resistances to all antibiotics and *Nigella Sativa* oil. 8(42.0%) of the isolates, (DWI 2, 9, 10, 11, 14, 15, 17, 18) were sensitive to various concentrations of

Nigella Sativa oil. While DWI 9, 10, 14 and 15 showed a concentration dependent effect (table 2).

In the diluted form of 200, 400 and 800 mg/ml, most (79% of each) of the isolates remained resistant as shown in table 3. However, 42% showed sensitivity at the concentration of 1000mg/ml. The mean zones of inhibition was also seen to increase as the oil concentration increased, showing a concentration dependent pattern of sensitivity. The results comparing the mean zone of inhibition between *Nigella sativa* (100%) and Other Antibiotics is presented in table 4. The results show that the zone of inhibition for *Nigella sativa* was more in most of the cases than the other antibiotics compared. The results are statistically significant.

DISCUSSION

The results obtained from the present study shows that *Staphylococcus aureus* remains a prominent etiological agent in pyogenic infections. That the bacterium was isolated from all the patients in the investigation, with 19(56%) of these exhibiting multidrug resistance, makes the organism an issue for concern. The trend of antibiotic resistance to a large number of commonly prescribed drugs as observed in the present study conforms to earlier findings (Adegoke and Komolafe 2009; Menichetti 2005; Van *et al.*, 2011; Sass *et al.*, 2012). They reported the upsurge in antibiotic resistance and were of the view that abuses of antibiotics as well as the high prevalence of self-medication were responsible for the antibiotic resistant strains (Adegoke and Komolafe 2009).

In the present investigation, the isolated *S. aureus* showed a high percentage of resistance to β -lactam antibiotics. Similar findings had been reported by Adegoke and Komolafe (2009). The high resistance to Amoxycillin (97%), Cloxacillin (97%) and Augmentin (88%), contributes to the record of sick individuals worldwide with β -lactam base resistant infection (Firkin *et al.*, 2003).

Bacterial resistance to multidrug has become a common feature associated with disease and wound infections (Firkin *et al.*, 2003).

Bacterial resistance to multidrug has become a common feature associated with disease and wound infections (Ahmed *et al.*, 2004; Jahan *et al.*, 2004). The effects of Multi-resistant strains of *S. aureus* will be devastating if remained untreated.

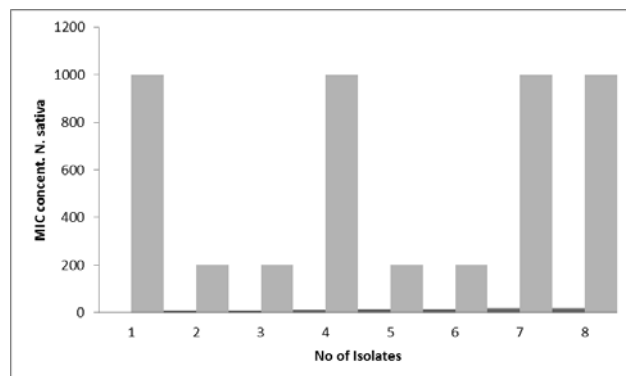


Fig. 3: MIC of oil extract of *Nigella sativa* on multi-drug resistant *Staphylococcus aureus*.

Nigella sativa oil (Black cumin), an easily available herb in Asian and Arabian countries with its known antibacterial activity, was evaluated against multidrug resistant *S. aureus* (Ankri and Mirelman 1999). Eight (42%) of the 19 isolates tested were sensitive to the oil extract while 11(58%) remained resistant. This is contrary to the findings of Alam *et al.*, 2010 who evaluated extracts of the seed and reported that the ethanol extract of the black cumin seeds was highly effective against *S. aureus* (Alam *et al.*, 2010). They expressed their view that since *S. aureus* was commonly implicated in infections, it would be interesting to guess that the extract could be used as an alternative in the treatment of wounds infected with this multidrug resistant bacterium. The fact that only forty-two percent of isolated *S. aureus* in the present investigation were sensitive to the oil extract at 100% concentration suggests that the Black cumin oil could be used in the treatment of multidrug resistant *S. aureus*. Again with the percentage of resistance high at 58%, the oil might not serve as an alternative in the treatment of multidrug resistant *S. aureus*.

The findings of the present study in which the bacteria under investigation was resistant to commonly prescribed drugs, makes it alarming particularly for a country like Nigeria where majority of its populace cannot afford appropriate treatment.

It is suggested that *Nigella sativa* Linn (Black cumin) oil extract can be beneficial as a part of treatment. More work however needs to be done as there is the possibility that there might be a new mutation of *Staphylococcus aureus* originating from this region of Nigeria.

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