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In vitro Evaluation of Antimicrobial Activity of *Nigella sativa* against Methicillin Resistant *Staphylococcus aureus* in Shendi Town, Sudan

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Augment of antibiotics resistance with development of new aggressive strains for the same disease is an international health problem. The ability of treating diseases is based on our ability to discover a new line of treatment with highly effectiveness and simple potentiality; so Herbal-Medicine is the line of choice. Traditional Arabic medicine point to the beneficial effect of *Nigella sativa* in curing a lot of diseases.
The Prophetic-Sunna point out that *Nigella sativa* has highly therapeutic properties for various diseases. Prophet Mohammed quoted as saying "There is healing in Black seed for all diseases except death".
Methods: A cross-sectional community and hospital-based study, was done from 10 Jan-2023 to 31 March-2023 in Shendi Town, River Nile State. 95 nasal and skin swabs were collected from people and a variety of hospitals in shendi town, then cultured on Manitol Salt Agar (MSA) for selectivity of *S.aureus* to be isolated, bacterial identification done by colonial morphology, Gram`s stain and biochemical tests. The antimicrobial susceptibility of S.aureus to *N. sativa* was carried out using agar well diffusion method.
Results: 48 pure culture of Methicillin resistant *Staphylococcus aureus* bacteria (MRSA) was isolated from 95 different clinical swabs specimens. *Nigella sativa* oil shows an effective inhibitory reactions against all MRSA bacterial isolates. All tested concentrations (50%, 25%, 13%) compared with Methicillin antibiotic inhibit 48(100%) of MRSA isolated bacteria.
Conclusions: Our results show the highly effect of *Nigella sativa* against MRSA so can be derived as strongly effectiveness herbal antimicrobial agent against it.

Keywords: Antimicrobial activity; Nigella sativa; MRSA.

1. INTRODUCTION

Methicillin resistance is a serious health concern since it has spread among *Staphylococcus aureus* [1]. *Nigella sativa* is a blessed seed, Prophet Mohammed was quoted as saying "There is healing in Black Seed for all diseases except death'' Sahih Al-Bukhari, 5687 [2]. It's also known as Black cumin, Black seed, Siyahaneh, Black caraway, Black coriander, Black onion seed, Charnushka, Roman coriander, kalonji [3]. The genus Nigella derived from Latin Niger "black" refer to seed color [3]. It's annual flowering plant in family Ranunuclaceae local in Cyprus, Turkey, Iraq, Iran, Bulgaria, Romania and many other countries in worldwide [3]. *N. sativa* seeds contain bioactive constituents of iso-quinoline alkaloids including (thymoquinone (TQ), thymohydroquinone (THQ) and di-thymoquinone) which have been reported to have many biological and immunological Activities [4]. It's also contained linoleic acid, oleic acid, palmitic acid, trans-anethole, nigellicine, nigellimine, nigellimine N-oxide, P-cymene, carvacrol, alpha & beta pinene, anethole and proteins [3]. *N. sativa* act as antibacterial anti-histamine, anti-inflammatory, anti-tumor, anti-fugual, promote lactation, strengthens the immune system [5]. Staphylococcus is included in the Micro-coccaceae family within the phylum Actino-bacteria [6]. *Staphylococcus aureus* is a gram positive, spherically shaped bacterium arranged in grape like clusters after division, which responsible for severe morbidity and mortality worldwide [7]. They are facultative anaerobe grow with/without need to oxygen [3]. Also known as "golden staph"due to the pigment produced and the golden yellow morphology with clear zones of hemolysis of its colonies on blood agar culture [3]. Some strains of S.aureus characterize with resistant to methicillin antibiotic which known as methicillin resistant *Staphylococcus aureus* [7]. The first semi-synthetic anti-staphylococcal penicillins were developed around1960 and methicillin resistant *S. aureus* (MRSA) was observed within 1 year of their first clinical use [8]. The organism concentrated in the hospital setting producing bloodstream and surgical wound infections [7]. MRSA leading to infections in bones, joints, abscesses and severe complications in the normal heart valves [7]. Its non-motile, non-spore former, and some strains are capsulated, the first isolation is carried out by Alexander Ogston [9]. Also reported to cause clinical diseases such as food poisoning, pyogenic endocarditis, suppurative pneumonia, otitis media, osteomyelitis, and pyogenic infections of the skin and soft tissues [9]. Antimicrobial resistance of MRSA is caused by chromosomal acquisition of the gene responsible of penicillin binding protein (PBP) [10]. Virulence factors of MRSA that play a role in causing infections are mecA is a

biomarker gene responsible for resistance to methicillin and other beta lactam antibiotics [3]. MSCRAMM which are types of micro surface components recognizing adhesive matrix molecules [11]. SSCCmec staphylococcal cassette chromosome mec is a genomic island of unknown origin containing the antibiotic resistance gene mecA, also it contained additional genes beyond mecA including cytolysin gene psm-mec, which suppress virulence in HA-MRSA strains [3]. ACME arginine catabolic mobile element, facilitate hypersensitivity by *S.aureus* and stable colonization of the skin, wounds infections, person to person transmission [3,12,13]. Isolation process of MRSA based on using culture media selective for *Staphylococcus aureus* such as blood agar, mannitol salt agar (MSA), and Baird-Parker agar which containing methicillin antibiotic [14]. Identification process of MRSA started with growing reaction on media mentioned above, Gram stain and biochemical tests such as catalase test are used [15]. Because Sativa has a group of pharmacological properties and because *S. aureus* is currently the most common cause of nosocomial infections, the *N. sativa* active organic oil was used against MRSA isolated from nasal and skin specimens of both hospitalized and community carriers[16].

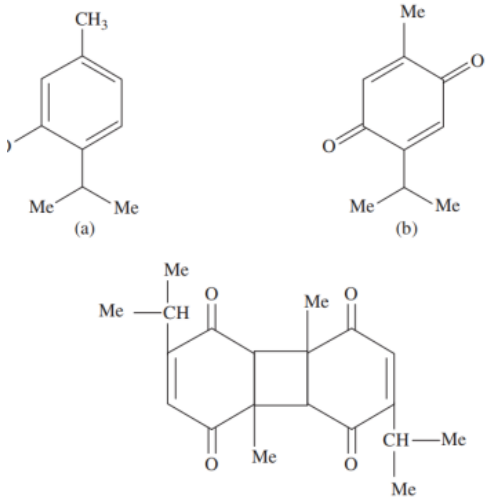


Fig. 1. Chemical structures of thymol (a), thymoquinone (b) and dithymoquinone (c) (Me: methyl group) [17]

2. MATERIALS AND METHODS

2.1 Study Design

A descriptive cross-sectional community and hospital-based study, was done from 10 Jan 2023 to 31 Mar 2023 in Shendi town - River Nile State. The samples were distributed between different Shendi hospitals and community.

2.2 Samples Collection

A number of 95 nasal, skin, axillar and hospitalized newborn nursery swabs specimens under aseptic status were collected (n=95), done at Shendi town, River Nile, Sudan.

2.3 Bacterial Isolation and Identification

Bacterial growth on Manitol Salt Agar (MSA) media, gives a yellow colonies that's indicate manitol ferment. The isolated bacteria identified by Colonial morphology, Gram stain and biochemical tests.Fully identification done by using of Gram staining technique and specific confirmatory biochemical tests. After identification,stock culture were made and then kept on refrigerator at optimum temperature.

2.3.1 Concentrations of *Nigella sativa* oil used

N. sativa oil was used in different concentrations 13%, 25%, 50 % against isolated MRSA organisms.

2.3.2 Bacterial Suspension

In each test tube, one ml of sterile physiologic normal saline was put, a number of purified bacteria was taken using a sterile loop and mixed with normal saline. Density of the suspension was compared with McFarland (0.5) standard solution.

2.4 *In vitro* Antimicrobial Activity of *Nigella sativa* Against MRSA

Bacterial suspension was distributed on the plates of MHA using swabbing technique. Different concentrations of *N. sativa* oil were put in the wells that made using a sterile Durham tube with diameter of 5mm. A methicillin antibiotic discs of 5 µg were used for making comparison. After completely distribution of the *N. sativa* oil in the plates, the inoculated plates of MHA were incubated for 24hours at 37 C* in the presence of oxy-gen. After incubation period done, the inhibition zones were observed and the diameters around the wells were obtained.

2.5 Data Collection and Analysis

Self-administrated questionnaire was used and supported with coding numbers to facilitate the sorting out of data.

Data were entered, check and analyzed using Microsoft Excel 2007, The final results were presented as frequencies and percentages.

3. RESULTS

Fifty (50) samples of *Staphylococcus aureus* bacteria (48 MRSA and 2 MSSA) have been isolated from 95 swabs specimens from different hospitals and community in Shendi town. The study population concerned with 39 (41.1%) males and 56 (58.9%) females (Table 1). Hospitalized and community specimens concerned with this study were 25 nasal swabs, 65 skin swabs, 1 axillar swab, 1 Incubator wall swab, 3 nursery drips swab in sequence (Table 2). Methicillin antibiotic(5µg) was used to confirm the identity of MRSA and to compare its activity with *Nigella sativa* activity (Table 3). A total of tested Methicillin-Resistant *Staphylococcus Aureus* (MRSA) bacteria were sensitive to the all tested concentrations of *Nigella sativa* oil (Table 4). Type of MRSA hospital acquired or community acquired (Table 5).

Table 1. Distribution of specimens based on gender

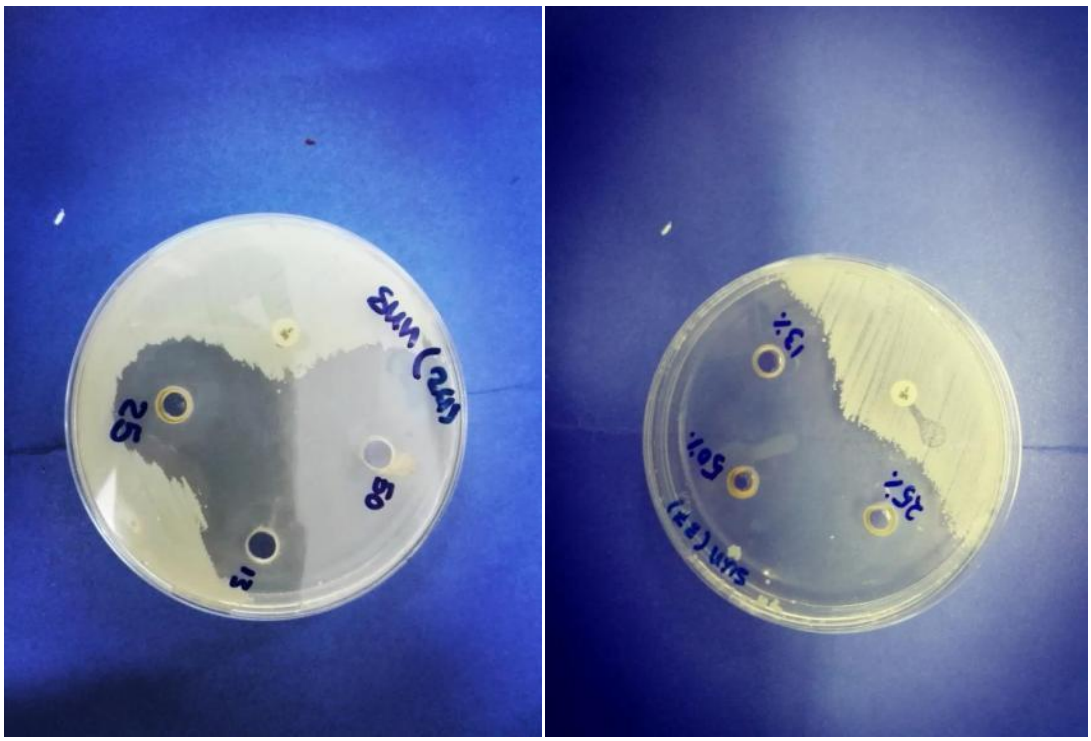
Gender	Frequent	Percent (%)
Male	39	41.1
Female	56	58.9
Total	95	100

Table 2. Distribution of specimens based on site of collection

Sample type	Frequent
Nasal swabs	25
Skin swabs	65
Axillar swab	1
Incubator wall swab	1
Nursery dripsswab	3
Total	95

Table 3. Antimicrobial activity of methicillin 5µg against Isolated Microorganism

Isolated bacteria	Sensitive	Resistant
S. aureus	2	48
Total	50	



Picture 1. Show the activity of *N. sativa* oil against MRSA

Table 4. Antimicrobial activity of different concentration of *Nigella sativa* oil against MRSA (frequency and percentage)

Isolated bacteria	*N. sativa* oil Concentrations	Sensitive (%)	Resistant
MRSA	13v/v	48 (100%)	0
	25 v/v	48 (100%)	0
	50v/v	48(100%)	0
MSSA	13v/v	2	0
	25v/v	2	0
	50v/v	2	0
Total		50	0

Table 5. Shows types of MRSA based on origin of specimens

Type of MRSA	Count	Percent (%)
HA-MRSA	23	46
CA-MRSA	27	54
Total	**50**	**100**

Table 6. Different concentrations *N. sativa* Oil and diameters of inhibition Zones

Diameter	Concentration	Inhibition zone
N. sativa Oil	13	12±4mm
	25	23±7mm
	50	42±8mm

4. DISCUSSION

Various studies were performed to approve the beneficial effects of *Nigella sativa* on the different disorders includes bacterial resistance. Bacteria isolated from all the patients and individuals in this investigation have multi drugs resistance properties which let us to focusing on using Herbal-Medicine. Herbal-Medicine is highly effectiveness with limited side effects and with simple potentiality. Antimicrobial action of *Nigella sativa* was tested by a groups of studies. *Nigella sativa* was used as spice and food preservative as well as a protective and health medication in traditional public medicine for the treatment of many disorders [17]. *Nigella sativa* has an antimicrobial effects due to presence of thymoquinone (TQ), thymohydroquinone (THQ) and di-thymoquinone bioactive components [4]. In this study we tested *Nigella sativa* antimicrobial activity using different concentrations (13%, 25%, 50%) against Methicillin Resistant *Staphylococcus aureus* (MRSA) bacteria isolated from different shendi town hospitals and community. *Nigella sativa* shows strongly inhibitory effects against all isolated MRSA (100%) at all concentrations. This sensitivity results agreed with study done by Hannan and his/her colleagues reported that *Nigella sativa* ethanol extract has effects against MRSA at concentration of 4mg/disc [7].Bessedik Amina and Allem Rachida at faculty of science, University of Chlef in agreement with our study when reported inhibition zone diameter with 12.6±0.03mm [18]. Ayse RuveydaUgur and her colleagues in agreement with our study shows that *Nigella sativa* oil has antibacterial effects against Gram positive cocci with MIC of 0.5 µg/mL [1]. Lorina I Badger-Emeka and her colleagues in agreement with our study, reported inhibitory effects of *Nigella sativa* at concentrations of (2.5, 5.0, 7.5, 10) [19]. Tara Faeq M. Salih and his partners at Sulaimani Polytechnic University in agreement with our study, reported strongly inhibitory effects of *Nigella sativa* oil against MRSA with Inhibition zone diameter of 22 ± 0.5mm [20]. A.D.H. Sudesh and his partners reported ihibitory effects of *Nigella sativa* oil against isolated strains of MRSA with inhibition zone diameter of42.33±0.58mm [21]. EmSutrisna, Sri Wahyuni and ArisFitrianiin agreement with our study reported inhibition zone diameter at Concentration of 50%vlv alcoholic extraction of *N. sativa* was 30.84mm [22]. Muhammad A.Randhawa, MastoorS.Al-Ghamdi also in agreement with our study reported the antibacterial effect of *N. sativa* aginst Gram positive *Staphylococcus aureus* [23]. HalaGali-Muhtasib, NahedElNajjar, Regine Schneider-Stock reported the inhibitory effects of *N. sativa* against Gram Positive Bacteria [19]. Lorina Badger Emeka , Promise Madu Emeka and Tahir Mehmood Khan are in agreement with our study reported Out of 19 *S. aureus* 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 [24]. In the

same study of Lorina Badger Emeka , Promise MaduEmeka and Tahir Mehmood Khan they disagree with our study in that Eleven (58%) of the isolates were completely resistant to all the oil concentrations [24]. In Table 1, females shows highly frequency (58.9%) compared with males (41.1%).

5. CONCLUSION

Nigella sativa oil shows highly antimicrobial activity against methicillin resistant *Staphylococcus aureus* at different concentrations.

6. RECOMMENDATIONS

In base of study results we recommended to use *Nigella sativa* oil as bio-active component in herbal medications against multi drug resistant bacteria (MDR-MRSA).

CONSENT

Patient's written consent has been collected.

ETHICAL APPROVAL

Permission given by College of Ethical Committee of Shendi University and Hospitals. Participators have been noticed and no coercion of any sort has be done and any information that may disclose the participators identity was not kept in consideration.

NOTE

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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