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In vitro Antibacterial activity of Nigella Sativa extract against clinical Isolates of Methicillin Resistant Staphylococcus Aureus

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Abstract: ***S. aureus is one of the most popular bacteria; it causes large number of infection for human and animal. Due to its prevalence and regular treatment, it gained resistance for many regular medications and become over concerning matter, so substitution drugs have been brought out in order to beat the bacteria resistant types. N sativa seem to have an antibacterial effect for so many years back in time. So this study aimed to confirm that effect on MRSA through culturing the s. aureus with different strains in different concentrations of N sativa extract. Inhibition activity seem to be found in high concentration of N sativa and decline with lower concentrations with finding small zones with increasing growth. N. sativa is used among popular medicine and has effectiveness through years of using, so it should be consider in modern medicine to set in treatment protocol.***
Key word: N. sativa, MRSA, antibacterial, strains

I. INTRODUCTION

Staphylococcus aureus is member of the family Micrococcaceae, is a Gram-positive coccus whose cells tend to occur either singly or if dividing cells do not separate, form pairs, tetrads and distinctive irregular "grape-like" structures [(1)]. S aureus is both a commensal bacterium and a human pathogen of significant importance because of its ability to cause a wide range of diseases and capacity to adapt to diverse environmental forms. Approximately 30% of the human population is colonized with S. aureus [(2-3-4)]. The organism colonizes skin, skin glands and mucous membrane, causing infections such as rashes, inflammations of bones and the meninges as well as septicemia [(5)]. Penicillin and its derivatives, including methicillin have been used for the treatments of infections caused by S. aureus [(6)]. However, certain strains of S. aureus developed resistance known as methicillin resistant Staphylococcus aureus (MRSA). At present, less than 90% of S. aureus strains are resistant to most penicillin derivatives [(7)]. Resistant strains typically produced an enzyme, called a β-lactamase, which inactivated the β-lactam, so penicillin derivatives that resistant. Efforts to β-lactamase hydrolysis were made in 1959 with the synthesis of methicillin, which had the phenol group of benzylpenicillin disubstituted with methoxy groups. The methoxy groups produced steric hindrance around the amide bond reducing its affinity for staphylococcal β-lactamases. But as soon as methicillin was used clinically, methicillin-resistant S. aureus (MRSA) strains were isolated [(1)]. Resistance was not due to β-lactamase production but due to the expression of an additional penicillin-binding protein (PBP2a), acquired from another species, which was resistant to the action of the antibiotic [(1)]. The use of different types of antibiotics over the years has led to the emergence of multi-resistant MRSA strains [(8)], the result of mutations in genes coding for target proteins and through the acquisition and accumulation of antibiotic resistance-conferring genes [(8)]. The rise in multidrug-resistant bacteria has become a serious health problem and major challenge in developing treatment options of infectious diseases [(9)]. Plants have always laid the foundation of pharmaceutical drug to develop and allow treating diseases on a greater and more efficient scale, 60% of currently available antimicrobial and antitumoral drugs are from plants [(10)].

N. sativa is one of the oldest documented herbal medicinal plants and has been used for centuries in traditional medicine [(11)]. Nigella sativa that belongs to family Ranunculaceae is commonly known as black seed or black cumin [(12)]. It has been shown to possess antimicrobial, immunomodulatory, anti-inflammatory, and antioxidant properties [(13)]. Herbal treatment with this plant is already commonly applied and well known for its safety [(11)]. N. sativa seeds contain oil, protein, carbohydrate, fiber, and saponin. The fixed oil is composed of arachidonic acid, linoleic acid, oleic acid, almitoleic acid, palmitic acid, stearic acid, myristic acid, steroles, and eicosadenoic acid [(14)], whereas the essential oil of N. sativa entails nigellone, thymoquinone, thymohydroquinone, thymol, carvacrol, α- and β-pinene, d-limonene, d-citronellol, and p-cymeme [(15-16)].

II. MATERIAL AND METHOD

This experimental cross sectional study, targeted clinical isolates which collected from saba teaching hospital and standard bacterial strain obtained from national center for research -Khartoum Sudan include American type culture collection (ATCC 25923).Total of 90 clinical isolates of S. aureus isolated from different clinical specimen and confirmatory tests conducted which include morphological and biochemical test (catalase, coagulase, DNase, mannitol fermentation on MSA media). Antimicrobial susceptibility processed in order to determine methicillin resistant s. aureus strain , The concentrations of antimicrobial sensitivity testing discs used and interpretation of sizes of zones of inhibition were in accordance to Performance Standards for Antimicrobial Susceptibility Tests, CLSI [(17)]. Nigella sativa plants were dried by air, ground to fine texture and then 70% ethanol added to 50 g of dried plants powder for extended period and the resultant extracts were concentrated, under reduced pressure each sample diluted with methanol for four concentrations as 200, 100, 50 and 25 $mgml^{-1}$. Out of the 90 S. aureus isolated random total of 9 clinical isolates of MRSA were included beside of and ATCC 25923. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test by paper disc diffusion method to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). Bacterial suspension was diluted with sterile physiological solution to 10^8CFU/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of ethanlic extract of plant.The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

III. RESULT

All MRSA and ATCC 25923 growth of N. sativa different concentrations have different size of zones i.e. inhibition of growth, but larger zones obtained at higher concentration (200), size reached 15 mm (inhibition of growth $\geq$ 12mm used as good inhibitory effect of extract [18]), then declines through other concentrations till lower one 25 in which small notable zones found indicator for growth of the bacteria as in table 1

Table 1: diameter of bacterial zones/mm in different concentrations of N. sativa

	Activity against different dilutions of extract of with NS			
no	200	100	50	25
Standard ATCC	14	12	11	7
778	15	11.5	11	9.5
972	15	13	12.5	11
552	14	13	12	11
335	15	13	12	11
403	15	12	11	10
206	14	12	11	10
764	13	12	11	10
384	15	14	11	9
474	13	12.5	12	7

IV. DISCUSSION

Nigella sativa is an annual flowering plant. It grows to 20–30 cm tall. The fruit of plant is large and inflated capsule composed of 3-7 united follicles, that each of them has numerous seeds. The black colored seeds are flattened, oblong and angular, funnel shaped, with the length of 0.2 cm and 0.1 cm wide [(19)]. Recently years number of studies has been carried out; acclaimed medicinal properties emphasized on different pharmacological effects of N. sativa seeds [20]such as anti-inflammatory and immunomodulatory and anti-tumor properties [(21-22)]. In this study, considering MRSA and N-sativa number of 10 S. aureus strains was cultured in different concentrations of N. sativa ethanol extracted, at 200 concentration sensitivity indicated by clear zones with diameters reach up to 15mm and decline zone diameters with lowering concentrations of N sativa. This in agreement with different studies conducted at the same manner, one of them different concentrations of Nigella sativa oil were tested for their antibacterial activity against different strains of Gram positive and Gram negative multi-drug resistant bacteria (MRSA and others) by using well

diffusion method, for the different strains of MRSA different zones of inhibition where obtained for all the different oil dilutions used. Bacterial growth was inhibited at 100%, 80%, 50%, 40%, 30% and 20% N [(23)]. Same inhibitory effects of N. sativa revealed in other studies at the same concern they were conducted, proof of how effective herbal treatment could be [(24-25-26)].

V. CONCLUSION AND RECOMMENDATION

Alternative medicine contains herbal and other plants for treatment of what considered a resistant, one of these plants N. sativa and it shown in vitro presence for MRSA as it inhibits growth of the bacterial with more high concentration of extract. Protocol of such medication should be tested enough and open for un resolved issues of resistance of drugs.

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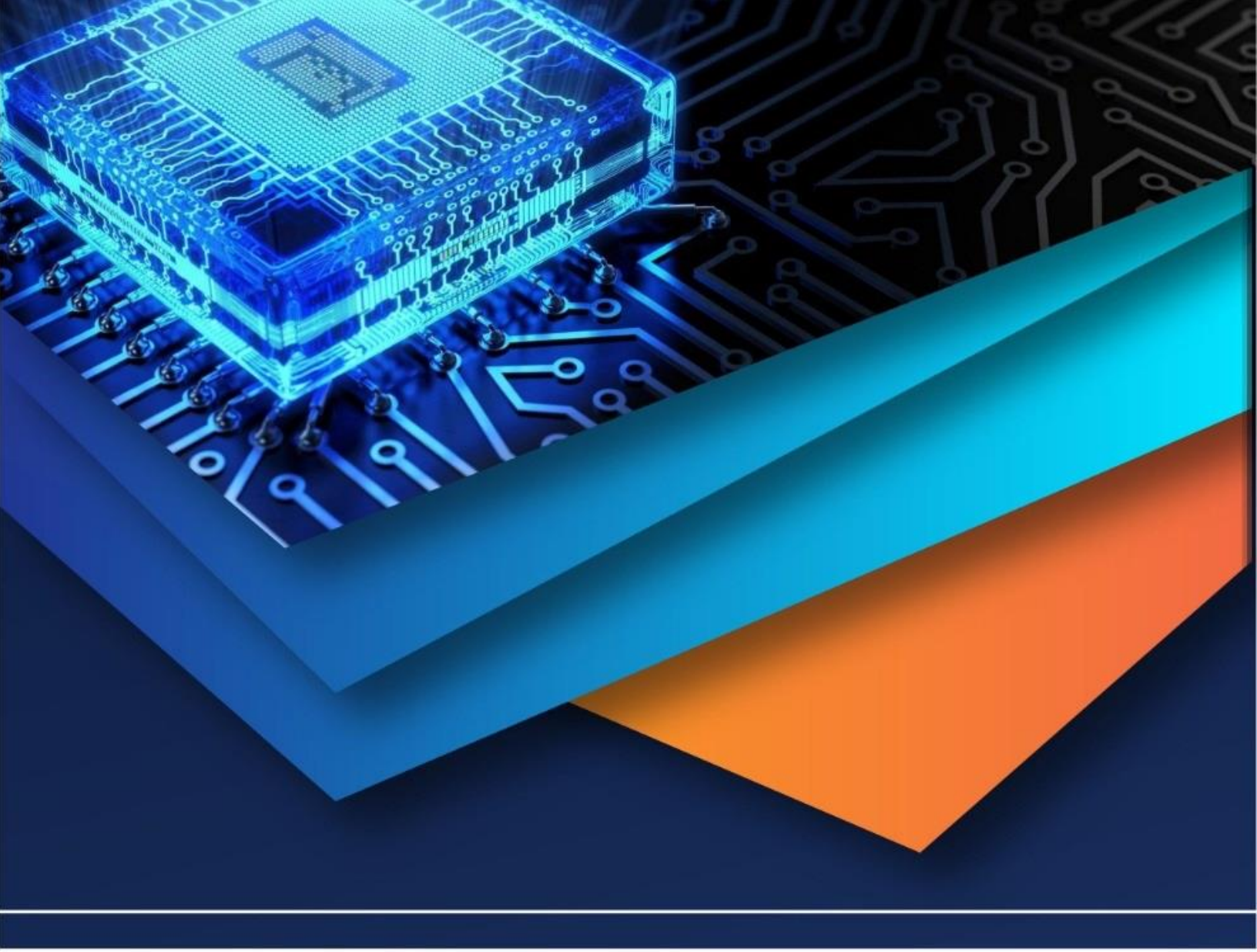

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