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Wide spectrum antibacterial activity of Nigella *Sativa L.* seeds.

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ABSTRACT: Crude extracts of *Nigella sativa* L. (black cumin) seeds were tested in varying dilutions against strains of *Bacillus cereus* 2156, *B. subtilis* 2920, *Escherichia coli* 2065, *Staphylococcus epidermidis* 2493, *Klebsiella pneumoniae* 2957, *Pseudomonas aeruginosa* 5029, *Salmonella typhmurium* 2501, *Enterobacter aerogens* 5139 using agar well diffusion technique in swabbed Muellar Hinton agar plates under standard laboratory conditions. Extract in ethanol and n-hexane showed remarkable dose dependant antibacterial activity against the tested strains as evident from the zones of inhibition. No activity of the extract was observed against *Pseudomonas aeruginosa* 5029 and *Enterobacter aerogens* 5139. The most sensitive strain was *S. epidermidis*. No cross resistance was noticed with any of the tested antibiotics.

I. INTRODUCTION

Microorganisms that were once thought to have been controlled by antibiotics are changing to new forms that are resistant to standard antibiotic therapies [1]. Various incidents of epidemics due to drug resistant microorganisms are now a common global problem causing enormous public health concerns [2]. Due to a large number of multi-drug resistant bacterial strains, current drugs effectiveness is getting limited for example methicillin-resistant staphylococci, pneumococci resistant to penicillin and macrolides, vancomycin-resistant enterococci as well as multidrug resistant gram-negative organisms [3]. There is an unmet medical need to find an alternative for the treatment of various diseases caused by various microbial agents. According to World Health Organization more than 80% of world population relies on traditional medicine for their primary health care needs. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. Vast knowledge of how to use the plants against different illness may be expected to have accumulated in areas where the use of plants is still of great importance [4]. The medicinal value of plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds [5]. Different pharmacological effects of *Nigella sativa L* (Ranunculaceae family) seeds have been reported. These include effects such as isulinotropic,hypoglycemic, anti inflammatory, antinociceptive, anticancer, hepatoprotective, neuroprotective ,antihistamine, antiulcer and bronchodilator [6-13]. However the antimicrobial effects of *N.sativa* for these bacteria has not studied yet. Therefore, in this study we evaluated the antibacterial activity of *N.sativa* seeds against both gram-positive and gram-negative bacteria.

II. MATERIAL AND METHODS

Bacterial strains

Eight pathogenic bacteria include, *Bacillus cereus* 2156, *B. subtilis* 2920, *Escherichia coli* 2065, *Staphylococcus epidermidis* 2493, *Klebsiella pneumoniae* 2957, *Pseudomonas aeruginosa* 5029, *Salmonella typhmurium* 2501 and *Enterobacter aerogens* 5139 were selected for testing antimicrobial activity of extracts of *N. sativa* seeds. All strains were purchased from National Chemical Laboratory (Pune, India).

Preparation of *Nigella Sativa* L extract

Dried seeds of *Nigella sativa* were weighed, grounded using mortar and pistle and extracted with ethanol and n-hexane. The crude extracts were filtered and concentrated by evaporating the solvents under reduced pressure. Stock solution of 50 mg/ml was prepared in dimethyl sulfoxide (DMSO) and aliquots stored at -80°C until used.

Culture of bacterial strains

Microorganisms were cultured according to the method described by NCCLS 2002 [14]. At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. Using a loop, single colony was picked and transferred into a tube containing 5ml of nutrient broth medium. The broth culture was incubated at 35°C until it achieved or exceeded the turbidity of the 0.5 McFarland standard (usually 2 to 6 hrs). The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standard. Bacterial suspension was then swabbed on Mueller–Hinton agar plates at three directions according to Clinical and Laboratory Standards Institute (CLSI).

Determination of antibacterial activity of *Nigella sativa* L extract

Antibacterial activities of crude extracts of *N. Sativa* seeds were evaluated by agar-well diffusion method. Petri dishes with 20 ml of Mueller Hinton agar were prepared and streaked with bacterial strains. Five mm wide wells were made and filled with 25 µl extract. The plates were then incubated for 24 hrs at 37°C. After incubation, diameter of the inhibition zone was measured and results were expressed in mm. Extract were tested at 1.5, 3.0, 4.5 and 6.0 mg/ml concentration. Antibacterial activity of *N. sativa* extracts was compared with standard antibiotics (erythromycin, tetracycline, ampicillin and ciprofloxacin)

Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

MIC of extracts for antibacterial activity was determined using the broth dilution bioassay. Overnight cultures of bacterial strains incubated at 37°C were diluted with Mueller-Hinton (MH) broth. The crude seed extracts were dissolved in DMSO to make 50 mg/ml. From the stock extract, required concentrations were prepared (1.5, 3.0, 4.0, 4.5, 6.0 mg/ml). Diluted bacterial strains were then treated with different concentrations of extracts in culture tubes for 24 hrs at 37°C. After treatment, strains were incubated on petriplates prepared with MH agar medium. MIC values were recorded as the lowest concentrations of extracts from where the inhibition in the growth had started. The MBC, a concentration where the colonies were not observed was also recorded.

III. RESULTS

The effect of ethanol extracts of *N.sativa* against both gram positive and gram negative bacteria is given in the Table 1. The extract showed pronounced activity against *S. epidermidis K. Pneumonia* followed by *B. Cereus, B. Subtilis, E. coli, S. Typhimurium* and no activity against *P. aerogenosa and E. aerogens. S. epidermidis* was the most sensitive strain showing inhibition upto 15 mm whereas *E. coli* was least sensitive, as is evident from Fig.1

	Extract concentration (mg/ml)			
	1.5	3.0	4.5	6.0
Bacteria	---------- Inhibition zone (mm) ----------			
B. cereus	5	8	9	8
S. epidermidis	7	9	10	15
S. typhimurium	5	7	8	9
B. subtilis	6	5	8	7
E. coli	5	6	6	ND
P. aerogenosa	R	R	R	R
E. aerogens	R	R	R	R

Table 1: Antibacterial activity of ethanol extracts of *Nigella sativa* seeds (ND = not determined) There was statistically significant difference between groups as determined by one way ANOVA (F (3, 28) =.288, p=.833). A Turkey post-hoc test revealed that the zone of inhibition was statistically significantly higher after taking concentration 4.50 mg/ml (p=.751) and 6.0 mg/ml (p=.833) compared to 3.0 mg/ml .There was no statistical significance between zone of inhibition by the concentration 3.0 mg/ml and 4.50 mg/ml (p=.916)

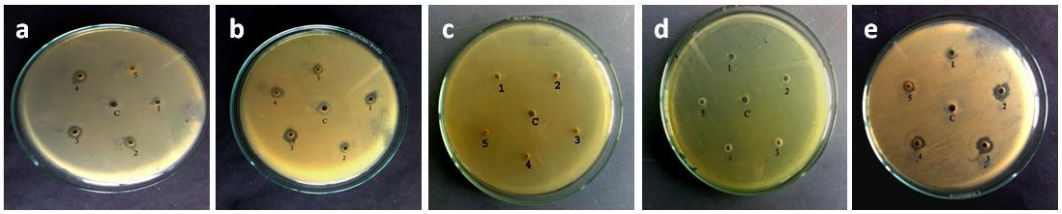

Fig: 1. Antibacterial activity of ethanol extract of N. sativa seeds. Different concentrations of extract were tested for antibacterial activity against (a) S. typhimurium, (b) B. subtilis, (c) E. coli, (d) P. aeruginosa and (e) B. cereus. (1: 1.5 mg/ml; 2: 3.0 mg/ml; 3: 4.5 mg/ml; 4: 6.0 mg/ml; 5: 7.5 mg/ml; C: control

The strain was also tested against the hexane extract of *N. Sativa* and the results were presented in the Table 2, Fig 2. The extract was most effective against *B. Subtilis* followed by *S. epidermidis* and *B. Cereus*. The extract showed no activity against *P. Aerogenosa, E. coli and E. aerogens.* The maximum zone of inhibition (26 mm) was observed in case of *B. Subtilis*.

	Extract concentration (mg/ml)			
	1.5	3.0	4.5	6.0
Bacteria	---------- Inhibition zone (mm) ----------			
B. cereus	8	10	13	11
S. epidermidis	14	16	20	19

S. typhimurium	R	R	R	R
K. pneumoniae	R	R	R	R
B. subtilis	15	22	20	26
E. coli	R	R	R	R
P. aerogenosa	R	R	R	R
E. aerogens	R	R	R	R

Table 2: Antibacterial activity of n- hexane extracts of *Nigella sativa* seeds. (R= resistant)

There was statistically significant difference between groups as determined by one way ANOVA (F (3, 28) =.108, p=.954). A Turkey post-hoc test revealed that the zone of inhibition was statistically significantly higher after taking concentration 3.0 mg/ml (p=.890) and 6.0 mg/ml (p=.934) compared to 4.50 mg/ml .There was no statistical significance between zone of inhibition by the concentration 3.0 mg/ml and 4.50 mg/ml (p=.934)

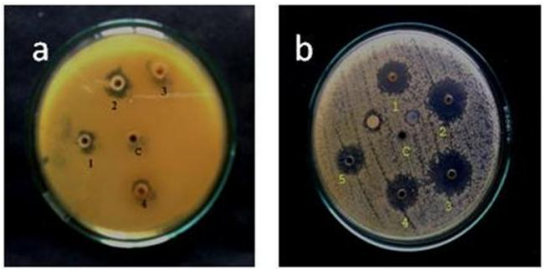

Fig: 2. Antibacterial activity of n-hexane extract of *N. sativa* seeds. Different concentrations of extracts were tested for activity against (a) *B. cereus* and (b) *S. epidermidis* (1: 1.5 mg/ml; 2: 3.0 mg/ml; 3: 4.5 mg/ml; 4: 6.0 mg/ml; 5: 7.5 mg/ml; C: control)

In this study, the extract was found to be more effective on Gram positive than Gram negative bacteria except *K. pneumonia* and *S. typhimurium* in which zone of inhibition was observed. The results are in conformity of a number of earlier studies [15, 16, 17]. A number of compounds derived from plants often show considerable activity against Gram positive bacteria but not against Gram negative species. Gram- negative bacteria have an effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphipathic compounds, and multidrug resistance pumps that extrude toxins across this barrier. It is possible that the apparent ineffectiveness of plant antimicrobials is largely due to the permeability barrier [18]

The MIC and MBC value of the Nigella sativa L ethanol extract against *S.epidermidis* was 1.0 and 4.0 mg respectively.

Fig 3. shows the antibacterial effect of various commonly used antibiotics on bacterial strains used for study.

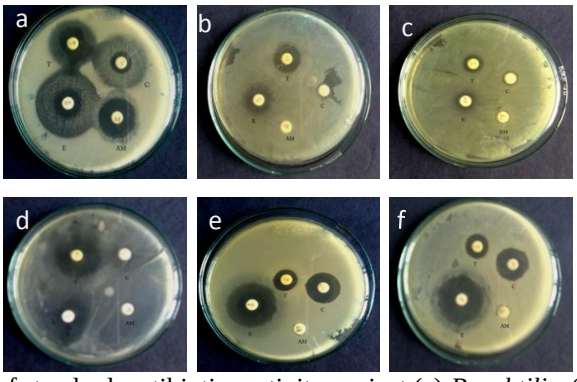

Fig 3. Antibacterial activity of standard antibiotics activity against (a) *B. subtilis*, (b) *E. coli*, (c) *P. aeruginosa* (d) *S. epidermidis* (e) *S. typhimurium* (f) *B.cereus* (T: tetracycline; C: ciprofloxacin; AM: ampicillin; E:erythromycin)

IV. DISCUSSION

The study shows a better alternative of common antibiotic drugs may be folk medicines. *Nigella sativa* is traditional medicine used from a very long time. Due to overuse of common antibiotics various cases of antibiotic resistance in microbes occurred. A large number of population depends on traditional medicines for cure from various microbial diseases. In our study we found *Nigella sativa* possess a very good antibacterial response as compared to various common antibiotics. It will be better for the mankind to rely on these traditional plants as they have very least side effect as compared to various antibiotics and chances of antibiotic

resistance due to overuse of antibiotics may be diminished. *N. sativa* ethanol extract showed pronounced activity against S. epidermidis and hexane extract against B. Subtilis. and no activity against P. aerogenosa and E. Aerogens. Similar to this hexane extract showed no activity against P. Aerogenosa, E. coli and E. aerogens. This confirms the study of Agarwal et al [19] who reported that the oil inhibited one strain of S. aureus even upto 1:100 dilution, the least concentration tested. However, the zones of inhibition observed in our study were larger which may be due to difference of the strains tested. The authors also reported activity of oil against E. coli. This may be because of the reason that N. sativa oil obtained from different commercial sources or isolated by different methods from the same seeds have been shown to vary significantly in their content of Thymoquinone, which has antibacterial activity and various storage conditions are expected to make a difference in the amounts of the quinone constituents of the oil, especially if the seed oil samples are exposed to heat and light [20]. Secondly, variability in the performance of Mueller- Hinton agars from different manufacturers has been shown to be statistically significant, especially when testing E. coli [21]. The size of inoculums used, depth of medium in the plates, inoculation technique and time period between inoculation and application of discs, incubation temperature and time of incubation will also cause differences in the results obtained.

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