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# Comparative in Vitro Study of the Antimicrobial Efficacy of *Nigella Sativa* Seed Oil (Black Seed Oil) Against Selected Microorganisms with Conventional Antibitics

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**Abstract:- This study investigates the antimicrobial activity of essential oil obtained from Nigella sativa seeds (Black seed oil). The objectives are to determine the antimicrobial efficacy of black seed oil against selected pathogens such as Pseudomonas aeruginosa (ATCC 27853), Methicillin Resistant Staphylococcus aureus (MRSA), Methicillin sensitive Staphylococcus aureus (MSSA), Staphylococcus pyogenes (ATCC 19615), Escherichia coli (ATCC 25922 and ATCC 35218) and clinically isolated Candida albicans and to compare the ability of microbial growth inhibition with conventional antibiotics that are used for the test organisms. Bacteria were inoculated on Mueller Hinton Agar and Sabouraud Dextrose Agar was used to inoculate C. albicans. Agar well diffusion technique was used to evaluate antimicrobial activity of the test organisms. As Dimethyl sulfoxide (DMSO) was served as the solvent for black seed oil, it was used as the negative control. Among the test organisms, staphylococci; MRSA, MSSA, S. pyogenes and C. albicans were sensitive to the oil. Both two strains of E.coli, P. aeruginosa were resistant to the oil while all test organisms were susceptible to the antibiotics used. The comparative study revealed that all three staphylococci are more susceptible to black seed oil than amoxicillin in tested concentration.**

***Keywords:-*** *Nigella Sativa; Black Seed Oil; Antimicrobial Activity; Antibitic Resistance.*

## I. INTRODUCTION

Emergence of resistance in pathogenic microorganisms to antibiotics that are used to treat serious infections causes considerably increased mortality [1]. After the introduction of antibiotics, the antibiotic resistance genes of both pathogens and commensals have been widespread throughout the environments like hospitals, communities etc. [2]. In addition, organisms have been acquiring multi-drug resistance during the past few years [3].Those multi-drug resistant bacteria can cause serious, incurable infections [4].

Novel alternative approaches should be introduced to face the challenge of antibiotic resistance. Development of new effective strategies against antibiotic resistant microorganisms can be used to delay the emergence of antibiotic resistance and as successful antimicrobials [5].

Pharmaceutical concerns invest resources for discovery programs of novel compounds with considerable antimicrobial activity [6]. There is a significant trend to determine the antimicrobial potential of untapped natural sources such as plants, animals and microorganisms for a new generation of antimicrobials [7].

Although there are 300,000 herbal species that present globally, only 15% plant species have been investigated for their pharmacological potential [8]. Among various plants that possess medicinal value, *Nigella sativa* L. (Black cumin or black seed in English) which belongs to the family Ranunculaceae has been considered as one of the most precious herb around the world [9]. Black cumin seed is widely used in traditional medicine for wide range of ailments [10].

This study was conducted with the aims of determining the antimicrobial effect of black seed oil against selected strains of *pathogenic bacteria and* yeast and to compare the antimicrobial efficacy with conventional antibiotics that are effective against the tested pathogens.

## II. MATERIALS AND METHODS

### *A. Sample Preparation*

- *Black seed oil test sample preparation*

Commercially available black seed oil (Manufacturer; Bio Extracts (Pvt) Ltd, Colombo 3, Sri Lanka) was mixed with DMSO (Dimethyl sulfoxide) to the ratio of (9:1) aseptically.

- *Microbial culture preparation*

*Pseudomonas aeruginosa* (ATCC 27853), Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin sensitive *Staphylococcus aureus* (MSSA), *Staphylococcus pyogenes* (ATCC 19615), *Escherichia coli (*ATCC 25922 and ATCC 35218) and clinically isolated *Candida albicans* were inoculated in sterilized test tubes containing Nutrient Broth separately and incubated overnight at 37ºC. After incubation, cultures were adjusted by adding sterilized

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peptone water until turbidity matched that of a McFarland 0.5 standard.

➢ *Antibiotic solution preparation*

- *Preparation of Amoxicillin*

Amoxicillin powder was dissolved in sterile distilled water aseptically to obtain the concentration of 10 mg/ mL

- *Preparation of Ciprofloxacin*

Ciprofloxacin powder was dissolved in sterile distilled water aseptically to obtain the concentration of 10 mg/mL

- *Preparation of Fluconazole*

Fluconazole powder was dissolved in sterile distilled water aseptically to obtain the concentration of 2.5 mg/mL

➢ *Inoculation of microbial lawns*

Aliquots (100 µL each) of turbidity adjusted broth cultures of *Pseudomonas aeruginosa*(ATCC 27853), Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin sensitive *Staphylococcus aureus* (MSSA), *Staphylococcus pyogenes* (ATCC 19615), *Escherichia coli (*ATCC 25922 and ATCC 35218) were pipetted out onto Mueller-Hinton agar plates separately and swabbed uniformly over the agar surface using sterile cotton swabs to obtain lawns.

100 µL aliquot of *Candida albicans* culture with adjusted turbidity was pipetted out onto Sabouraud Dextrose Agar and inoculated evenly as mentioned above.

*B. Screening antimicrobial activity of black seed oil and antibiotics*

Agar well diffusion method was used to determine the antimicrobial efficacy of black seed oil and antibiotics. Three wells (diameter 8 mm) were made on each inoculated agar plate using a sterile cork borer. Wells were labeled as "BSO", "+" and "-". 50 µL of black seed oil test sample was added to all wells labeled as BSO. Ciprofloxacin (50 µL; 10 mg/mL) was added to the wells that are named as "+" of *P. aeruginosa* inoculated agar plates. Amoxicillin (50 µL; 10 mg/mL) was added to the "+" labeled wells of the agar plates inoculated with Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin sensitive *Staphylococcus aureus* (MSSA), *Staphylococcus pyogenes* (ATCC 19615), *Escherichia coli (*ATCC 25922 and ATCC 35218). "+" wells of the *C. albicans* inoculated plates were added with fluconazole (50 µL; 2.5 mg/mL). All the wells named as "-" were filled with 50 µL aliquots of DMSO. The test was triplicated for all microorganisms used.

Agar plates were incubated at $37^0$C for 24 hours. Antimicrobial efficacy of black seed oil and antibiotics were evaluated using the inhibition zone diameter around the respective wells that indicated as clear zones. Average was taken from the all three diameters of each triplicate.

## III. RESULTS AND DISCUSSION

Bactericidal activity of Black seed oil was assessed for six strains of bacteria and one strain of yeast. The antimicrobial activity and the potency of black seed oil and antibiotics were quantitatively assessed by the presence or absence of inhibition zone and zone diameter. According to the obtained results, black seed oil was effective against all tested Staphylococci while it was unable to affect the growth of two *E.coli* strains and *P. aeruginosa*. The growth of the yeast was also affected by black seed oil. All strains used were susceptible to the respective antibiotics. DMSO which was used as the negative control did not exhibit any inhibition against the tested organisms. The results were recorded in Table 1.


Fig 1:- Sensitivity testing showing clear zones on Mueller Hinton Agar inoculated with MRSA

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| Organism | Inhibition zone diameter in mm | |
|---|---|---|
| | ***Black seed oil*** | ***Antibiotic*** |
| MRSA | 42.33±0.58 | 12.33±0.58 |
| MSSA | 37.67±0.58 | 28.27±0.58 |
| *S. pyogenes* | 38.33±0.58 | 34.33±0.58 |
| *P. aeruginosa* | Nil | 34.33±0.58 |
| *E. coli* (ATCC 25922) | Nil | 36.33±0.58 |
| *E. coli* (ATCC 35218) | Nil | 36.00±0.58 |
| *C. albicans* | 21.00±0.00 | 35.00±0.00 |

Table 1:- Antimicrobial activity of black seed oil and antibiotics

DMSO (Dimethyl Sulfoxide) was used as the solvent to dissolve Black seed oil. Therapeutic substances that are insoluble in water are probably soluble in DMSO and its high diffusibility [11] helps to penetrate the test sample through the agar medium. To check whether there is an effect of DMSO towards microbial growth, it was used as the negative control which confirmed that it does not affect the growth of tested organisms.

Black seed oil exhibited significant inhibitory action against MRSA compared to the standard amoxicillin. This result indicates that the antimicrobial action of black seed oil is not interfered with the antibiotic resistance. It might possess different modes of action regarding inhibition on test organism.

Although growth of Methicillin sensitive *staphylococcus aureus* (MSSA) was inhibited by both Amoxicillin and black seed oil, when considering the extent of inhibition, black seed oil exhibited higher capability of inhibition.

Clinically isolated *Candida albicans* strain was found to be sensitive to Black seed oil. However, quantitatively anticandidal activity of fluconazole was higher than that of black seed oil.

Both two *E.coli* strains showed no susceptibility to black seed oil. They were sensitive to Amoxicillin exhibiting approximately similar zone diameters.

*S. pyogenes* showed to be sensitive to both black seed oil and Amoxicillin. Inhibition zone diameter of black seed oil is larger than the diameter of the antibiotic which indicates higher inhibitory action of black seed oil on *S. pyogenes.*

*P. aeruginosa* (ATCC 27853) was found to be resistant to black seed oil while it was susceptible to ciprofloxacin.

Almost all antibiotics are secondary metabolites of soil bacteria and fungi [12].Evaluation of the potency of plant derived products is particularly relevant to the discovery of new antimicrobial drugs.

Antimicrobial activity of black seed oil may be due to the presence of active phytochemical substances such as thymol [13], thymoquinone [14] and thymohydroquinone [15] that is known to possess antimicrobial effectiveness.

## IV. CONCLUSION

Black seed oil possesses considerably higher antibacterial effect against MRSA, MSSA and *S. pyogenes* (ATCC 19615) than the tested antibiotics. It also affects the growth of clinically isolated *C. albicans* strain but the reference antibiotic is more effective. Black seed oil does not affect the growth of *Escherichia coli (*ATCC 25922 and ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 27853). Although black seed oil is used for typical application in traditional medicine, further studies should be carried out to evaluate the systemic use and safety of use.

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