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Calculation of the Zone of Inhibition and Sensitivity Pattern of *Fenugreek* Seeds Extract and *Negila sativa* Seeds Extract on ESBL *E. coli* Growth Plates

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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: The reason behind the poor outcomes in treating bacterial infections is mainly the limited treatment options. The strains of ESBL are generally resistant to broad-spectrum penicillin, monobactams, and third-generation cephalosporins. Literature is currently highlighting the efficacy of herbs against many resistant organisms. Hence, the study aims to identify the sensitivity pattern and zone of inhibition of *Fenugreek* seed extract and *Nigella sativa* Extract against *ESBL E. Coli.*
Methodology: The calculated sample size was n = 40. The *ESBL E. coli* growth plate samples were recruited from an associated lab which were identified by performing biochemical tests on appropriate media i.e. Mueller-Hinton agar was used. Agar dilution methods were performed to examine the antibacterial effects of fenugreek seed extract (FGSE) and *Nigella sativa* seed extract (NGSE) against ESBL *E. coli.* wells of 8mm diameter were punched in the inoculated plates by using sterile cork borer, 100µl of different concentrations of Fenugreek seed extract (30, 40, 50, 60,

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and 80mg/ml) and *Nigella sativa* extract (30, 40, 50, 60, and 80mg/ml) were added to wells and the plates and the plates were incubated for 24 to 48 hours at 37°C. After 48 hours presence or absence of zones of inhibition were investigated.
Results: Total 40 samples were calculated for each concentration of the solution of extracts i.e. FGSE group and NGSE group. The effective concentration of FGSE was found to be 40mg/ml and for NGSE was found to be 60mg/ml that showed maximum. Both the extracts showed inhibition of growth of organism at different concentrations table 2 shows the zone of inhibition.
Conclusion: The *Fenugreek seed* extract at 40mg/ml and *Nigella sativa* seed extract at 60mg/ml have highest sensitivity and are effective in inhibiting the growth of ESBL *E. coli* on growth plates.

Keywords: Fenugreek seed; Nigella sativa; sensitivity; ESBL E. coli.

1. INTRODUCTION

In the last two to three decades, infections caused by extended-spectrum β-lactamase (ESBL)- producing bacteria are significantly increasing over time. ESBLs can hydrolyze the oxyimino-β-lactam component of antibiotic drugs which is essential for their therapeutic effects in humans and animals, thus producing resistance [1]. Most ESBL infections spread through bodily fluids of an infected person, like blood, phlegm, or urine [2]. Contaminated surface contacts also contribute to the spread of ESBL-*E. coli*. The ESBL producing bacteria, especially *E. coli,* are frequently been reported in livestock, companion animals, and food chain [3]. The transmission of ESBLs through livestock animals to humans is now considered a potential threat. These ESBL-*E. coli* strains may cause various ailments among which the most common are community-acquired urinary tract infection, pneumonia, and septicemia [4]. The reason behind the poor outcomes is mainly the limited treatment options. The strains of ESBL are generally resistant to broad-spectrum penicillins, monobactams, and third-generation cephalosporins. Furthermore, a lot of ESBL-producing strains of *E. coli* show co-resistance to the other antimicrobial agents such as tetracycline, aminoglycosides, and fluoroquinolones [5].

Drugs that are stable against hydrolysis by ESBL are required to treat such infections produced by ESBLs [6]. Due to resistance produced by many broad-spectrum antibiotics and cephalosporins, the choice of drugs for ESBLs are carbapenems, including ertapenem, meropenem, and imipenem [7]. These drugs are reserved for less-typical moderate to severe infections showing resistance to other common antibiotics [8]. The bactericidal activity of carbapenems is a result of its binding to penicillin-binding proteins that inhibits bacterial cell wall synthesis [9]. The limitations of carbapenems use include that these drugs usually produce common adverse effects caused by other antibiotics such as pruritus, rash, injection site reaction, and diarrhea [10]. Another potential weakness of carbapenems use is the high cost of these antibiotics that may lead to loss of compliance [11].

In the last few years, many strains of carbapenems-resistant bacteria have been diagnosed. In 2017, the World health organization (WHO) ranked carbapenem-resistant *Pseudomonas aeruginosa*, carbapenem-resistant Enterobacteriaceae (CRE), and carbapenem-resistant *Acinetobacter baumannii* in the highest priority and critical category [12]. This urges the medical researchers to find out a novel, economical, and non-resistant alternative treatment for such infections. *Trigonella foenum-graecum* commonly known as *Fenugreek* is a traditional plant, is used in herbal medicines for a variety of ailments. Multiple studies have reported anti-parasitic, anti-diabetic, and especially antimicrobial effects of *fenugreek* seed extract [13]. The second plant, *Nigella sativa* is a bioactive plant, which is also one of the most extensively used medicinal plants due to its diverse pharmacological actions [14]. Plenty of literature is available on the pharmacological and biological effects of *Nigella sativa* seeds extract on neurological illness, diabetes, cancer, infertility, and cardiovascular diseases. It has been also in use for many parasitic, viral, bacterial, and fungal infections [15]. Therefore, the study aims to identify the sensitivity pattern and zone of inhibition of *Fenugreek* seed extract and *Nigella sativa* Extract against *ESBL E. Coli.*

2. METHODOLOGY

It was a preclinical experimental study conducted at Baqai Medical and Dental College Karachi., from July - November 2021. The calculated

sample size was n = 40. The *ESBL E. coli* growth plate samples were recruited from an associated lab which were identified by performing biochemical tests on appropriate media i.e. Mueller-Hinton agar was used. *Fenugreek* seed and *Nigella sativa* seeds were purchased from the local market and authentication numbers i.e. Specimen vouchers 53 and 96 were allotted. 1000-gram of both the seeds were soaked in 2500ml of 90% ethanol for 30 days after washing and grinding to powder. The filtrate was then filtered with Whitman filter paper that was further processed at 60^{o}C by using a water bath. The mixture was then dried at 50^{o}C until a well-concentrated extract was produced. The extract was kept in an airtight bottle and stored in a refrigerator till usage. The extracts were diluted in different concentration i.e. 20, 30, 40, 50 and 60 mg/ml of DMSO. Agar dilution methods were performed to examine the antibacterial effects of fenugreek seed extract (FGSE) and *Nigella sativa* seed extract (NGSE) against ESBL *E. coli.* wells of 8mm diameter were punched in the inoculated plates by using sterile cork borer, 100µl of different concentrations of FGSE (30, 40, 50, 60, and 80mg/ml) and NGSE (30, 40, 50, 60, and 80mg/ml) were added to wells and the plates and the plates were incubated for 48 hours at 37°C. After 48 hours presence or absence of zones of inhibition were investigated. A diameter scale was used to measure and compare the zones of inhibition. ANOVA followed by post hoc Tukey was applied as a test of significance, <0.05 p-value was considered as significant at 95% confidence interval.

3. RESULTS

Total 40 samples were calculated for each concentration of the solution of extracts i.e. FGSE group and NGSE group. The effective concentration of FGSE was found to be 40mg/ml and for NGSE it was found to be 60mg/ml table 1 shows the mean inhibitory concentration of both the seeds. Both the extracts showed inhibition of growth of organism at different concentrations table 2 shows the zone of inhibition.

4. DISCUSSION

Misuse of conventional antibiotics is an issue of serious concern these days. To tackle the rise of new infectious diseases, there is a need to find new antibacterial agents having novel properties. As plants are the major contributors to traditional medicines since ancient times, around the globe. That shows that these plants provide us with a wide range of chemical compounds having multiple biological activities [16]. But, in the last few decades, extensive work has been done on the specific antibacterial role of various plant compounds [17]. That is why there is a significant improvement in the extraction of novel plant chemicals that contribute to the elimination of various diseases caused by pathogenic bacteria.

Seeds, leaves, and roots of both the plants of our study, that are *Fenugreek* and *Nigella sativa*, have proven their medical importance in various studies as potential anti-inflammatory, antibacterial and anticancer agents [18]. Infections caused by extended-spectrum-β-lactamase producing *E. coli* is a serious issue of concern for many years, especially in immune-compromised patients [19]. The severe morbidity and increased mortality rate are directly connected to the delay in the treatment and depending upon already-resistant conventional antibiotics due to the presence of drug-resistant strains of *E. coli* [20]. In infections caused by ESBL-*E. coli*, the somehow effective antibiotics like carbapenems have a major issue of being uneconomical and are also associated with the major adverse effects of standard antibiotics [21].

Table 1. Sensitivity pattern of FGSE and NGSE against ESBL *E. Coli samples*

Concentration mg/ml	FGSE Sensitivity pattern	NGSE Sensitivity pattern
30	20	20
40	35	20
50	28	26
60	30	37
80	29	32

Table 2. Zone of inhibition identified at maximum sensitivity pattern

	FGSE	NGSE
Maximum Sensitive concentration	40 mg/ml	60 mg/ml
Zone of inhibition in mm	10.24 ± 1.3	12.16 ± 1.9

The results of our study report that the most effective concentration of *Fenugreek* seed extract (FGSE) is 40mg/ml out of various concentrations ranging from 30mg/ml to 80mg/ml. This concentration of 40mg/ml of FGSE shows a maximum sensitivity pattern that is 35. At this maximum sensitivity pattern, the zone of inhibition identified in our study is 10.24mm±1.3. In comparison, another study conducted at Sudan University of Science and Technology showed the mean of inhibitory zones of *Fenugreek* oil against *E. coli* (15.8mm), *K. pneumoniae* (15.1mm), and *P. aeruginosa* (15.1mm) that are quite significant. This study also reported the antibacterial activity of *Fenugreek* oil against standard isolates of *E. coli* that is *E. coli ATCC25922*. Against standard isolate, the zone of inhibition was reported 25mm.

The maximum sensitive concentration of our second plant, *Nigella sativa*, was recorded 60mg/ml among multiple concentrations ranging from 30mg/ml to 80mg/ml. The maximum sensitive pattern was recorded at 37 at the concentration of 60mg/ml. The mean of inhibitory zones was recorded at 10.24mm ± 1.3 which denotes a significant amount of antibacterial activity of *Nigella sativa* against *E. coli* strain.

A study done in Prince Sattam Bin Abdulaziz University, Saudi Arabia showed similar resistance by *E. coli* strains to multiple antibiotics including the drugs of choice for *E. Coli* infections, like imipenem and meropenem and also gentamicin. In the same study ethanolic extract of *Nigella sativa* seeds, activity was tested against multi drug resistant strain of ESBL-*E. coli* with multiple concentrations (10, 20, 30, 40 and 50µL). It was done by filter paper impregnation method, which showed dose-dependent antimicrobial activity. Out of various multi-drug resistant strains, *Nigella sativa* showed a 15.3mm±1.3 zone of inhibition against *E. coli* strain. The antimicrobial activity was also measured by the Cork Borer disk diffusion method. By this method, the concentration of 30µL showed a 25mm±1.3 inhibitory zone denoting a significant antibacterial activity by *Nigella sativa* against ESBL-*E. coli*. Same as the results showed a strong growth inhibitory activity of *Nigella sativa*, the minimum inhibitory concentration (MIC) for *E. coli* was recorded 6.25mg/ml against tobramycin (positive control) that was recorded as 25mg/ml [22].

Another study done in 2018 reported that the strains treated with ampicillin, cefuroxime, and levofloxacin showed 5mm, 7mm, and 6mm of inhibitory zones, respectively. Contrarily, when the same strains were treated with essential oil of *Nigella sativa*, they showed 11mm, 15mm, and 13mm of inhibitory zones showing a significant increase in growth inhibition of drug-resistant *E. coli* strains [23].

5. CONCLUSION

The Fenugreek seed extract at 40mg/ml and Nigella sativa seed extract at 60mg/ml have highest sensitivity and are effective in inhibiting the growth of ESBL E. coli on growth plates. Furthermore, the phytoconstituents of FGSE and NGSE should be evaluated for possible antibacterial properties against resistant bacteria.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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