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IN- VITRO ANTIBACTERIAL EFFICACY OF *NIGELLA SATIVA* AND FENUGREEK SEED EXTRACTS AGAINST *PSEUDOMONAS AERUGINOSA* AND *E. COLI*

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ABSTRACT

Background: *Pseudomonas aeruginosa* and *Escherishia coli* are virulent organisms, their multi drug resistant strains are become hallmark to treat. Various herbal remedies have shown antibacterial activity against both of these organisms out of them *Fenugreek* seed extract and *Nigella sativa* seed extracts have shown predominance.

Objective: To evaluate the efficacy at different concentrations of Fenugreek seeds and *Nigella sativa* extract against *Pseudomonas aeruginosa, E. coli* and their MDR strains and to compare their efficacy with Amikacin and Ciprofloxacin.

Methodology: It was an in-vitro cross sectional study, conducted in October – December 2019 after the ethical approval, in department of Microbiology, Baqai University Karachi. Different concentration of seed extracts was prepared and their antibacterial effects were observed on growth plate and efficacy was measured by zone of inhibition in millimeters.

Results: N. sativa when compared against fenugreek shown high efficacy at 3mg/ml concentration against both organisms nevertheless ciprofloxacin preserved greater (56mm) zone of inhibition in P. aeruginosa.

Conclusion: *N. sativa* Seed extract at 3mg.ml concentration have superior efficacy then fenugreek seed extract at either concentration when analyzed against *P. aeruginosa* and *E coli* but ciprofloxacin remained highly sensitive against *P. aeruginosa*.

Keywords: *Fenugreek*, *N. Sativa*, Comparative efficacy, control drugs

INTRODUCTION

Pseudomonas aeruginosa is a versatile bacterium metabolically, which is responsible for variety of opportunistic infections in people with underlying metabolic disorders. It causes soft tissue infections, UTI, bacteremia in immunocompromised patients, diabetic foot and respiratory infections **[1, 2]**. On the other hand, *E. coli* is the member of the strains which are known variously as enterohaemorrhagic, verocytotoxin-producing, or Shiga-toxin-producing organisms **[3]**, it is responsible for infections such as non-bloody and bloody diarrhea, UTI and hemolytic uremic syndrome which is responsible for acute renal failure **[4]**. Many therapeutic options were available to treat these vulnerable pathogens but due to development of resistance against antibiotics we are left with few options along with multi drug resistant strains of both of these bacteria **[5-8]**. Various researchers are trying to conquer these pathogens by intervening with new therapeutic options either medicinal **[9, 10]** or herbal.

Fenugreek seeds are used in folk medicines due to antibacterial, antifungal, gastric stimulant, dyslipidemic, and antidiabetic properties **[11]**. Antidiabetic anti-cholesteric properties of fenugreek are studied and proven to be highly significant however multiple studies have shown that fenugreek possess antibacterial and anti-cancer properties **[12]**. Its antibacterial activity has been checked against *Pseudomonas aeruginosa, E. coli* and other microorganisms which is proven significant and Minimum inhibitory concentration has been recorded significantly satisfactory in previous researches **[13-15]**. Furthermore, *Nigella sativa* commonly known as black seed is used in the herbal medicines for the treatment of asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza **[16]**. Despite its multi-dimensional role for various diseases its antibacterial properties are also evaluated and proven to be effective against *Pseudomonas aeruginosa* and *E. coli* **[17]**.

According to Global Surveillance Around 700,000 mortality rates are recorded worldwide each year and estimated to increase up to 10 million by 2050 **[9]**. Due to increase in resistance clinicians are facing deadlock in choosing a resistance less drugs to treat nosocomial infections around the globe **[18]**. In coming years, it is predicted that the next challenge to deal in medical sciences will be drug resistance rather than an epidemic disease **[19]**. This is the reason why researchers are looking for new medicine or alternative options to treat the various organisms **[20]**. Both the herbs individually are tested against these susceptible pathogens but their combined efficacy and comparison with conventional antibiotics has not been assessed yet. The objectives of our study are to evaluate the efficacy at different concentrations of *Fenugreek* seeds and *Nigella sativa* extract against *Pseudomonas aeruginosa, E. coli* and their MDR strains and to compare their efficacy with Amikacin and Ciprofloxacin.

MATERIALS AND METHODS

It was an in-vitro cross sectional study, conducted in October – December 2019 after the ethical approval, in department of Microbiology, Baqai University Karachi. The effect of the extract at different concentration and applied drugs were analyzed on six growth plates of each bacterium. Zone of inhibition was calculated in each plate in millimeters and compared with the commercially used drugs. Data was analyzed by using SPSS version 20. Anova test was applied to compare the inter group variation. For preparation of seed extract and growth of bacterial strains following methods were applied.

Preparation of Alcoholic Extract of Test Compound

Fenugreek and *Nigella sativa* seeds were purchased from local market of Karachi. Seeds were washed and dried under shelter for five to six days. Dried Seeds were placed in the oven at 38°C and then crushed to powder form with mortar and pestle. After crushing, 50 ml of methanol and 10 grams of each respective compound seed was mixed with vortex mixture. After 24 hours, methanol extracts were filtered using Whitman filter paper (No. 1) and dried at temperature below 45°C for methanol removal to obtain the dense extract and they were stored in sterile bottles for further testing. The stock preparation was done using 100mg/ml in methanol. Two commonly used Commercial antibiotic (Amikacin and Ciprofloxacin) discs were used as controls.

Growth of Bacterial strains

P aeruginosa (ATCC 27853) and *E. coli* (ATCC 25922) strains were cultured on blood Agar. Biochemical identification was done according to CLSI Guideline 2019. Inoculum was prepared in 5 mL Mueller Hinton broth (Oxoid, UK).

RESULTS

When analyzed for *P. aeruginosa* fenugreek extract at either concentration did not show significant findings the same was observed for *Nigella Sativa* extract at 2mg concentration however, when the later was compared at 3mg concentration with Amikacin it gave significant (p-value = 0.03) findings. Furthermore, no statistically significant findings were observed when ciprofloxacin was compared to seed extracts at different concentrations **(Table 1, Figure 1)**.

When zone of inhibition was evaluated for *E. coli*, *Fenugreek* extract when compared to control did not displayed significant results but intergroup comparison of *fenugreek* extract at 3mg/ml concentration gave relatively significant data when compared to 2mg/ml concentration of *Nigella sativa* seed extract. Intergroup comparison of *nigella sativa* 3mg/ml concentration was found to be significantly (p-value = 0.001) superior than control drugs **(Figure 2)**.

Table 1: Mean zone of inhibition at different concentrations of the seed extract and control drugs.

Test compound	Concentration (mg/Well)	Zone of inhibition (in mm) Test organisms for *P.aeruginosa*	Zone of inhibition (in mm) Test organisms for *E. coli*
Fenugreek	**1 mg**	**-**	**-**
(Trigonellafoenum-	**2mg**	**10.5 ± 3**	**8.1 ± 2.5**
graceum)	**3mg**	**14.5 ± 4**	**16.2 ± 4.5**
Nigella sativa	**1mg**	**-**	**-**
	2mg	**15.5 ± 3.2**	**12.5 ± 3.5**
	3mg	**28.0 +- 4.5**	**35.1 ± 5**
Amikacin		**24 ± 4**	**30 ± 5.5**
Ciprofloxacin		**56 ± 5**	**33 ± 4**

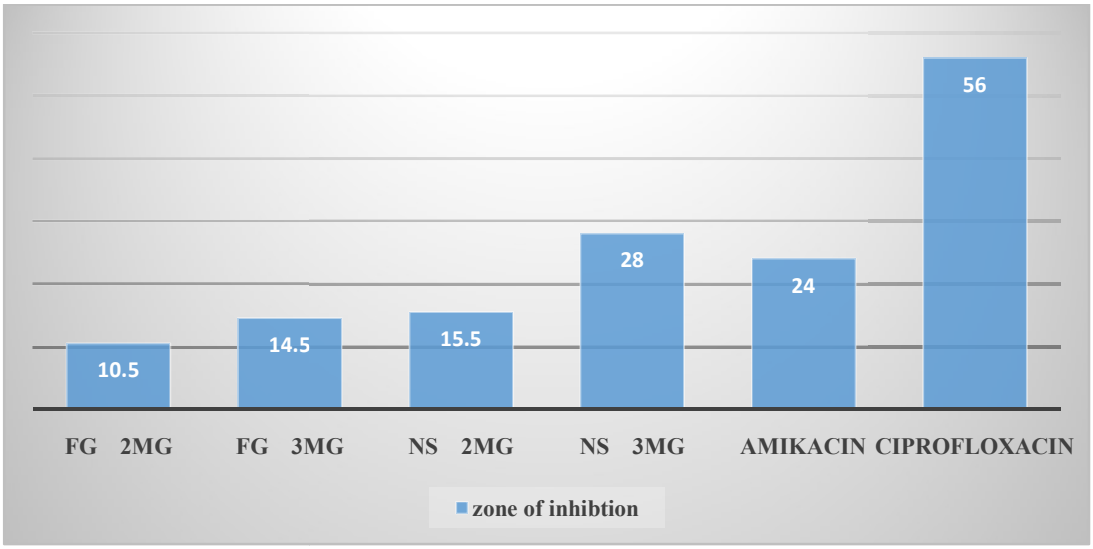

Figure 1: Mean zone of inhibition of *P. aeruginosa*

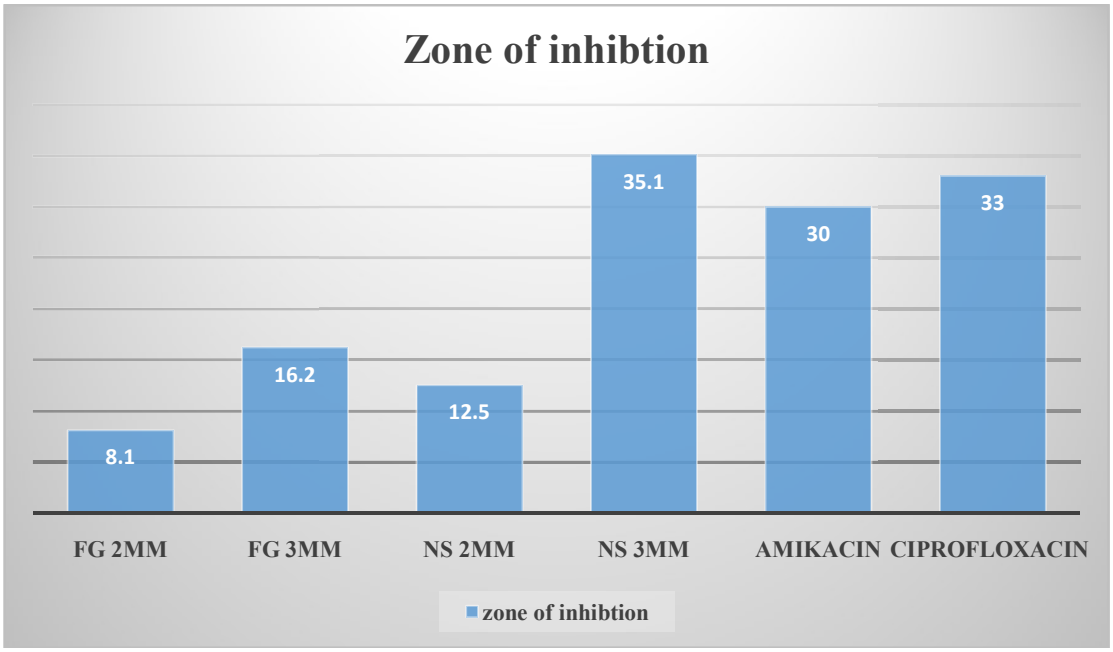

Figure 2: Mean zone of inhibition of *E. coli*

DISCUSSION

For treatment of life threatening infections antimicrobial drug resistance (ADR) is a challenging phenomenon. In our study *N. sativa* has shown significantly larger zone of inhibition when compared to fenugreek seed extract for *P. aeruginosa*, antibacterial efficacy of *N. sativa* against *P. aeruginosa* is found to be parallel with multiple studies **[21, 22]**. Furthermore, when analyzed individually fenugreek seed extract at 3mg/ml concentration also shown not

significant in terms of states but efficacy against *P. aeruginosa* which is in accordance to Jha *et al* **[23]**. Our study has highlighted that the antibacterial activity of N. sativa is superior than *Fenugreek* seed extract when compared against *P. aeruginosa*. When compared to Amikacin 3mg concentration of N. Sativa zone of inhibition for *P. aeruginosa* was significantly larger, though Ciprofloxacin remained highly sensitive when it was compared against amikacin and different concentrations of both the seed extracts. On the other hand, 3mg/ml concentration of *N. sativa* seed extract was found to have significantly (0.001) larger zone of inhibition (35.1 mm) when compared to other regimens against *E. coli. Fenugreek* seed extract and *N. sativa* seed extract both have shown sensitivity against *E.coli* which is similar to **[24, 26]**. In current scenario Chinese herbal medicines, Indian ayurveda and Arabic herbal remedies has shown promising results in treating various organisms **[27]**. Multiple studies are available in parallel to antibacterial efficacy of both mentioned herbal extracts the same contribution is by our study which has highlighted the superior efficacy *N. sativa* against both virulent organisms i.e. *P. aeruginosa* and *E. coli* however, the need of time is to formulate new drugs with appropriate concentrations from these herbal remedies to overcome the resistance of organisms against antibiotics along with that it is also recommended that animal based trial followed by clinical trials should be carried out to market the cheap medications to population.

CONCLUSION

N. sativa Seed extract at 3mg.ml concentration have superior efficacy then fenugreek seed extract at either concentration when analyzed against *P. aeruginosa* and *E coli* but ciprofloxacin remained highly sensitive against *P. aeruginosa*.

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