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IN VITRO SYNERGISTIC EFFECT OF ESSENTIAL OIL AGAINST PSEUDOMONAS AERUGINOSA.

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Abstract
Background*: Pseudomonas aeruginosa* is one of the leading causes of nosocomial infections and is associated with high mortality. Infections caused by *Pseudomonas* are difficult to treat as the repertoire of useful antipseudomonal agents is limited. Moreover, *Pseudomonas* exhibits remarkable abilities to acquire resistance to these agents.
Method*:* In vitro synergistic effect of *Cuminum cyminum* (*C. cyminum*) essential oil (EO) against *Pseudomonas aeruginosa* PAO1. The *C. cyminum* EO was used for determination of minimum inhibitory activity (MIC) and synergistic effect of *C. cyminum* EO and chloramphenicol against *P. aeruginosa* PAO1.
Results*:* The present study investigated the *C. cyminum* EO increasing the antibacterial efficacy at the level of 6.25%. Similarly, Chlorombinical alone inhibited the growth of bacteria at the concentration of 0.6mg/ml. The combination between *C. cyminum* EO (0.097%,v/v) and Chlormbinical (0.1mg/ml) effectively inhibited the growth of *Pseudomonas. aeruginosa.*
Conclusion*:* According to the study's findings, P. aeruginosa-caused bacterial infections can be treated with a combination of C. cyminum (EO) and antibiotics.
Keywords: *Pseudomonas aeruginosa*,Chloramphenicol, *Curminum cyminum, Essential oil, Synergistic effect,* environment.
Running Title*: Evaluate the synergistic effect essential oil*
Type of study*: In vitro synergistic effect of essential oil against Pseudomonas aeruginosa*

INTRODUCTION:
Pseudomonas aeruginosa, also known as P. aeruginosa, is a saprophytic, adaptable bacterium that can cause infections like cystic fibrosis. Chronic pulmonary colonization with mutants of P. aeruginosa that produce mucoid alginate is a major cause of morbidity and mortality and is a fascinating example of host-bacteria interaction and microbial adaptation (Bouhdid et al., 2010).According to Karlowsky et al. (2005), pseudomonas aeruginosa resistance to antibiotics is a significant therapeutic concern. P. aeruginosa may exhibit chromosomal or plasmid-mediated antibiotic resistance. Changes to the intracellular environment or cellular membrane may potentially impact resistance to P. aeruginosa. Primarily a nosocomial, P. aeruginosa typically colonizes urine and respiratory secretions (Frank, 2012).
Every year that goes by, the treatment of infectious diseases gets harder. This is particularly true for infections brought on by Pseudomonas aeruginosa, an opportunistic organism that can quickly acquire resistance to a variety of antibiotic classes (Jones et al., 2005; Frank, 2012). The most challenging issue with P. aeruginosa is its potential to quickly build resistance during the course of treating an infection, despite the fact that the import of resistance mechanisms on mobile genetic elements is always a problem (Frank, 2012; Hauser and Rello, 2012). Particularly pertinent to this therapeutic challenge are the multidrug efflux pumps, the outer membrane porin OprD, and the chromosomally encoded AmpC cephalosporinase (Karlowsky et al., 2003).

The essential oil Curminum cyminum, often known as C. cyminum, is an annual plant that has a variety of medicinal, nutraceutical, and pharmacological qualities. It is also one of the most widely used seed species and one of the oldest and most cultivated fragrant and herbaceous natural products. It is extensively utilized in the food, beverage, pharmaceutical, toiletry, and perfume sectors (Chamkouri et al., 2021). Many bacterial infections can be effectively treated with the antibiotic chloramphenicol.This involves using as an ointment to the eyes to relieve conjunctivitis. It is taken orally or intravenously to treat cholera, typhoid fever, meningitis, and plague.It is only advised to administer it intravenously or orally when safer antibiotics are not an option (Weber, 1968).Our team has extensive knowledge and research experience that has translate into high quality publications(Krishnamurthy *et al*., 2009; Abdul Wahab *et al.*, 2017; Eapen, Baig and Avinash, 2017; Ravindiran and Praveenkumar, 2018; Subramaniam and Muthukrishnan, 2019; Anita *et al*., 2020; Kumar *et al.*, 2020; Rajasekaran *et al*., 2020; Arumugam, George and Jayaseelan, 2021; Dhanraj and Rajeshkumar, 2021)

The ambition of this study was to generate the in vitro synergistic effect of essential oil against *Pseudomonas aeruginosa*.

MATERIALS AND METHOD:

Bacterial culture and culture conditions

Florida University in the United States' Dr. Kalai Mathee gifted P. aeruginosa PAO1. Luria Bertani (LB) broth was used to subculture the culture, and the tubes were incubated for 24 hours at 370C.

Essential Oil

The original agents used in this study were: essential oil of Cumin (*C. cyminum*) and antibiotic Chloramphenicol. *C.cyminum* was extracted from AROMA

Minimum inhibitory concentration:

Briefly put, the test oils were vortexed in room temperature sloppy agar to create successive two fold dilutions (10% - 0.01 %, v/v). Using P. aeruginosa PAO1, a sterile 96-well microtiter plate was prepared. Similarly, each well loaded Luria bertani broth and at different concentrations of antibiotic suspension were prepared and loaded into the respective wells. Overnight culture of *P. aeruginosa* was loaded into the wells. The dish was incubated at 37^0C for 24 h. Each well was filled with the antibiotic and essential mixture, and the plate was incubated for 24 hours at 370C. Following a 24-hour incubation period, 10 µl of TTC (1 mg/ml) solution were added, and the plates were then incubated for 20 minutes at 370C.

RESULT AND DISCUSSION:

The addition of control strain in three rows of micro wells namely A, B and C, where A shows presence of *Cuminum cyminum*, B shows presence of combination of *C. cyminum* and Chloramphenicol and C shows presence of Chloramphenicol (Figure1). The minimum inhibitory concentration was calculated for each of the components and the inferences were drawn and tabulated below in table 1. The present study investigated the *C. cyminum* EO increasing the antibacterial activity has a concentration of 6.25%, v/v. Similarly, Chlorombinical alone inhibited the growth of bacteria at the concentration of 0.6mg/ml. The combination between *C. cyminum* EO (0.097%,v/v) and Chlormbinical (0.1mg/ml) effectively inhibited the growth of *P. aeruginosa* thereby the synergistic effect of P.aeruginosa and Chloramphenicol showed better control of growth of *P. aeruginosa*. According to Pereira et al the minimum inhibition con- centration (MIC), can be defined as the minimum concentration of antimicrobial agent that is able to inhibit the visible bacterial growth after one incubation period of 18–24 h(Pereira *et al.*, 2014). This method has been used and is considered a fundamental instrument for the determination of susceptibility of microorganism to antimicrobial agents.In our study the MIC was 6.25%, but in study conducted by Gilles et al,the value of MIC ranged between 625 μg/mL and 2500 μg/mL for the organism *P.aeruginosa(Gilles et al., 2010)* .Similar values were obtained in an earlier study with methanolic and dichloromethane extracts from Eucalyptus camaldulensis and Euca- lyptus torrelliano leaves against *P. aeruginosa*, being 313.0 μg/mL the minimum value of MIC observed (methanolic extract of E. calam- dulensis) and 2500.0 μg/mL (methanolic extract of E. torrelliano) the highest(Tiwari *et al.*, 2017) . In other previous studies ethanolic and hexane extracts of *E. olida* and *E. staigeriana* leaves were more effective against *P. aeruginosa* than the ones used in the present study, with MIC values of 62.5 μg/mL–250.0 μg/mL for the methanolic extracts and of 250.0 μg/mL–313.0 μg/mL for the ethanolic extracts (Dupont et al., 2006). However, in the study of Badrunnisa et al. with ethanolic and methanolic extracts of E. tereticornis, tested against *P. aeruginosa*, the values obtained were higher for the methanolic extract (10,000 μg/mL) and lower for the ethanolic extract (2000 μg/mL). On the other hand, in other previous studies that evaluated several bacterial species, the value obtained was lower obtaining results of MICs for the aqueous extract against *P. aeruginosa* of 32 μg/mL . In the study of Niranjan et al. that tested the activity of E. globulus leaves extracts (n-hexane) against two Gram-positive bacteria, were reported MIC values ranging between 30 and 80 μg/mL(Niranjan, Koushal and Jain, 2017),being 313.0 μg/mL the minimum value of MIC observed (methanolic extract of E. calam- dulensis) and 2500.0 μg/mL (methanolic extract of E. torrelliano) the highest(Tiwari *et al.*, 2017) . The MIC values for the methanolic extract of E. torrelliano and E. calam-dulensis were found to be 2500.0 μg/mL and 313.0 μg/mL, respectively (Tiwari et al., 2017). The ethanolic and hexane extracts of E. staigeriana and E. olida leaves were found to be more effective against P. aeruginosa in other studies. The MIC values for the methanolic extracts were 62.5 μg/mL–250.0 μg/mL, while the ethanolic extracts were 250.0 μg/mL–313.0 μg/mL (Dupont et al., 2006). However, when ethanolic and methanolic extracts of E. tereticornis were tested against P. aeruginosa in a study by Badrunnisa et al., the results showed that the methanolic extract (10,000 μg/mL) produced higher values than the ethanolic extract (2000 μg/mL). Conversely, yet, in In other earlier research that assessed many bacterial species, the MIC values for the aqueous extract against P. aeruginosa were found to be 32 μg/mL, which was a lower value. The MIC values of E. globulus leaf extracts (n-hexane) against two Gram-positive bacteria were found to range from 30 to 80 μg/mL in the study conducted by Niranjan et al. (Niranjan, Koushal and Jain, 2017).

Table1*:* *C. cyminum* EO increasing the antimicrobial activity at the concentration of 6.25%, v/v. Similarly, Chlorombinical alone inhibited the growth of bacteria at the concentration of 0.6mg/ml. The combination between *C. cyminum* EO (0.097%,v/v) and Chlormbinical (0.1mg/ml)

Table 1:

Components	Organism (*Pseudomonas aeruginosa)*	Minimum inhibitory concentration
Essential oil	Cuminum cyminum	6.25%
Antibiotic	Chloramphenicol	0.6 mg/ml
Antibiotic + Essential oil	Cuminum cyminum+ Chloramphenicol	0.097%, 0.1 mg/ml

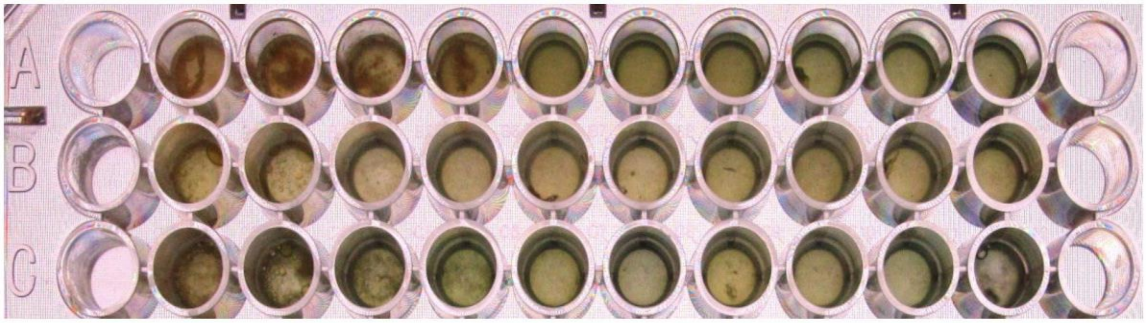

Figure1: The addition of control strain in three rows of micro wells namely A, B and C, where A shows presence of *Cuminum cyminum*, B shows presence of combination of *C. cyminum* and Chloramphenicol and C shows presence of Chloramphenicol.

In our study, the MIC was lowest when *C.cyminum* and Chloramphenicol were added together. In previous studies conducted by Sankar Ganesh et al, All of the plant extracts that were first screened for antimicrobial activity reduced the growth of the tested bacterial species. The generation of violacein in C. violaceum (CV12472) has been considerably inhibited by the methanol extract of T. bellerica (0.0625e0.5 mg/ml) at the sub-minimum inhibitory concentration (Ganesh, Sankar Ganesh and Ravishankar Rai, 2015).

CONCLUSION:

In the current study, the MIC value was highest for *Cuminum cyminum* and lowest for antibiotic chloramphenicol. However, the combination of both essential oil and antibiotic shows the least MIC of 0.097% hence showing synergism. From all these inferences, *Pseudomonas aeruginosa* can be controlled with the synergistic effect of *Cuminum cyminum* and chloramphenicol. However, more research is required to determine which antimicrobial substances in the EO are responsible for the synergistic impact so that in vivo investigations can proceed in the future.

AUTHORS CONTRIBUTION:

PSG designed the study. SK did all the experiments. The data collection was done by PSG and SK. SK wrote the manuscript and it was edited and revised by PSG. SK, PSG, ASSG and JVP approved the submission of the manuscript.

CONFLICT OF INTEREST:

The authors declared that there is no conflict of interest.

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