SYNERGISTIC STUDIES OF PEPTIDE, PLANT EXTRACT AND GRAM POSITIVE AND GRAM NEGATIVE BACTERIA.

A Thesis Submitted For the partial fulfillment for the award of degree of Bachelors of Biotechnology

By

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CERTIFICATE
Candidate's Declaration

We hereby declare that the project work entitled "**SYNERGISTIC STUDIES OF PEPTIDE, PLANT EXTRACT AND GRAM POSITIVE AND GRAM NEGATIVE BACTERIA**", submitted to Jaypee University of Information Technology, Waknaghat, Solan is a record of original work done by us under the guidance of Dr. Gopal Singh Bisht, Associate Professor, Department of Biotechnology and Bioinformatics JUIT Solan India. This project work is submitted in the partial fulfillment of the requirements of the reward of the degree of Bachelor of Technology in Biotechnology. The results embodied in this thesis have not been submitted to any other university or institute for the award of any degree or diploma.

Sakshi Gupta, 151808

This is to certify that the above statement made by the candidates is true to the best of my knowledge.

Dr. Gopal Singh Bisht, MSc, Phd

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DECLARATION OF SCHOLAR

I hereby declare that the project titled "**SYNERGISTIC STUDIES OF PEPTIDE, PLANT EXTRACT AND GRAM POSITIVE AND GRAM NEGATIVE BACTERIA**" submitted towards fulfilment for the award of degree of Bachelor of Technology in Biotechnology from Jaypee University Of Information Technology is based on the results of studies carried out under the supervision of Dr. Gopal Singh Bisht. This work, in part or in whole, has not been submitted anywhere else for award of any degree or diploma. I am responsible for the contents of this report.

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Abstract

There is an alarming increase in antimicrobial resistance worldwide and India is also facing this similar problem. The high accessibility of antibiotics over the country has majorly contributed in the high antimicrobial resistance observed. Although therapeutic industries have produced a number of new antibiotics in the last few decades, still bacteria manage to attain resistance against these drugs. Such facts are cause for distress, because due to new multi- resistant microorganisms number of patients in hospitals have repressed immunity. Synergistic studies are one of the approach used to deal with antimicrobial resistance. So in this we have used combination of peptide with antibiotics and antibiotics with various plant extracts for their antimicrobial synergy against gram negative and gram positive bacteria.

In this project we have used different antibiotics (gentamycin, vancomycin and ampicillin) and synthetic peptide (O3S) along with combination of different plant extracts (*Cestrum nocturnum* and *Witharnia somnifera*) to check whether they show synergistic activity against microorganism. As in synergistic response, the combination of antibiotics work together to produce more effective response than the each antibiotic produce individually.

Chapter 1

1.1 Introduction

Antimicrobial resistance may be adaptive or developed. Intrinsic resistance implicates the capability of the pathogen to resist the antibacterial treatment because of intrinsic basic or practical properties, while adaptive resistance denotes the ability of the bacteria to adjust to non-lethal conditions by rapidly modifying their transcriptomes in response to a traumatic environment (Natalia et al., 2017).

Antimicrobial peptides (AMPs) are short peptic particles produced by most living organisms. They help unicellular creatures to effectively contend for nutrients with other living beings sharing their natural niche, while AMPs are structural part of the resistant framework of multicellular animals. All AMPs share basic characteristics, such as small size, with cationic and hydrophobic arrangements inside a straight or cyclic structure. They belong to a large class of antimicrobial compounds that are active at low pH (Martin I et al., 2018). AMPs can repress or on the other hand annihilate microorganisms at micro molar fixations, frequently by non-explicit mechanisms; henceforth the resistance to these antimicrobials is uncommon. Besides, AMPs can inhibit or annihilate anti-biotic resistant microscopic organisms, such as *Acinetobacter baumannii* and the methicillin-safe *Staphylococcus aureus*. In the close future AMPs, due to their properties, can be represented as an alternative to antibiotics to control pathogenic microorganisms and keep up the current human life anticipation.

Discovery of antibiotics was a fundamental part in battling bacterial diseases that once wrecked mankind. Distinctive anti-toxins practice their inhibitory action on diverse pathogenic life forms. The improvement and spread of opposition to at present accessible anti-infection agents will be an overall concern. The expanding wonder of securing of opposition among microorganisms to antimicrobial drugs is credited to the aimless and ill-advised use of current antimicrobial drugs. Today, clinically critical microscopic organisms will be portrayed not just by single medication obstruction, however likewise by numerous anti-infection obstruction - the inheritance of past decades of antimicrobial use and abuse. Medication obstruction presents an ever expanding

worldwide wellbeing risk that includes all major microbial pathogens and antimicrobial drugs. These will be troublesome to treat and will be dependable for an assortment of irresistible sicknesses. For over a decade, the pace of advancement of new antimicrobial specialists has backed off while the commonness of obstruction has developed at a galactic rate. The rate of development of anti-toxin safe microscopic organisms is not coordinated by the rate of advancement of new anti-toxins to battle them.

Antibiotics that work today may not work tomorrow. It will be basic to examine more up to date drugs to which there will be lesser opposition. As opposition to old anti-infection agents spreads, the improvement of new antimicrobial operators has to be assisted if the issue will be to be contained. Anyway, the past record of quick, broad rise of opposition to recently presented antimicrobial operators demonstrates that even new families of antimicrobial specialists will have a short life hope. The relentlessly expanding bacterial obstruction to existing drugs will be a genuine issue, and in this manner there is a critical need to look for new classes of antibacterial substances, particularly from normal sources. Not at all like engineered drugs, will antimicrobials of plant source be not related with side impacts and have an extraordinary helpful potential to mend numerous irresistible infections. Some of the time the use of single anti-microbial does not produce the wanted viable inhibitory impacts and to defeat this, a blend of drugs frequently practices their synergistic impact which outperforms their individual execution. The synergistic impact may be due to certain complex arrangement which moves toward becoming more compelling in the hindrance of a specific species of microorganisms either by restraining the cell divider combination or by causing its lyses or passing.

Antibiotics will be generally characterized as regular mixes, created by microorganisms, with particular antibacterial movement that does not have any solid side impacts on human. Their mechanism of activity is either through killing the microbes (bactericidal impact) or by hindering bacterial development (bacteriostatic impact). The revelation of anti-infection agents had destroyed the diseases that once attacked mankind. Be that as it may their unpredictable use has driven to the improvement of multidrug-safe

pathogens. Around 90– 95% of Staphylococcus aureus strains overall will be safe to penicillin and in most of the Asian nations 70– 80% of the same strains will be methicillin safe. The presentation of penicillin cleared the path for the investigation of different regular mixes, with distinctive targets in the bacterial cell. Penicillin assaults microorganisms by repressing the cell divider biosynthesis, making the cell divider a powerless spot and causing cell lysis. Other substances target diverse destinations inside the microbes and have distinctive impacts counting hindrance of DNA replication, RNA blend and protein synthesis. Different tranquilize opposition in human pathogenic microorganisms has been created due to unpredictable use of commercial antimicrobial drugs usually utilized in the treatment of irresistible infections. The improvement of anti-toxin obstruction will be multi factorial, including the explicit nature of the relationship of microscopic organisms to anti-infection agents, the use of antibacterial specialist, have qualities what's more, ecological factors. This circumstance has constrained researchers to scan for new antimicrobial substances from different sources as novel antimicrobial chemotherapeutic specialists, however the cost creation of manufactured drugs will be high and they produce unfriendly impacts contrasted with plant determined drugs.

These antimicrobial substances will be of characteristic beginning, and it will be thought that their impacts on the condition will be few and can be utilized as organic control specialists. Anyway, some therapeutic herbs for some reasons have not found more extensive application and some of the time will be alluded as 'overlooked plants'. Indeed in spite of the fact that pharmacological businesses have delivered a number of new anti-infection agents in the last three decades, obstruction to these drugs by microorganisms has expanded. In general, microscopic organisms have the hereditary capacity to transmit and get opposition to drugs, which will be used as remedial specialists.

The discovery of penicillin about 90 years back altered the treatment of bacterial ailment. Since that time, various other anti-infection agents have been found from microscopic organisms and growths, or created by substance blend and have turned out to be viable chemotherapeutic choices. Anyway, the abuse

of anti-toxins has diminished the adequacy of numerous usually utilized anti-infection agents (Matthew J. et al., 2017).

The rise of safe strains of microscopic organisms has truly restricted our capacity to treat bacterial ailment, and new anti-infection agents are frantically required. Since the disclosure of penicillin, most anti-microbial improvement has concentrated on the revelation of new anti-infection agents got from microbial sources, or on the combination of new mixes utilizing existing anti-toxin platforms to the inconvenience of other lines of disclosure. Both of these techniques have been productive. Surely, the number of recently created anti-infection agents has diminished drastically in ongoing years. Rather, a reevaluation of customary meds has progressed toward becoming more normal and has as of now gave a few new anti-microbials. Customary prescription plants will be likely to give further new anti-infection agents in the future. However, the use of plant separates or unadulterated normal mixes in mix with regular anti-microbials may hold more noteworthy guarantee for quickly giving moderate treatment choices. Surely, some combinational anti-infection treatments will be as of now clinically accessible. This ponder audits the late writing on combinational anti-toxin treatments to feature their potential and to control future look into in this field (Matthew J. et al., 2017).

The revelation of various peptide anti-infection agents has come about in a novel territory of inquire about into antimicrobial specialists. In specific, ribosomally orchestrated (normal) peptides, due to their antimicrobial power, may speak to a novel remedial approach for the treatment of contaminations. Antimicrobial peptides are delivered in nature as a major segment of the normal have resistance atoms of a wide go of creatures, plants and bacterial species. Studies have exhibited that this bunch of peptides has a wide range of antibacterial movement, the site of activity of which will be the cytoplasmic layer. Different systems have been proposed to clarify the mode of activity of these mixes on the layers of microorganisms. These systems 2incorporate the restricting of monomers to the layer and inclusion into the film to structure a particle channel pore that ranges the layer; and the cover model in which peptide atoms soak the surface of the film earlier to broadly disturbing the porousness obstruction. The deadly occasion

that happens at the cytoplasmic layer will be not completely comprehended; be that as it may, it has been shown that peptides cause direct development in the cytoplasmic layer, coming about in cell demise. In expansion, it has been indicated that peptides permit maximal section of a few hydrophobic substrates into the cell and apply synergistic impacts with lipophilic and amphiphilic specialists, including rifampin, macrolides,and novobiocin.

Chapter 2

Aim

Following is the aim of this project work:

- To collect the plant *Cestrum nocturnum* and *Withernia somnifera.*
- To obtain plant extract using different extraction techniques.
- To determine the minimum inhibitory concentration.
- To do the synergistic study of synthetic peptide.

Chapter 3

Review of literature

Broad writing survey was taken to gain knowledge into the work done by different scientists.

Rosato *et.al. 2018* - Mentha piperita L. basic oil (EO) was utilized for outside use as antipruritic, astringent, rubefacient and antiseptic. A few investigations exhibited its critical antiviral, antifungal and antibacterial properties. The point of this work was the investigation of the synergistic impacts of M. piperita EO with antibacterials and antifungals that are generally accessible and as of now recommended in treatments against infections. The observed solid cooperative energy may comprise a potential new way to deal with counter the expanding marvel of multidrug resistant microscopic organisms and growths. In vitro viability of the affiliation M. piperita EO/drugs was assessed against an expansive board of Gram-positive and Gram-negative microorganisms and yeast strains. The antimicrobial impacts were contemplated by checkerboard microdilution strategy. The synergistic impact of M. piperita EO with gentamicin brought about a solid development restraint for all the bacterial species under investigation. The synergistic impact watched for M. piperita EO and antifungals was less articulated.

***Priscila Gava Mazzola et.al. 2009* -** Behavior of selected microorganism was studied and compared, they were submitted to insignificant inhibitory focus (MIC). The MIC interims, which decreased microorganisms populaces more than 6 log10, were: 59 to 156 mg/L of quaternary ammonium mixes (QACs); 63 to 10000 mg/L of chlorhexidine; 1375 to 3250 mg/L of glutaraldehyde; 39 to 246 mg/L of formaldehyde; 43750 to 87500 mg/L of ethanol; 1250 to 6250 mg/L of iodine in polyvinyl-pyrolidone edifices, 150 to 4491 mg/L of chlorine-discharging operators (CRAs) and 469 to 2500 mg/L of hydrogen peroxide. Chlorhexidine indicated non inhibitory action over growing spores. A. calcoaceticus indicated protection from most of the operators tried, trailed by E. cloacae and S. marcescens.

Sangeetha *et.al.* 2014 – *in vitro* activity of antibiotics was determined, including arbekacin, cefminox, fosfomucin, and biapenem which are still unavailable in India, against gram negative clinical isolates. In general 925 separates were incorporated; 211 E. coli, 207 Klebsiella spp., 153 P. aeruginosa, and 354 Acinetobacter spp. The MIC50 and MIC90 were

high for cefminox, biapenem and arbekacin for all pathogens yet interpretative criteria were not accessible. The MIC50 was ordered as vulnerable for two or three anti-infection agents, including piperacillin/tazobactam, carbapenems and amikacin, for E. coli, Klebsiella spp. what's more, P. aeruginosa. In any case, for Acinetobacter spp., the MIC50 was sorted as helpless just for colistin. Then again, fosfomycin was the main anti-microbial that repressed 90% of E. coli and Klebsiella spp. confines, while 90% of P. aeruginosa secludes were restrained just by colistin. At long last, 90% of Acinetobacter spp. segregates were not restrained by any anti-toxin tried.

Kogah H *et.al.* 2014 – New developments were done for improving the understanding of both MIC and MFC as endpoints of antifungal susceptibilities, and for standardizing methods for determining the MFC. In this overviewing of MFC and MIC was done for tropical drugs as endpoints of antifungal susceptibility and a novel test was described based on the standardized broth micro dilution method combined with the trans-well system and neutral red, which was recently developed in their laboratory for directly measuring the MFC.

Bing Li *et.al.* 2018 - An aggregate of 197 M. abscessus strains were isolated from sputum and bronchoalveolar lavage liquid samples amid the period from January 2012 to December 2016. Of these, 163 strains were Mycobacterium abscessus subsp. abscessus and 34 strains were Mycobacterium abscessus subsp. massiliense. The MICs of bedaquiline against M. abscessus clinical secludes went from 0.007 to 1 mg/liter, with a MIC50 and MIC90 of 0.062 and 0.125 mg/liter, separately. This outcome proposed that bedaquiline showed a high in vitro executing action against M. abscessus confines**.**

Meredith A. Hackel *et.al.* - Disk diffusion and MIC quality control (QC) ranges were resolved for nafithromycin, another lactone-ketolide, following the consummation of a nine-research center, Clinical and Lab Guidelines Establishment (CLSI) record M23-characterized level 2 contemplate. Five QC strains consistent with the range of movement of nafithromycin were tested: Staphylococcus aureus ATCC 25923 (plate just), S. aureus ATCC 29213 (juices just), Enterococcus faecalis ATCC 29212 (soup just), Streptococcus pneumoniae ATCC 49619 (circle and juices), and Haemophilus influenzae ATCC 49247 (plate and juices). Nafithromycin plate dissemination QC ranges were resolved to be 25 to 31 mm for S. aureus ATCC 25923, 25 to 31 mm for S. pneumoniae ATCC 49619, and 16 to 20 mm for H. influenzae ATCC 49247. Nafithromycin MIC QC ranges were resolved to be 0.06 to 0.25

μg/ml for S. aureus ATCC 29213, 0.016 to 0.12 μg/ml for E. faecalis ATCC 29212, 0.008 to 0.03 μg/ml for S. pneumoniae ATCC 49619, and 2 to 8 μg/ml for H. influenzae ATCC 49247.

Koeth LM *et.al.* 2017 - This study was directed to decide the impact of testing parameters on the in vitro movement of gepotidacin, another triazaacenaphthylene antibacterial specialist for *the treatment of customary and biothreat pathogens. CLSI strategies, and varieties of those* techniques, were utilized to test 10 Staphylococcus aureus, 10 Streptococcus pneumoniae, 10 Haemophilus influenzae, and 5 Escherichia coli disconnects by MIC and 30 S. aureus, 15 S. pneumoniae, and 15 S. pyogenes detaches by plate dispersion (DD) strategies. Levofloxacin and linezolid were tried as comparator operators for MIC and DD techniques, individually. Soup microdilution (BMD), macrodilution (MD), and agar dilution (Promotion) strategies were thought about. Variations in media, temperature, brooding time, CO2 level, and inoculum fixation were tried by all strategies, and variation in pH, calcium, magnesium, zinc, potassium, thymidine, and polysorbate 80 levels were tried by BMD and DD. No distinctions in MIC results among the three broth manufacturers were observed. The outcomes for telithromycin, the control specialist, were within the CLSI control limits for each QC living being tried on every day of testing.

Chapter 4

Materials and methods

3.1 Materials

Table 1: Chemicals/Reagents required

S.No.	Name of chemical	Source/Company
1.	Nutrient agar (NA)	Himedia
2.	Nutrient broth (NB)	Himedia
3.	Luria broth (LB)	Himedia
4.	Ethanol	Sigma chemicals
5.	Vencomycin	Auromedics
6.	Gentamycin	Intogen
7.	Ampicillin	Ampiwell
8.	O3S peptide	JUIT repository

Table 2: Apparatus and equipments required

S.No.	Apparatus and equipments
1.	Shaking incubator
2.	Spectrophotometer
3.	96 well plates
4.	Petri plates

3.1.1. Microorganisms required

E.coli, *Salmonella typhi* and *S.aureus* were obtained from JUIT repository.

3.2 Methods

3.2.1. Plant extraction

Fresh sample of *Cestrum nocturnum* and *Withernia somnifera* plants were collected. Then the sample was washed using tap water and then dried in an oven for 4 hours at 44.5°C. Dried leaves were grinded in a small grinder to lower the particle size so that surface contact between sample and solvent will be increased (Azwanida et al., 2015). Powdered leaves transferred to a glass sealed can and placed in refrigerator. Then two ways were opted for making plant extract.

1. 5g of leaves powder were blended in 50ml of distilled water. Mixture was taken into 250ml Erleand theeyer flask, then sterile cotton was used to plug it and kept in shaking incubator for 24 hours at 150 rpm. Muslin cloth was used to filter the extract. Than process was repeated (Raja Nagappan et al., 2012).
2. Hot water extract 10g weighed plant leaves powder was soaked in 100ml boiled water. Mixture was boiled for 30 min into a conical flask and put for 24 hours. Then extract was filtered (Tushar Dhanani et al., 2017).

3.8. Antimicrobial assay

3.8.1. Agar well diffusion method

Agar well diffusion method was performed by spreading 50 ml of diluted inoculum of test organism (*S.typhi* and *S.aureus*) all over the nutrient agar (NA) plates. Then 3 wells of diameter 1 cm each were punched into the agar plate for different concentration of plant extracts (*Withania somnifera* and *Cestrum nocturnum*). Ampicillin was used as positive control. Then the plates were incubated for 18 h at 30 °C. After incubation period diameter of zone of inhibition was measured (Ahmad et al., 2001).

3.8.2. Determination of minimum inhibitory concentration (MIC)

MIC determination was done by broth dilution method (Meiji co., Japan). The inoculum was prepared by the growth method with which the test bacteria were grown on non-selective culture media and incubated overnight. Then, 4–5 colonies were taken from that plate and suspended into 10 ml of nutrient broth and incubated for 2 hours. The bacterial inoculum was adjusted to 0.1McFarland Standard. Then, 25 µL of the inoculum was added into 12 mL of

luria broth and 50 µL of the mixture was inoculated into each plate. Finally, the inoculated plates were incubated at 35±2°C in ambient air for 20–24 hours for *E.coli* or 16–20 hours for the other bacteria (Sangeetha rajendra et al., 2014).

3.8.3. Synergistic activity

To measure the interactive inhibition of synergy between synthetic peptide and antibiotics chequerboard method was performed. Synergistic mixtures were prepared using the synthetic peptide (O3S) and antibiotics to which the bacterial strains were resilient. Two fold serial dilution of antibiotic and synthetic peptide (O3S) was done. The effects of combination were evaluated by calculating the fractional inhibitory concentration index (FICI).

FIC of agent A = (MIC of agent A in combination)/ (MIC of agent A alone)

ΣFIC = FIC of agent A + FIC of agent B (Hideki et al., 2017).

If FIC value is less than 0.5 (FIC <0.5) then mix of compound is showing synergistic activity. An FICI between 0.5 - 4.0 shows that there is no interaction between the compounds. An FICI > 4.0 shows that there is antagonism between the two agents.

Chapter 5

Results

4.1 Plant extract results

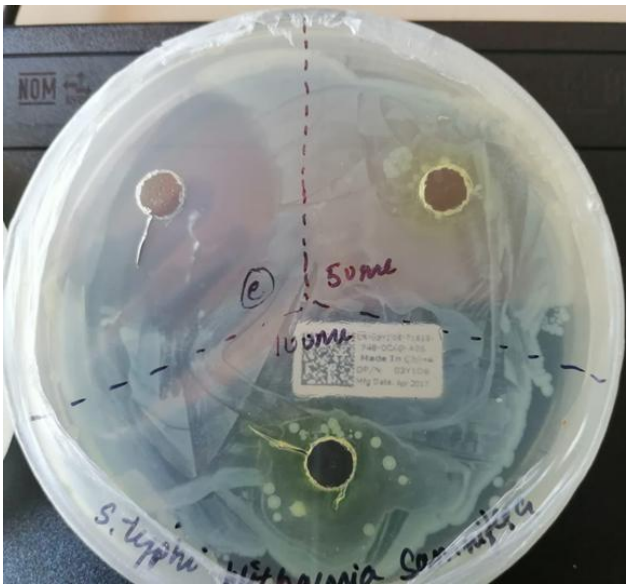

Fig 4.1

Zone of inhibition for *Withernia somnifera* against *S.typhi* was 1.1cm for 100ml and 0.7 cm for 50 ml cm, whereas zone of inhibition for control is 1.6 cm for 100ml.

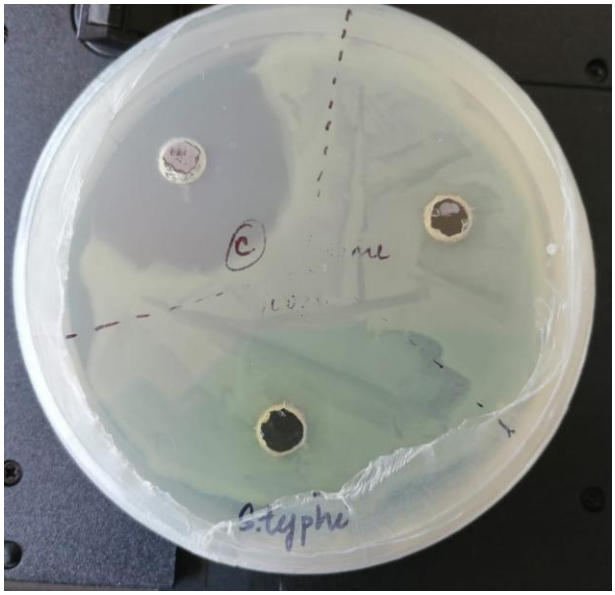

Fig 4.2

Zone of inhibition for *Cestrum nocturnum* against *Salmonella typhi was* 0.7 cm for 100ml and 0.5 cm, whereas zone of inhibition for control was 1.4.

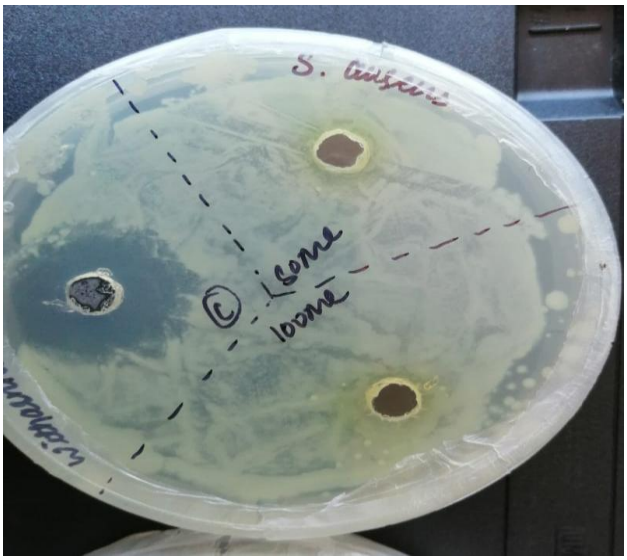

Figure 4.3

Zone of inhibition for *Withania samnifera* against *S.ausreus*

Zone of inhibition for 100 µl concentration *was* 0.6 cm and 0.5 cm for 50 µl, whereas zone of inhibition for control (ampicillin) was 1.4 cm.

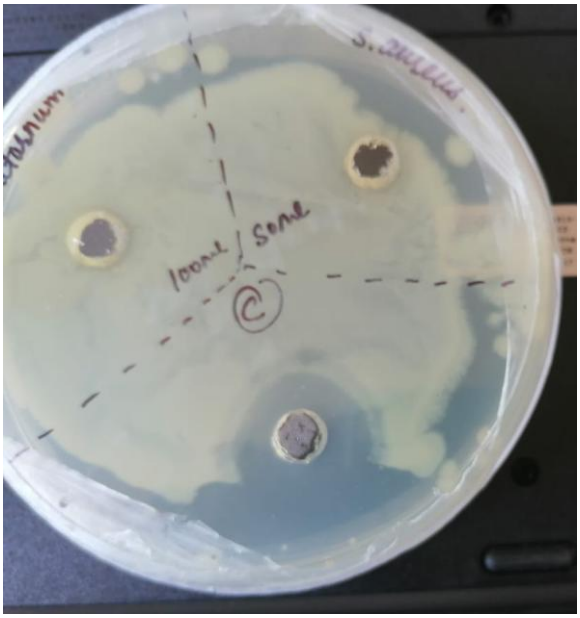

Fig 4.4

Zone of inhibition for *Cestrum noctrnum* against *S.aureus*

Zone of inhibition was 100 µl 0.7 cm and 0.5 cm for 50 µl, whereas zone of inhibiotion for control (ampicillin) was 1cm.

Fig 4.5

Zone of inhibition for mixture of *Witharnia samnifera* and *Cestrum nocturnum against S.aureus*

Zone of inhibition for 100 µl was 0.2 cm and 0.1cm for 50 µl, whereas zone of inhibition for control (ampicillin) was 1.5 cm.

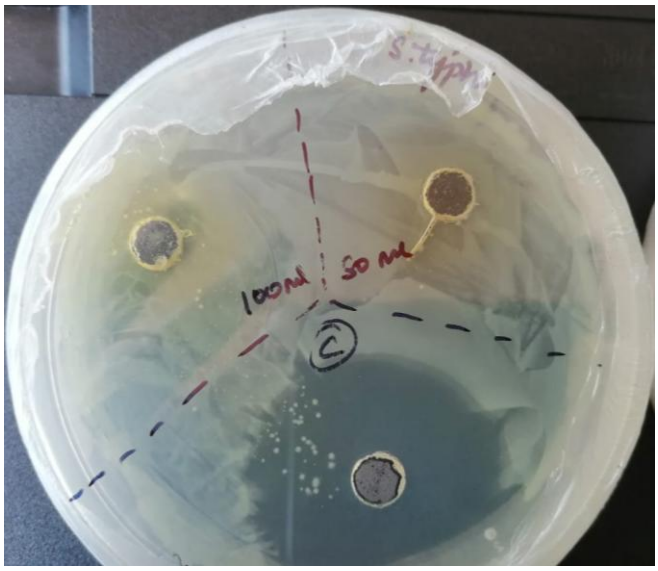

Fig 4.6

Zone of inhibition for mixture of *Cestrum nocturnum and* Witharnia *samnifera* against *Salmonella typhi*

Zone of inhibition for 100 µl *was* 0.6 for and 0.3 cm for 50 µl, whereas zone of inhibition for control (ampicillin) was 2 cm.

4.2 MIC results

1. Results of antibacterial activity are shown in table 4.1.

	0.5	**0.25**	**0.12**	**0.06**	**0.03**	**0.01**
Gentamycin	0.278	0.181	0.147	0.131	0.113	0.252
Vancomycin	0.095	0.109	0.245	0.343	0.553	0.859

Table 4.1 Antimicrobial activity of vancomycin and gentamycin against *E.coli*.

2. Results of antibacterial activity are shown. Then FIC was calculated.

	0.5	**0.25**	**0.12**	**0.06**	**0.03**	**0.01**
Vancomycin	0.225	0.051	0.052	0.072	0.089	0.154
O3S	0.596	0.444	0.318	0.191	0.121	0.115
Vancomycin + O3S	0.489	0.309	0.199	0.122	0.103	0.090

Table 4.2 Antimicrobial activity of vancomycin and O3S and their mixture against *E.coli*.

1. ΣFIC 1= 2.97, as it is greater than 0.5 and less than 4.0 that means there is no interaction between compounds.
2. ΣFIC 2= 6.6, as it is greater than 4.0 that means there is antagonism between two compounds
3. ΣFIC 3= 4.4, as it is less than 4.0 that means there is no interaction between compounds.
4. ΣFIC 4= 4, as it is equal 4.0 that means there is no interaction between compounds.
5. ΣFIC 5= 2, as it is less than 4.0 that means there is no interaction between compounds.
6. ΣFIC 6= 1.2, as it is less than 4.0 that means there is no interaction between compounds.

3. MIC of 96 well plate was taken and results were observed. Then FIC was calculated.

	0.5	0.25	0.12	0.06	0.03	0.01
Gentamycin	0.055	0.262	0.414	0.414	0.489	0.819
O3S	0.130	0.423	0.267	0.176	0.117	0.064
Gentamycin + O3S	0.435	0.303	0.201	0.128	0.064	0.115

Table 3.3 Antimicrobial activity of gentamycin and O3S and their mixture against *E.coli*.

1. ΣFIC 1= 11.2, as it is greater than 4.0 that means there is antagonism between compounds.
2. ΣFIC 2= 1.86, as it is greater than 0.5 and less than 4.0 that means there is no interaction between compounds.
3. ΣFIC 3= 1.23, as it is greater than 0.5 and less than 4.0 that means there is no interaction between compounds.
4. ΣFIC 4= 1, as it is greater than 0.5 and less than 4.0 that means there is no interaction between compounds.
5. ΣFIC 5= 0.67, as it is greater than 0.5 and less than 4.0 that means there is no interaction between compounds.
6. ΣFIC 6= 1.84, as it is greater than 0.5 and less than 4.0 that means there is no interaction between compounds.

4. Data interpreted

S.no.	ΣFIC Trial 1	ΣFIC Trial 2	(ΣFIC1 + ΣFIC2)/2
1.	2.97	11.2	7.08
2.	6.6	1.86	4.23
3.	4.4	1.23	2.81
4.	4	1	2.5
5.	2	0.67	1.33
6.	1.2	1.84	1.52

Table 3.4

Chapter 6

Conclusions

In conclusion, from this project the average ΣFIC obtained from ΣFIC 1 and ΣFIC 2 as represented in table 3.4 shows that none of the ΣFIC obtained was less than 1 so, hence there was no synergistic effect observed.

The maximum zone inhibition observed for *Withernia somnifera* against *Salmonella typhi* was 1.1 cm and against *S.aureus* was 0.7 cm for 100 µl concentration of the extract and the maximum zone of inhibition observed for *Cestrum nocturnum* against *Salmonella typhi* was 0.7 cm and against *S.aureus* was 0.7cm for 100 µl concentration of extract. Whereas the maximum zone of inhibition observed for the mixture of *Withernia somnifera* and *Cestrum nocturnum* against *Salmonella typhi* was 0.6 cm and against *S.aureus* was 0.2 cm for 100 µl concentration of the extract.

If zone of inhibition of individual plant extracts and combination of both plant extracts was compared than zone of inhibition of individual plant extracts were greater than the zone of inhibition obtained from their combination that shows no synergistic activity was observed.

Chapter 7
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