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Use of Piper Nigrum (Black Pepper) In Combination With Standard Antibiotics In Anti-Microbial Chemotherapy

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Abstract:

Overuse, misuse, and underuse of antibiotic and other drug molecules is a big problem. Self-medication always a keen interest in human beings. This lead to the development of complicated multidrug-resistant bacteria. Normal antibiotics having less efficacy against them. Natural plant products used for therapeutic purposes from ancient times. It has been observed that conjugation of these antibiotics with certain plant products increases the efficacy of the drug against the pathogen. Black pepper is a well-known spice used in Indian cuisine and has antimicrobial properties. In presented work, we have used *Piper nigrum* along with standard antibiotics available in the market to check the efficacy of the combination against *E.coli, Bacillus subtilis* and *S. aureus.*

Keywords: Antimicrobial activity, Multidrug resistance, *Piper nigrum*, Antibiotics, Synergism

Introduction

The plant kingdom is a well-known source of bioactive compounds that can be utilized as therapeutic and pharmaceutical purposes. There are several natural plant products are available which can be utilized for various purposes from ancient times. These chemicals are having antimicrobial properties, antioxidant properties, and gastro-protective nature. Natural plant products and secondary metabolites used for therapeutic purposes due to their antioxidant properties and antimicrobial property [1]. *Piper nigrum* is a well-known spice used in various Indian cuisine. They are widely utilized to prevent food spoilage and restrict the growth of the foodborne pathogens [3]. Black pepper contains piperine as a bioactive compound, it is also having other volatile oil, flavonoids, and alkaloids. Black Pepper also has a free radical scavenging activity which is helpful for the prevention of tumor growth [2]. The synergistic effect of these plant products along with standard drugs

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is a very important phenomenon now a day to combat the new and more harmful pathogens. The previous researcher found out that phenolic compounds that are found on fresh pepper may inhibit the microbial growth of several pathogenic bacteria such as *E. coli, S. aureus, Bacillus, Salmonella typhimurium* [4]. Piperine is the main bioactive component found on black pepper, it is responsible for the synergistic effect shown by black pepper along with other known therapeutic agents such as vaccines, drugs and nutritional components by increasing their bioavailability [5,6]. Piperine is act as an analgesic, antidepressant and anti-inflammatory compounds.

Multidrug resistance is a well-known problem in recent days. In this phenomenon, when infected cell exposed to the drug against any infection, they develop the resistance to other drugs which are structurally as well as functionally different from the initial drug. This resistance is intrinsic or acquired in nature [7]. Drug use, misuse, and overuse are potential problems that may develop the drug-resistant species. Plant-derived natural products are increasingly used in medical treatments due to their efficacy and almost negligible side effects [8]. Antibiotics related to resistant microorganisms are already widespread in the environment [9]. Treatment of the infection caused by multidrug-resistant microorganisms is very difficult and having limited access to the public [10].

2 Proposed methodologies

2.1 Collection of microorganisms

Bacterial strains were *Escherichia coli* (MTCC 40), *Bacillus substilis (MTCC 121)* and *Staphylococcus aureus* (MTCC 7461*) c*ultures were obtained from the microbial type culture collection of Institute of microbial technology Chandigarh in pure form.

2.2 Sources of chemicals

Drugs like streptomycin, gentamicin, penicillin G, neomycin, tetracycline, ciprofloxacin, amoxicillin were obtained from Himedia laboratories, Mumbai, India.

2.3 Plant Material and Preparation of extract

Plant parts i.e. leaves of *Syzgium Aromaticum* (Clove), *Ocimum Santalum* (Tulsi), *Azadirachta Indica* (Neem), fruits of *Piper nigrum* (Black Pepper) and rhizomes of *Zingiber Officinale* (Ginger), *Curcuma Longa* (Turmeric) were purchased and collected from the market and localities of Jalandhar, India. The spices were obtained from the market, they were cleaned, descaled when necessary and washed in sterile distilled water, surface-sterilized using methanol then methanol was allowed to evaporate in a sterile laminar flow chamber. In order to obtain the spice extracts, about 50gm of each dried spice was crushed with Soxhlet apparatus. The powdered sample was extracted with ethyl

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acetate. The extract was filtered by a Whatman filter paper. The collected filtrates were concentrated by evaporation; the resulted extracts were stored at low temperature in the refrigerator and used for evaluation of in-vitro antibacterial activities.

2.4 Antibacterial activity

2.4.1. Preparation of the bacterial suspension

The bacterial suspension was prepared by transferring 100 µl in 30ml in nutrient broth and incubated at 37 ℃ for 24 h.

2.4.2 Preparation of plate

Muller Hinton Agar medium was sterilized at 15lbs/ cm^2 pressure for 20 min in an autoclave. Then 15 ml of medium was poured in each Petri plate under sterile condition.

2.4.3 Antibacterial assay

After pouring the media into the Petri plates add 20 µl of each of the cultures and spread it with the help of the spreader. The antibiotic disk was kept in the different dilution of the extract for 25-30 minutes. After spreading the bacterial culture onto the plates disk were kept in the center of the Petri plates. Each disk is prepared in the replica of triplicates. Then Petri plates were incubated at 37 ℃ for 24-48 h for growth of the microorganisms.

3 Result and discussion

S.NO.	ANTIBIOTIC DISC	ZONE OF INHIBITION WITH *PIPER NIGRUM* EXTRACT (IN cm)				REMARK
		CONTROL	10%	15%	20%	
1.	Gentamycin	2.9	3.6	3.5	3.5	POSITIVE
2.	Streptomycin	2.0	2.8	4.0	5.0	POSITIVE
3.	Ciproflaxacin	3.0	3.8	4.5	4.7	POSITIVE
4	Neomycin	1.9	2.8	2.9	3.1	POSITIVE
5	Tetracycline	2.8	4.1	4.0	4.6	POSITIVE

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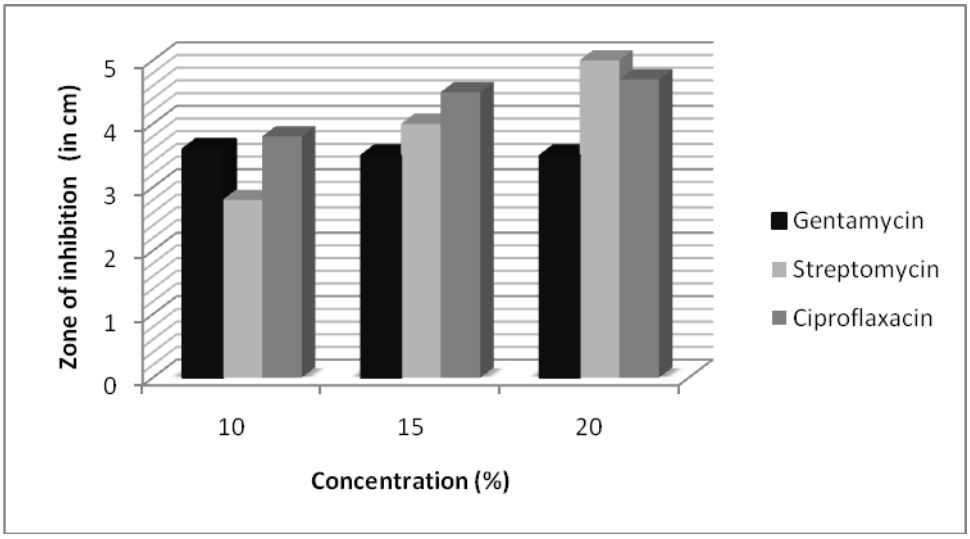
6	Amoxicillin	1.4	2.2	2.4	2.6	POSITIVE

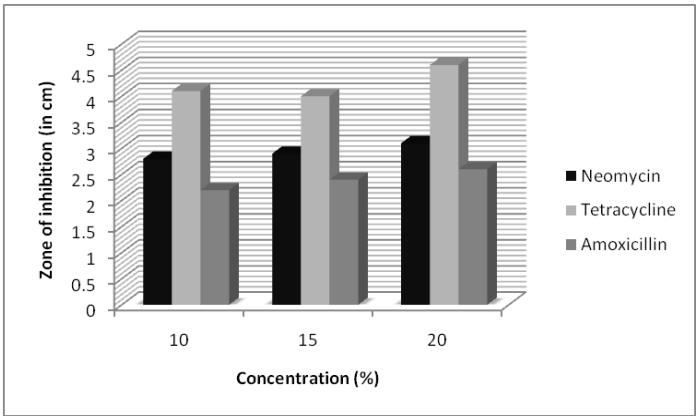
Table 1 *Piper nigrum* extracts in conjugation with Antibiotics against *E.coli.*

From table 1 we observed that *Piper nigrum* extracts in conjugation with streptomycin showed the higher zone of inhibition against *Escherichia coli* at the concentration of 20%. It showed 5.0 cm of the zone of inhibition while *Piper nigrum* extract in conjugation with ciprofloxacin showed the highest zone of inhibition of 4.1 cm against *Escherichia coli* at 20% concentration. Streptomycin showed much better results than ciprofloxacin.



Graph 1 Comparison of Streptomycin, Gentamycin and Ciproflaxacin against *E.coli.*

From Graph 1 we observed that *Piper nigrum* extracts in conjugation with Streptomycin showed better antibacterial activity as compared to gentamycin.



Graph 2 Comparison of Neomycin, Tetracycline, and Amoxicillin.

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From Graph 2, we observed that *Piper nigrum* extracts in conjugation with tetracycline showed better antibacterial activity as compared to Amoxicillin.

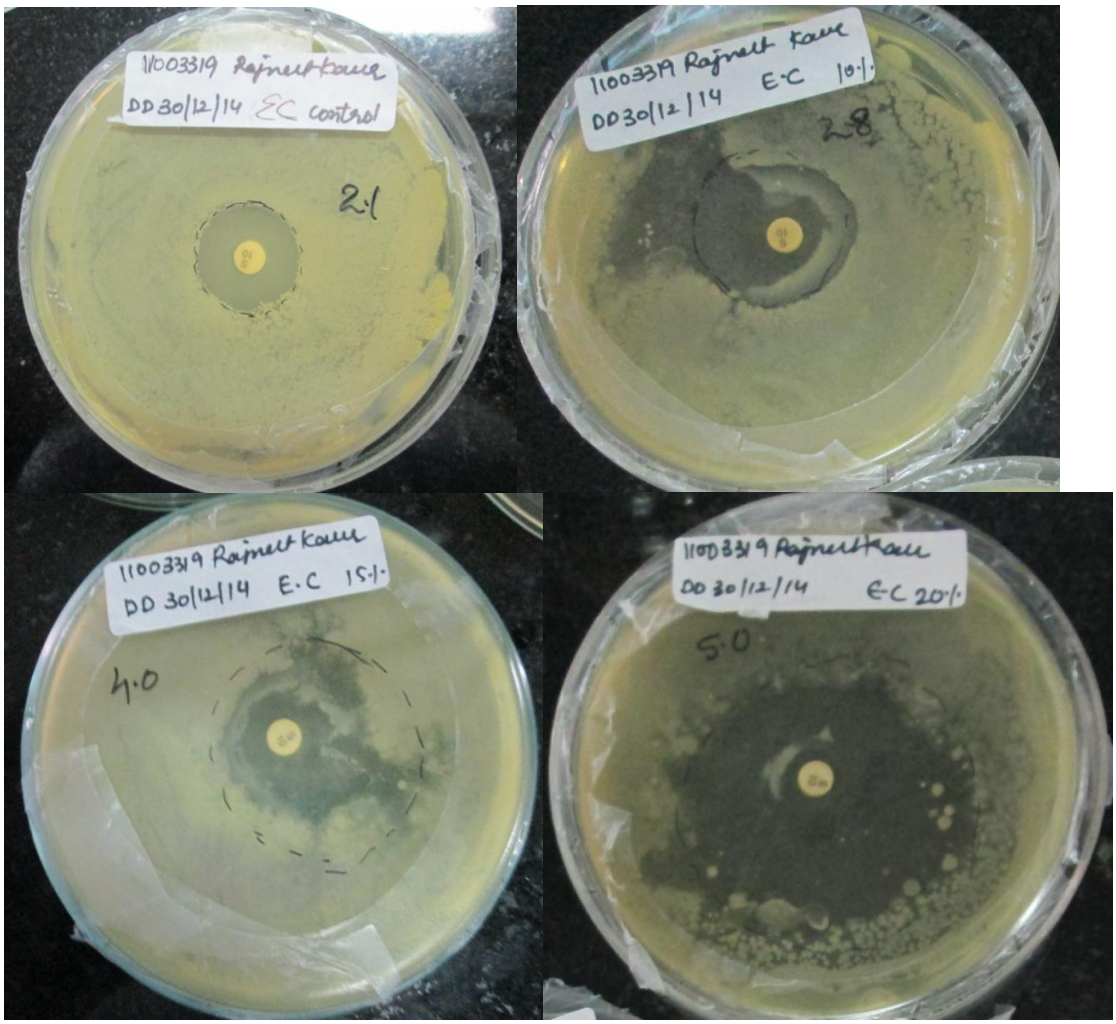

Image 1: Inhinition zone of streptomycin against *E.coli* at control, 10%,15% and 20% dilutions of *Piper nigrum*

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S.NO.	ANTIBIOTIC DISC	ZONE OF INHIBITION WITH *PIPER NIGRUM* EXTRACT (IN cm)				REMARK
		CONTROL	10%	15%	20%	
1.	Gentamycin	1.9	2.0	2.0	2.1	POSITIVE
2.	Streptomycin	2.5	2.7	3.9	5.9	POSITIVE
3.	Ciproflaxacin	2.3	2.7	2.8	2.8	POSITIVE
4	Neomycin	2.8	2.8	5.0	5.4	POSITIVE
5.	Tetracycline	2.5	3.5	3.6	4.0	POSITIVE
6.	Amoxicillin	1.2	2.1	2.3	2.5	POSITIVE

Table 2 *Piper Nigrum* extracts in conjugation with Antibiotics against *Bacillus Substilis.*

From table 2, we observed that *Piper Nigrum* extracts in conjugation with streptomycin showed the higher zone of inhibition against *Escherichia coli* at the concentration of 20%. It showed 5.5 cm of the zone of inhibition while *Piper nigrum* extract in conjugation with ciprofloxacin showed the highest zone of inhibition of 2.8 cm against *Escherichia coli* at 20% concentration. Streptomycin showed a much better result than Ciprofloxacin.

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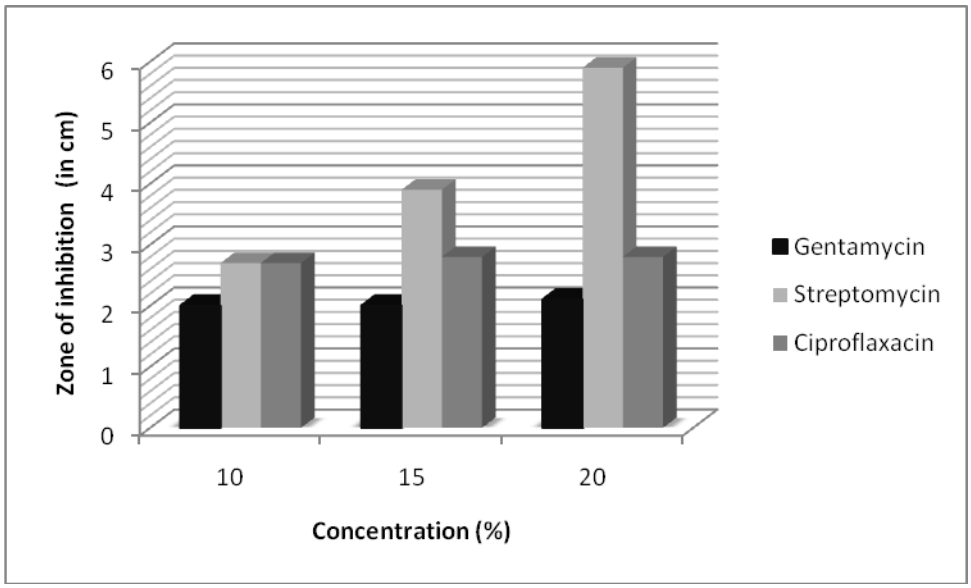
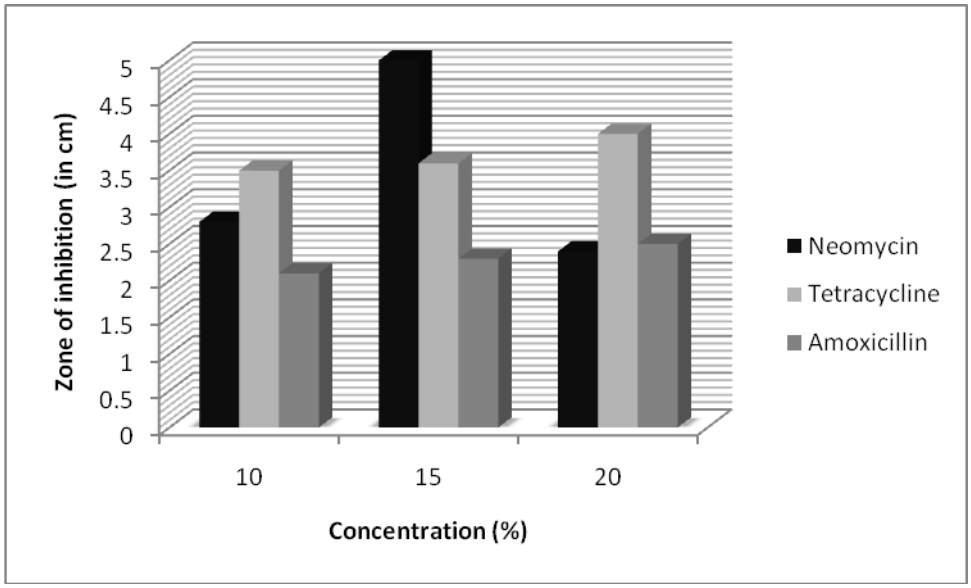


Graph 3 Comparison of Streptomycin, Gentamycin and Ciproflaxacin against *B.substilis.*

From Graph 3, we observed that *Piper Nigrum* extracts in conjugation with Streptomycin showed better antibacterial activity as compared to Gentamycin.



Graph 4 Comparison of Neomycin, Tetracycline, and Amoxicillin.

From Graph 4, we have observed that *Piper Nigrum* extracts in conjugation with Tetracycline showed better antibacterial activity as compared to Amoxicillin.

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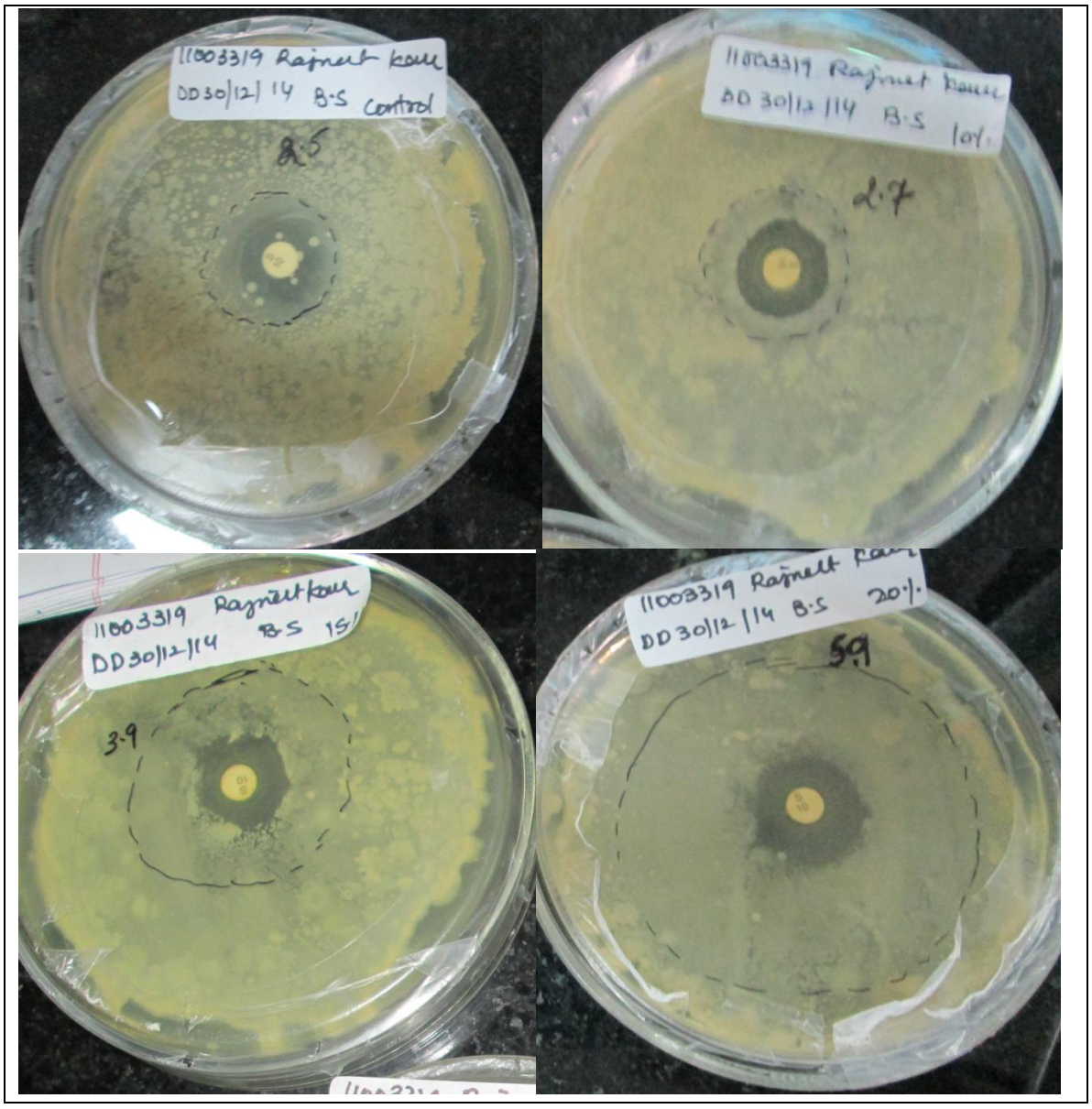

Image 2: Inhibition zone of streptomycin against *B.Substilis* at control, 10%,15% and 20% dilutions of *Piper Nigrum*

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S.NO.	ANTIBIOTIC DISC	ZONE OF INHIBITION WITH *PIPER NIGRUM EXTRACT* (IN cm)				REMARK
		CONTROL	10%	15%	20%	
1.	Gentamycin	2.0	3.1	3.1	3.0	POSITIVE
2.	Streptomycin	24	3.7	3.7	4.0	POSITIVE
3.	Ciproflaxacin	3.4	4.0	4.2	4.1	POSITIVE
4	Penicillin	1.1	1.9	2.5	2.6	POSITIVE
5	Tetracycline	3.2	2.5	3.3	3.5	POSITIVE
6	Amoxicillin	1.2	2.1	2.3	2.5	POSITIVE

Table 3 *Piper Nigrum* extracts in conjugation with Antibiotics against *S.Aureus.*

From table 3, we observed that *Piper Nigrum* extracts in conjugation with ciprofloxacin showed the higher zone of inhibition against *Escherichia coli* at the concentration of 20%. It showed 4.1 cm of the zone of inhibition while *Piper Nigrum* extract in conjugation with gentamycin showed the highest zone of inhibition of 3.0 cm against *Escherichia coli* at 20% concentration. Ciprofloxacin showed a much better result than Gentamycin

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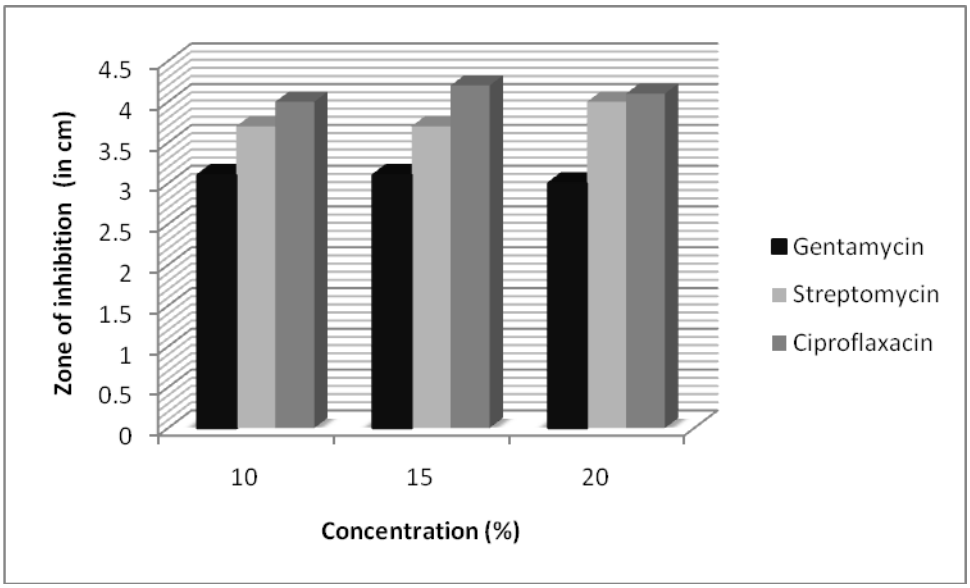
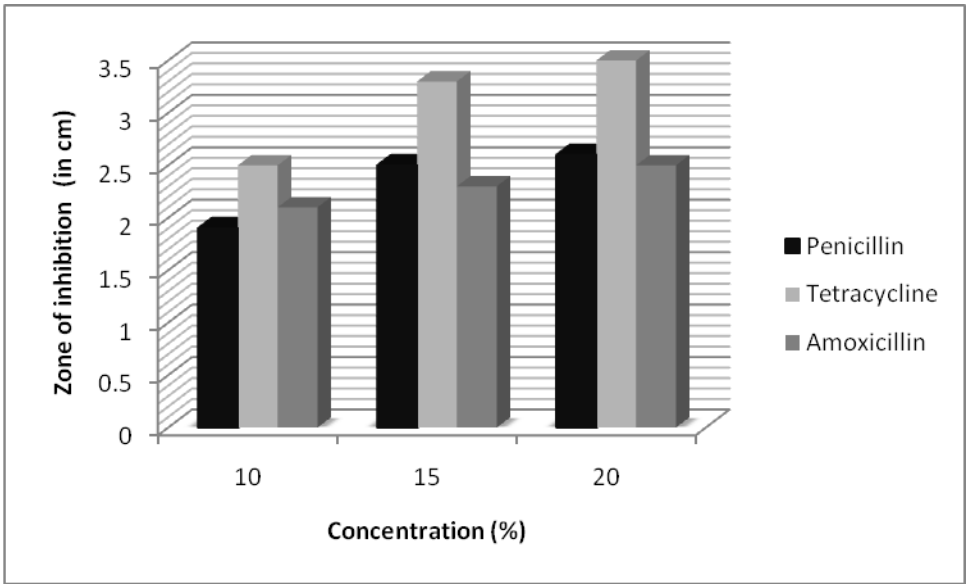


Graph 5 Comparison of Streptomycin, Gentamycin, and Ciprofloxacin against *S.Aureus*
From Graph 5 we observed that *Piper Nigrum* extracts in conjugation with ciprofloxacin showed better antibacterial activity as compared to gentamycin.



Graph 6 Comparison of Penicillin, Tetracycline, and Amoxicillin against *S.Aureus*

From Graph 6 we observed that *Piper Nigrum* extracts in conjugation with tetracycline showed better antibacterial activity as compared to amoxicillin and penicillin.

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4. CONCLUSION

The test for various antibiotics in conjugation with plant products against a gram-positive and gram-negative bacteria showed positive results. The antibiotics showed a positive zone of inhibition on bacterial growth. The microbes did not show any resistance towards the antibiotics that were conjugated with the plant extracts. Due to the misuse of antibiotics in recent times the microbes have become resistant towards antibiotics. This has become a challenge in recent times to combat the diseases caused by microbes. To find an alternative to this problem we can use the plant extracts in conjugation with the antibiotics to resist the growth of the microbes. Black pepper showed positive results against *E. coli, Bacillus subtilis,* and *S. aureus.*

The antibiotics in conjugation with the plant extracts increase the area of the antimicrobial resistance caused due to the antibiotics. The positive results show that the antimicrobial activity of the antibiotics increases if the plant extracts are added to the antibiotics. Synergistic effects of natural plant products along with the standard antibiotics gain interest from scientific communities. Multidrug-resistant bacteria are difficult to treat due to non-availability of the suitable methods, time as well as cost. Further research required in this area to make the optimum dosage of the plant extract along with standard drugs.

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