Enhancing Antibacterial Efficacy: Exploring the Synergistic Effects of Medicinal Plant Extracts and Antibiotics against *E. coli*

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Abstract
In this study, the focus was on exploring the antibacterial properties of selected medicinal plants and their combined effect with antibiotics. The research utilized five different isolates of Escherichia coli (E. coli). The findings revealed that methanol extracts from most tested plants hindered the growth of bacteria. Notably, black pepper exhibited the most significant inhibition with a zone diameter of 19 mm, followed by clove at 18 mm and thyme at 17 mm.

It shows that when plant extracts are combined with antibiotics, they show a synergistic effect, effectively inhibiting the targeted organisms. Particularly, the synergy with black pepper extract proved to be more potent in inhibiting antibiotic-resistant bacteria, followed by clove and thyme, in descending order of effectiveness.

Keywords: *E. coli*, black pepper, clove, thyme, antibacterial efficacy.

Introduction

Antibiotics, while extensively used for microbial infections, face a growing challenge due to antibiotic resistance. The increasing occurrence of new and re-emerging infectious diseases emphasizes the urgency in discovering novel antimicrobial compounds. Plant extracts exhibit promising antimicrobial activity, producing diverse compounds with varied biological effects[5]. Antimicrobial compounds from plants demonstrate efficacy against both plant and human pathogenic microorganisms. Plant extracts targeting sites distinct from antibiotics hold potential against drug-resistant microbial pathogen.

The increasing problem of bacterial resistance raises uncertainty about the future of antibacterial drugs. Plants offer a rich source of biologically active molecules with antibacterial properties. These plant-derived compounds can directly combat bacteria and enhance the effectiveness of antibiotics by modifying antibiotic resistance. *In vitro* studies have demonstrated the synergistic activity of plant extracts with antibiotics against multidrug-resistant bacteria.

However, the mechanisms behind these synergies remain insufficiently explored, necessitating further investigation. To advance this field, future research should prioritize understanding these mechanisms, along with comprehensive testing of the activity, toxicity and bioavailability of plant-derived antibacterial agents *in vivo*[10]. While the development of plant-origin antibacterial agents holds promise for infection treatment, additional studies on the bioavailability, pharmacodynamics and mechanism of action are crucial.

In impoverished nations, the unavailability and high prices of modern pharmaceuticals, exacerbated by poor economic conditions, have led to a continual rise in drug prices. Consequently, emerging nations heavily depend on derivatives of medicinal plants that are more affordable and culturally acceptable to the local population.[12] Plants have been discovered to harbor a wide array of bioactive compounds including peptides, aldehydes, alkaloids, as well as substances soluble in water, ethanol, chloroform and methanol. Additionally, they contain essential oils and phenols, which contribute to their diverse biological activities and potential therapeutic properties. These components have demonstrated effectiveness in treating bacterial, fungal and viral infections in humans[6]. The presence of these diverse compounds highlights the potential of plant-based remedies for combating a range of infections in the medical field.

Natural compounds particularly those derived from plants are being explored for their potential as antibiotics. Synergism, a positive interaction between two agents, is identified as a key mechanism through which plant-derived compounds exert their antibiotic properties. Research has demonstrated that combining plant extracts with antimicrobials can significantly reduce the minimum inhibitory concentrations (MICs) of antibiotics for bacterial strains.[1]

This research focuses on the potential of medicinal plants as alternative or complementary treatments, especially in combination with antibiotics, to address the challenge of multidrug-resistant microorganisms. The specific emphasis is on investigating the antibacterial effects of combining certain medicinal plants with antibiotics against multidrug-resistant *E. coli*. This study addresses the global health challenge of antibiotic resistance, emphasizing the necessity for innovative strategies against antibiotic-resistant bacteria. Its objective is to explore the potential of combining medicinal plants with antibiotics as an alternative approach[7].

Overall, the research aims to contribute valuable insights into combating multidrug-resistant *E. coli* through the

strategic utilization of medicinal plant extracts in conjunction with antibiotics.

The rise of bacterial resistance to commonly used antibiotics has prompted a search for alternative antibacterial agents or a combination of drugs to effectively address emerging resistant pathogenic bacteria. Hence, this study aimed to assess the antimicrobial properties of specific medicinal plants. Some studies have shown that combining antimicrobial drugs with crude plant extracts can enhance their effectiveness against certain pathogenic species[4].

Escherichia coli (E. coli) is a bacterium commonly found in the human body as part of the normal flora. It can exist in non-pathogenic, commensal, or pathogenic forms[9]. Pathogenic *E. coli* is known to cause various infections including urinary tract infections, systemic infections and enteric infections[10]. The increased use of antimicrobial agents has led to the development of resistance in *Escherichia coli*. In response to this challenge, medicinal plant extracts have been explored for their antimicrobial properties against *E. coli*[2]. These extracts have demonstrated effectiveness, particularly against enteropathogenic strains found in food materials[8]. This approach seeks alternative solutions to combat antibiotic resistance, highlighting the potential of natural compounds from medicinal plants in addressing *E. coli* infections.

In this study, 5 plants from diverse families were chosen to assess their efflux pump inhibitory (EPI) activity. The investigation focused on the methanolic extracts of these plants and their potential synergistic effects with commonly used antibiotics such as ciprofloxacin, gentamycin, erythromycin and tetracycline. Multidrug resistance (MDR) poses a significant challenge in treating bacterial infections, particularly due to the role of efflux mechanisms[3]. The study addresses the challenge of multidrug resistance associated with efflux pumps, particularly those belonging to the RND family, in Gram-negative bacteria[5]. Efflux pump overproduction is identified as a significant factor contributing to increased resistance to antimicrobial agents like tetracycline and ciprofloxacin.

Recognizing the pressing issue of antibiotic resistance, this study delves into the potential of addressing the challenge by combining extracts from specific medicinal plants, extracted using methanol as a solvent, with three different selected antibiotics. The research utilizes the disc diffusion sensitivity method to evaluate the activity against *E. coli* isolates, comparing the effectiveness of the plant extracts in conjunction with the three standard antibiotics. The goal is to explore synergistic effects that may offer a promising approach in combating antibiotic-resistant strains of bacteria.

Material and Methods

Tested Microorganisms: Five isolates of *E. coli* were isolated from soil, milk and sprouts.

Plant Material: A diverse assortment of plant materials was gathered for investigation, sourced from various families. The collection process involved acquiring plants from both the local markets in Nagpur and the Green Heaven India Herbal unit in Hingna, Nagpur. A total of 10 plants, representing distinct families, were selected for screening. The objective was to assess their synergistic activity in combination with various antibiotics against *E. coli*.

During the collection, emphasis was placed on obtaining only the necessary plant parts which were then stored in sterile containers. The storage conditions were maintained at 4°C and the collected plant materials were stored either in a dry or fresh state. This meticulous approach aimed to preserve the integrity of the plant samples for subsequent analysis and experimentation.

Preparation of plant extract: To prepare the plant extracts, the selected plant parts underwent a systematic cleaning process. Initially, these plant parts were washed with tap water to eliminate surface impurities. Subsequently, a thorough wash with 0.1% $HgCl_2$ was carried out to further remove any potential contamination. Following this, a final rinse with distilled water was performed. The washed plant parts were then left to air-dry for a period of 4 to 5 days in the shade.

After the drying process, the plant parts were finely powdered using a mortar and pestle. The resulting plant powder, obtained through this grinding process, was carefully stored at a temperature of 4°C until it was ready for use in subsequent procedures. This method ensured the cleanliness and proper preparation of the plant extracts for further analysis and experimentation.

Soxhlet extraction: The powdered plant material underwent extraction using a Soxhlet apparatus, employing methanol as the solvent. Methanol was chosen due to its widespread use as a solvent, facilitated by its ability to easily dissolve various compounds present in plant material. Additionally, methanol's efficient evaporation properties were advantageous for separating it from the extract. Its cost-effectiveness and ready availability further contributed to its selection.

The extraction process involved dissolving 1 gram of plant extract in 10 ml of methanol and the apparatus was operated for a duration of 18-24 hours to obtain the final concentrated slurry. Subsequently, the extract was transferred to a china dish. The evaporation of methanol from the extract was achieved through incubation at a temperature range of 35-38°C. The resulting powder was then weighed and stored in sterile vials at 4°C until needed for further use, following the methodology described by Stefanovic[14].

The investigation of the combined effects of plant extract and antibiotics was conducted using the well diffusion method, following the protocol outlined by the National

Committee for Clinical Laboratory Standard in 1993. To measure antibacterial activity, bacterial cultures were inoculated and allowed to incubate overnight at 37°C. The inoculum size was adjusted according to the 0.5 McFarland standard, prepared by dissolving 99.5 mL of 1% H_2SO_4 and 0.5 mL of 1.175% $BaCl_2$, stored in the dark at room temperature.

Antibiotics used in this study: In this study, three antibiotics were employed: tetracycline (at a concentration of 10 mcg), Ampicillin (also at 10 mcg) and ciprofloxacin (at 5 mcg).

Study of combined effect of extract and antibiotics using well diffusion method (NCCLS, 1993): Nutrient agar plates were then prepared and sterilized swabs were dipped into a standardized bacterial suspension with an inoculum size of 1.5 x 108 cfu/mL. Excess culture was removed by turning the swab against the side of the tube and the inoculum was evenly spread over the entire surface of the nutrient agar plates. After allowing the plates to dry for at least 15 minutes, wells with a diameter of 7 mm were punched into the agar.

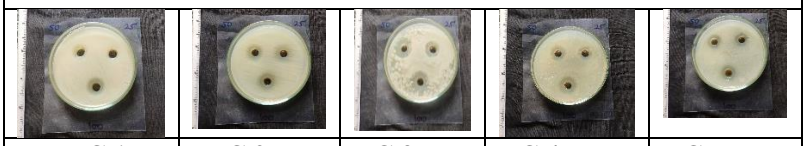
These wells were filled with 40 µL of plant extract at three different concentrations (25 µg/mL, 50 µg/mL and 100 µg/mL) as well as antibiotics alone. Additionally, 40 µL of both plant extract and the antibiotic were added to specific wells. Each plate was replicated three times. Following well preparation, the plates were incubated at 37°C for 24 hours to observe and to assess the inhibitory effects of the plant extract and antibiotics on bacterial growth.

Table- Antibacterial activity of black pepper extract (5 mg/ml)

Isolates	25 µl	50 µl	100µl
EC 1	NI	14 mm	15 mm
EC 2	16 mm	16 mm	17 mm
EC 3	15 mm	19 mm	19 mm
EC 4	14 mm	15 mm	16 mm
EC 5	13 mm	14 mm	15 mm

***Note: -** EC- *Escherichia coli*, NI- No inhibition

Table- Antibacterial activity of black pepper extract (5 mg/ml)



EC 1	EC 2	EC 3	EC 4	EC 5

Table- Synergistic Activity of black pepper extract (5 mg/ml) isolate EC1

EC 1	PE	AB	AB + PE	DMSO
CIP	NI	34 mm	36 mm	NI
AMP	NI	10 mm	12 mm	NI
TET	NI	25 mm	31 mm	NI

***Note: -** EC- *Escherichia coli*, NI- No inhibition, CIP- Ciprofloxacin, AMP- Ampicillin, TET- Tetracycline, PE- Plant extract, AB- Antibiotic, DMSO- Dimethyl Sulfoxide

Table- Synergistic Activity of black pepper extract (5 mg/ml) isolate EC1

EC 1 CIP	EC 1 AMP	EC 1 TET

Table- Synergistic Activity of black pepper extract (5 mg/ml) isolate EC2

EC 2	PE	AB	AB + PE	DMSO
CIP	10 mm	28 mm	32 mm	10 mm
AMP	NI	10 mm	NI	NI
TET	NI	19 mm	28 mm	NI

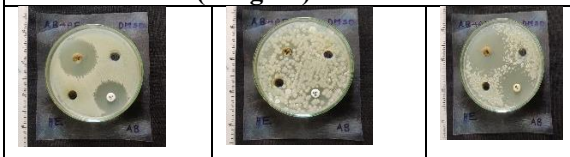
Table- Synergistic Activity of black pepper extract (5 mg/ml) isolate EC2

EC 2 CIP	EC 2 AMP	EC 2 TET

Synergistic Activity of black pepper extract (5 mg/ml) isolate EC3

EC 3	PE	AB	AB + PE	DMSO
CIP	10 mm	23 mm	29 mm	10 mm
AMP	NI	10 mm	10 mm	NI
TET	NI	28 mm	34 mm	NI

Synergistic Activity of black pepper extract (5 mg/ml) isolate EC3



EC 3 CIP	EC 3 AMP	EC 3 TET

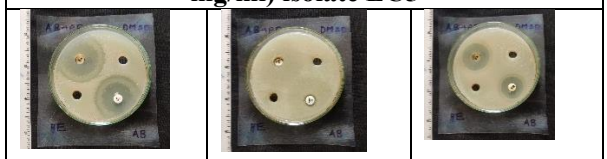
Synergistic Activity of black pepper extract (5 mg/ml) isolate EC4				
EC 4	**PE**	**AB**	**AB + PE**	**DMSO**
CIP	NI	25 mm	29 mm	NI
AMP	NI	NI	NI	NI
TET	NI	18 mm	25 mm	NI

Synergistic Activity of black pepper extract (5 mg/ml) isolate EC4

EC 4 CIP	EC 4 AMP	EC 4 TET

Synergistic Activity of black pepper extract (5 mg/ml) isolate EC5				
EC 5	**PE**	**AB**	**AB + PE**	**DMSO**
CIP	NI	27 mm	31 mm	NI
AMP	NI	10 mm1	10 mm2	NI
TET	NI	19 mm	24 mm	NI

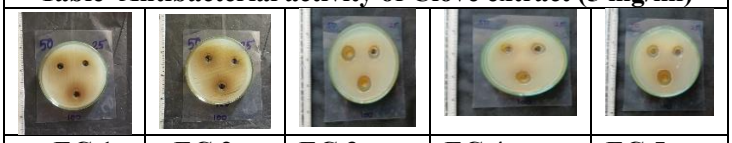
Synergistic Activity of black pepper extract (5 mg/ml) isolate EC5



EC 5 CIP	EC 5 AMP	EC 5 TET

Table- Antibacterial activity of Clove extract (5 mg/ml)			
Isolate	**25µl**	**50 µl**	**100 µl**
EC 1	NI	NI	NI
EC 2	11mm	11mm	12mm
EC 3	13mm	16mm	16mm
EC 4	13mm	12mm	16mm
EC 5	13mm	15mm	18mm
***Note: - EC- *Escherichia coli*, NI- No inhibition**			

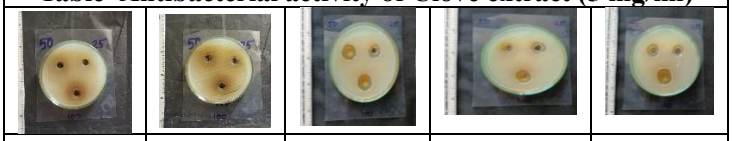
Table- Antibacterial activity of Clove extract (5 mg/ml)



EC 1	EC 2	EC 3	EC 4	EC 5

Table- Antibacterial activity of Clove extract (5 mg/ml)			
Isolate	**25µl**	**50 µl**	**100 µl**
EC 1	NI	NI	NI
EC 2	11mm	11mm	12mm
EC 3	13mm	16mm	16mm
EC 4	13mm	12mm	16mm
EC 5	13mm	15mm	18mm
***Note: -** EC- *Escherichia coli*, NI- No inhibition			

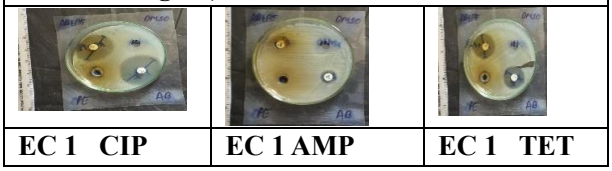
Table- Antibacterial activity of Clove extract (5 mg/ml)



EC 1	EC 2	EC 3	EC 4	EC 5

Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 1				
EC 1	**PE**	**AB**	**AB+ PE**	**DMSO**
CIP	12mm	32mm	28mm	NI
AMP	11mm	NI	10mm	NI
TET	12mm	19mm	21mm	NI
***Note: -** EC- *Escherichia coli*, NI- No inhibition, CIP- Ciprofloxacin, AMP- Ampicillin, TET- Tetracycline, PE- Plant extract, AB- Antibiotic, DMSO- Dimethyl Sulfoxide				

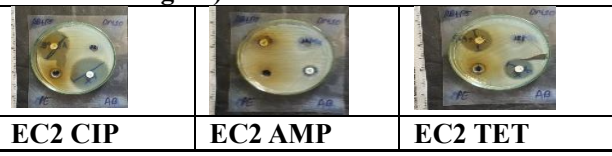
Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 1



EC 1 CIP	EC 1 AMP	EC 1 TET

Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 2				
EC 2	**PE**	**AB**	**AB+PE**	**DMSO**
CIP	12mm	34mm	30mm	NI
AMP	NI	12mm	11mm	NI
TET	12mm	21mm	22mm	NI

Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 2



EC2 CIP	EC2 AMP	EC2 TET

Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 3

EC 3	PE	AB	PE+AB	DMSO
CIP	NI	20mm	20mm	NI
AMP	NI	10mm	NI	NI
TET	11mm	30mm	24mm	NI

Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 3

EC 3 CIP | EC 3 AMP | EC 3 AMP

Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 4

EC 4	PE	AB	AB+ PE	DMSO
CIP	NI	32mm	NI	NI
AMP	10mm	10mm	11mm	NI
TET	NI	NI	NI	NI

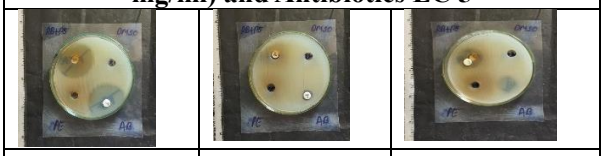
Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 4

EC 4 CIP | EC 4 AMP | EC 4 TET

Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 5

EC 5	PE	AB	AB+ PE	DMSO
CIP	NI	32mm	32mm	NI
AMP	NI	10mm	NI	NI
TET	NI	40mm	17mm	NI

Table- Synergistic Activity of Clove extract (5 mg/ml) and Antibiotics EC 5



EC 5 CIP | EC 5 AMP | EC 5 TET

Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotics EC 1

EC 1	AB	PE	AB + PE	DMSO
CIP	31 mm	NI	35 mm	NI
AMP	27 mm	17 mm	32 mm	NI
TET	19 mm	NI	29 mm	NI

Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotics EC 1

EC 1 CIP | **EC 1 AMP** | **EC 1 TET**

Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotics EC 2

EC 2	AB	PE	AB + PE	DMSO
CIP	34mm	NI	31mm	NI
AMP	10mm	NI	NI	NI
TET	16mm	NI	16mm	NI

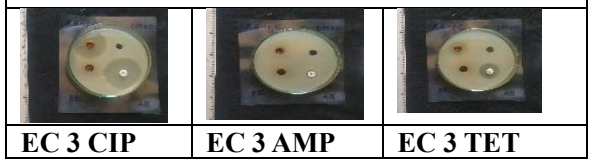
Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotics EC 2

EC 2 CIP | **EC 2 AMP** | **EC 2TET**

Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotics EC 3

EC 3	AB	PE	AB+PE	DMSO
CIP	32mm	NI	32mm	NI
AMP	20mm	NI	NI	NI
TET	18mm	NI	16mm	NI

Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotics EC 3

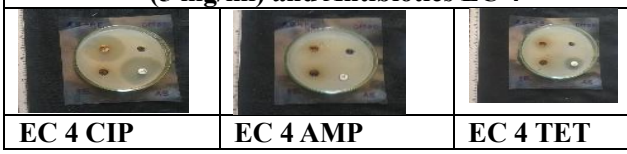


EC 3 CIP | **EC 3 AMP** | **EC 3 TET**

Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotics EC 4

EC 4	AB	PE	AB+PE	DMSO
CIP	35mm	NI	33mm	NI
AMP	10mm	NI	NI	NI
TET	17mm	NI	17mm	NI

Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotics EC 4

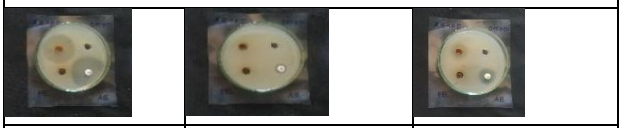


EC 4 CIP | **EC 4 AMP** | **EC 4 TET**

Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotics EC 5

EC 5	AB	PE	AB+PE	DMSO
CIP	35mm	NI	32mm	NI
AMP	10mm	NI	11mm	NI
TET	14mm	NI	13mm	NI

Table- Synergistic Activity of Ginger extract (5 mg/ml) and Antibiotic EC 5



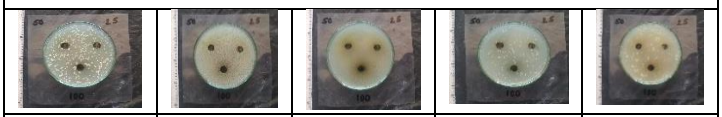
EC 5 CIP	EC 5 AMP	EC 5 TET

Table- Antibacterial activity of Sage extract (5 mg/ml)

Isolates	25 µl	50 µl	100 µl
EC 1	NI	NI	NI
EC 2	NI	NI	NI
EC 3	NI	NI	11 mm
EC 4	NI	NI	NI
EC 5	NI	NI	NI

***Note: -** EC- *Escherichia coli*, NI- No inhibition

Table- Antibacterial activity of sage extract (5 mg/ml)



EC 1	EC 2	EC 3	EC 4	EC 5

Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 1

EC1	PE	AB	AB + PE	DMSO
CIP	NI	30 mm	36 mm	NI
AMP	NI	11 mm	NI	NI
TET	NI	17 mm	20 mm	NI

***Note: -** EC- *Escherichia coli*, NI- No inhibition, CIP- Ciprofloxacin, AMP- Ampicillin, TET- Tetracycline, PE- Plant extract, AB- Antibiotic, DMSO- Dimethyl Sulfoxide

Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 1

EC 1 CIP	EC 1 AMP	EC 1 TET

Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 2

EC 2	PE	AB	AB + PE	DMSO
CIP	NI	31 mm	34 mm	NI
AMP	NI	11 mm	NI	NI
TET	Ni	16 mm	18 mm	NI

Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 2

EC 2 CIP	EC 2 AMP	EC 2 TET

Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 3

EC 3	PE	AB	AB + PE	DMSO
CIP	NI	28 mm	32 mm	NI
AMP	NI	11 mm	NI	NI
TET	NI	19 mm	25 mm	NI

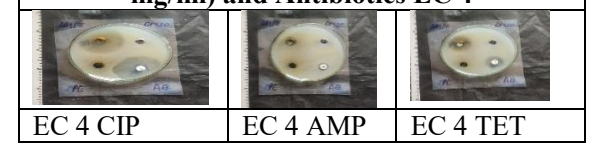
Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 3

EC 3 CIP	EC 3 AMP	EC 3 TET

Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 4

EC 4	PE	AB	AB + PE	DMSO
CIP	NI	35 mm	36 mm	NI
AMP	NI	12 mm	14 mm	NI
TET	NI	17 mm	22 mm	NI

Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 4



EC 4 CIP	EC 4 AMP	EC 4 TET

Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 5				
EC 5	**PE**	**AB**	**AB + PE**	**DMSO**
CIP	NI	32 mm	34 mm	NI
AMP	NI	13 mm	11 mm	NI
TET	NI	16 mm	23 mm	NI

Table- Synergistic Activity of Sage extract (5 mg/ml) and Antibiotics EC 5

EC 5 CIP | EC 5 AMP | EC 5 TET

Table- Antibacterial activity of Thyme extract (5 mg/ml)			
Isolates	**25 µl**	**50 µl**	**100 µl**
EC 1	12 mm	11 mm	15 mm
EC 2	10 mm	11 mm	17 mm
EC 3	NI	NI	NI
EC 4	NI	NI	13 mm
EC 5	NI	NI	12 mm
***Note: - Ec- *Escherichia coli*, NI- No inhibition**			

Table- Antibacterial activity of Thyme extract (5 mg/ml)

EC 1 | **EC 2** | **EC 3** | **EC 4** | **EC 5**

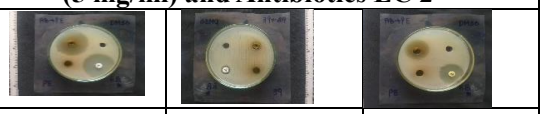
Table- Synergistic Activity of Thyme extract (5 mg/ml) and Antibiotics EC 1				
EC 1	**PE**	**AB**	**AB + PE**	**DMSO**
CIP	11 mm	37 mm	35 mm	NI
AMP	12 mm	11 mm	12 mm	NI
TET	12 mm	29 mm	30 mm	NI

Table- Synergistic Activity of Thyme extract (5 mg/ml) and Antibiotics EC 1

EC 1 CIP | **EC 1 AMP** | **EC 1 TET**

Table- Synergistic Activity of Thyme extract (5 mg/ml) and Antibiotics EC 2				
EC 2	**PE**	**AB**	**AB + PE**	**DMSO**
CIP	10 mm	31 mm	35 mm	NI
AMP	10 mm	11 mm	10 mm	NI
TET	NI	22 mm	22 mm	NI

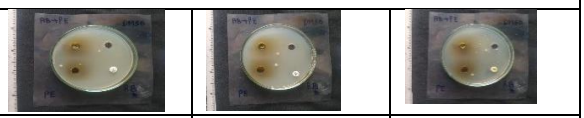
Table- Synergistic Activity of Thyme extract (5 mg/ml) and Antibiotics EC 2



EC 2 CIP | **EC 2 AMP** | **EC 2 TET**

Table- Synergistic Activity of Thyme extract (5 mg/ml) and Antibiotics EC 3				
EC 3	**PE**	**AB**	**AB + PE**	**DMSO**
CIP	NI	NI	NI	NI
AMP	NI	NI	NI	NI
TET	NI	NI	NI	NI

Table- Synergistic Activity of Thyme extract (5 mg/ml) and Antibiotics EC 3



EC 3 CIP | **EC 3 AMP** | **EC 3 TET**

Table- Synergistic Activity of Thyme extract (5 mg/ml) an Antibiotics EC 4				
EC 4	**PE**	**AB**	**AB + PE**	**DMSO**
CIP	11 mm	34 mm	32 mm	NI
AMP	NI	10 mm	NI	18 mm
TET	11 mm	22 mm	22 mm	NI

Table- Synergistic Activity of Thyme extract (5 mg/ml) and Antibiotics EC 4

EC 4 CIP | **EC 4 AMP** | **EC 4 TET**

Table- Synergistic Activity of Thyme extract (5 mg/ml) and Antibiotics EC 5				
EC 5	**PE**	**AB**	**AB + PE**	**DMSO**
CIP	12 mm	40 mm	38 mm	NI
AMP	NI	12 mm	11 mm	NI
TET	11 mm	22 mm	25 mm	NI

Table- Synergistic Activity of Thyme extract (5 mg/ml) and Antibiotics EC 5

EC 5 CIP | **EC 5 AMP** | **EC 5 TET**

Results and Discussion

The majority of methanolic plant extracts have demonstrated the ability to inhibit the growth of five isolates of *E. coli* bacteria. This indicates that these extracts contain bioactive compounds capable of hindering the growth of bacteria. In this experiment, the majority of plant extracts exhibited a zone of inhibition with diameters ranging from 10mm to 19mm. The plant extract with a concentration of 100ug/ml demonstrated the maximum zone of inhibition against the *E. coli* isolates. Among these, black pepper extract exhibited the highest antibacterial activity, with a zone of inhibition measuring 19mm. Following closely behind were clove, babul and thyme extracts which showed zone of inhibition measurements of 18mm, 17mm and 17mm respectively against the five isolates of *E. coli*.

Synergistic activity of plant extract in combination with antibiotics: Synergistic effect refers to a phenomenon where the combination of chemical substances or biological structures results in an effect that surpasses the sum of their individual effects. This concept is particularly relevant in the realm of antimicrobial agents where the combined action of two or more substances can yield a more potent antimicrobial effect than what each substance could achieve alone. This synergy often leads to enhanced solubility and broader antimicrobial activity

In the recent investigation, various plant extracts demonstrated diverse levels of synergy in inhibiting the growth of microorganisms. Notably, when combined with antibiotics, these plant extracts showcased a remarkable ability to enhance the inhibition zones against the tested microorganisms, surpassing the effects of antibiotics used alone. This suggests that while the plant extracts might not possess significant antimicrobial properties individually, their concurrent administration with antibiotics amplifies the efficacy of these drugs, highlighting their role as synergistic enhancers.

In recent years, there has been growing interest in exploiting the synergistic potential of combining different antimicrobials with plant extracts. This approach offers a unique strategy to bolster the antimicrobial activity of these substances[2]. By harnessing the complementary mechanisms of action inherent in diverse antimicrobial agents and plant extracts, researchers aim to create more effective treatments against microbial infections. This synergy-based approach holds promise for addressing the challenges posed by antimicrobial resistance and expanding the arsenal of antimicrobial therapies available to combat infectious diseases.

Five isolates of *E. coli*: The research aimed to assess the synergistic antimicrobial effects by combining three different antibiotics with a plant extract. The combination of ginger plant extract with antibiotics exhibited the most potent collaborative effect. When ginger plant extract was applied independently, it did not display any inhibition zone. On its own, tetracycline demonstrated a 19mm inhibition zone. However, when ginger plant extract was combined with tetracycline, the inhibition zone diameter increased significantly by 10mm, from 19mm to 29mm. This suggests a noteworthy enhancement in antibacterial activity when the two agents were used together compared to their individual effects.

The findings were consistent with those observed for ginger plant extract. When utilized independently, the black pepper extract did not produce any inhibition zone. However, when combined with tetracycline antibiotics, there was a notable increase in the inhibition zone diameter. Specifically, the zone of inhibition for tetracycline, when used alone, measured 19 mm. Yet, in conjunction with the black pepper plant extract, this zone expanded to 28mm, marking a significant 9 mm increase in inhibition zone diameter. This underscores the substantial enhancement in antibacterial effectiveness achieved through the combined application of the plant extract and antibiotics compared to their separate utilization.

Conclusion

This study underscores the significance of plant extracts in combating microbial growth, suggesting their potential as alternatives to traditional antibiotics. Specifically, the findings highlight the promising antimicrobial properties of leaf extracts from ginger, black pepper and sage, particularly when combined with antibiotics. Further exploration into the active constituents of these plants is essential to fully understand their mechanisms of action which could greatly contribute to the development of novel pharmaceuticals. By combining plant extracts with antibiotics, new treatment options for infectious diseases emerge, presenting plant extracts as potential agents for enhancing the effectiveness of antibiotics.

The study suggests that combining tetracycline with plant extracts can enhance its effectiveness in inhibiting efflux pumps in *E. coli* bacteria, potentially providing a novel method for combating bacterial resistance. The strongest activity was observed when tetracycline was used in combination with plant extracts, followed by ampicillin. This finding implies that the synergy between tetracycline and plant compounds may offer a promising strategy for overcoming antibiotic resistance mechanisms in bacteria like *E. coli*. Further research into this combination therapy could lead to the development of new treatments for bacterial infections.

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