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INVITRO ACTIVITY OF CERTAIN DRUGS IN COMBINATION WITH PLANT EXTRACTS AGAINST Staphylococcus aureus, Klebsiella pneumonia AND Epidermophyton floccosum

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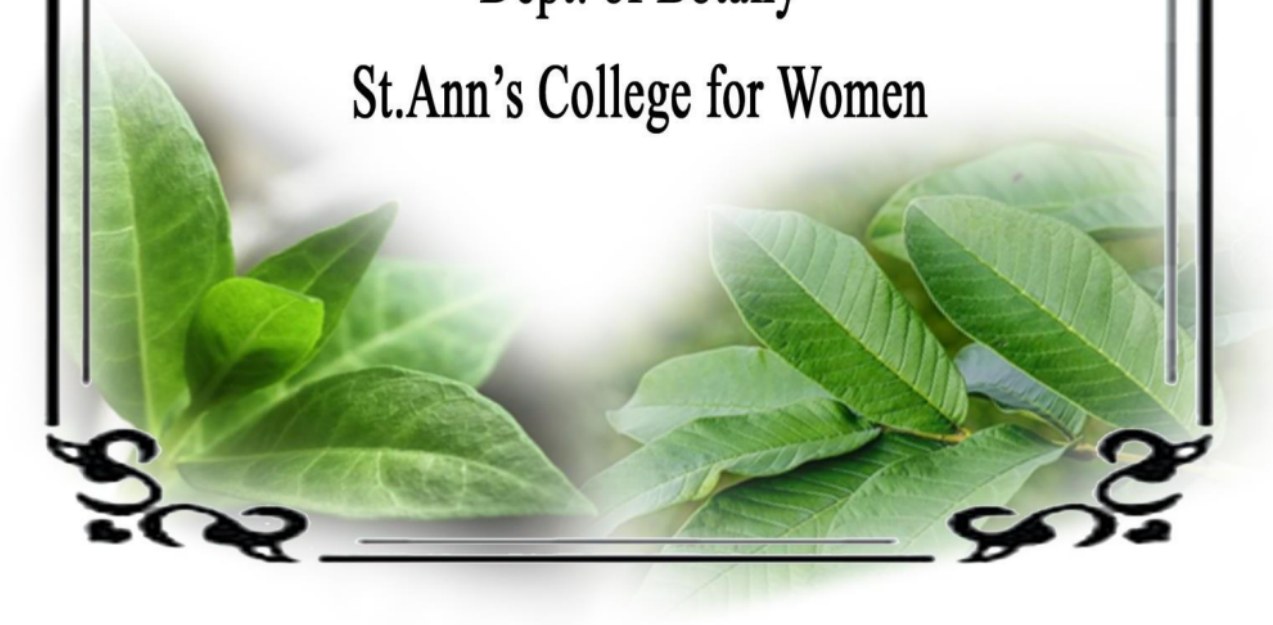

ABSTRACT

This is a study conducted to assess the interconnection between the methanolic draw out of *Lawsonia inermis* (leaf) and *Psidium guajava* (leaf) in combination with antimicrobial drugs including Ciprofloxacin and Amoxicillin against different microorganisms i.e.
Staphylococcus aureus, *Klebsiella pneumonia* and *Epidermophyton floccosum*.

The well diffusion method is used to assess the interaction between methanolic extracts and antimicrobial agents.

The experiment showed that methanolic extracts help increase the inhibition zones ofCiprofloxacin and Amoxicillin against the microbial strains taken.

This study evaluates the inhibition zones and also concludes the synergistic activity of antimicrobial drugs combined with plant extracts which may show a possible wayto treat infections caused by *S.aureus*, *K.pneumonia* and *E.floccosum*.

KEYWORDS: Methanol extracts, synergism, ciprofloxacin, Amoxicillin, *Staphylococcusaureus*, *Klebsiella pneumonia*, *Epidermophyton floccosum*.

INTRODUCTION

World's population sees deaths due to infections every single day. Infections are caused by many known and unknown pathogens invading human systems which may cause symptoms, pain and ultimately death. Although several drugs have been developed by pharmaceutical companies in past years, opposition to these drugs by bacteria has gone up over time which is now a major concern. [1] Microbial refusal to accept to classical antibiotics and their fast increment has brought up serious thinking in the cure of spreading diseases. Microbial spread are the reason for so many deaths each year worldwide. The happening of the advancement of resistance is responsible for the available antibacterial drugs to becoming less powerful or even ineffective. To get control of the resistance of prescription, many strategies have beensuggested; one of them includes a mix of other molecules with imperfect antibiotics which restores the beneficial antibacterial activity.[2]

Plants are widely known for their therapeutic properties and from centuries of being used in the field of medicine can play a key role in eradicating such clinical infections. Plant extracts have been used in many experiments for such desirable results as they contain many phytochemical compounds which may add strength in resistance against the microorganisms.

Its fact, bacteria have the genetic power to pass on and have resistance to drugsused as therapeutic representative. *S.aureus* (Gram-positive bacterium) and *K.pneumonia* (Gram-negative bacterium) are recognized as the foremost reason of infections in people happening in both the community and hospitals. *S.aureus* and *K.pneumonia* are responsible for common respiratory infections while *E.floccosum* is a filamentous fungus that is responsible for skin-related infections.

Drug synergism in the middle of studied antimicrobial agents and bioactive plant extracts is a great concept that has been used widely now. [3] This study was carried out to synthesize methanolic extract using *Lawsonia inermis* and *Psidium guajava* were analyzed for their antibacterial activity along with antimicrobial agents against pathogenic bacteria in laboratory conditions.

Synergism is a new concept in developing agents for antimicrobial activity. The new

approach is a therapy or mix of synergistic therapy in opposition to resistance microorganisms which make way to new ways of handling infectious diseases.[4]
In present study, we reported the inhibition zones and synergistic work of the methanolic extracts of *Lawsonia inermis* (leaf) and *Psidium guajava* (leaf) in combinationwith antimicrobial drugs including Ciprofloxacin and Amoxicillin against different microorganisms i.e. *Staphylococcus aureus*, *Klebsiella pneumonia* and *Epidermophyton floccosum*.

Lawsonia inermis (L.)

Psidium guajava (L.)

Methods and Materials

PLANT MATERIAL:

Dried leaves of *Lawsonia inermis* and *Psidium guajava* are gathered from the neighbourhood of Visakhapatnam, Andhra Pradesh, India. The plant bits were rinsed and air-dried at room temperature.

PLANT EXTRACT PREPARATION:

The put together plant materials are cut into small pieces, shade dried and powdered in a Willy mill. This material was put to weigh measure and extracted with Methanol along with a soxhlet extractor for 5-6 hours at a temperature not going beyond the boiling point that the solvent possess. For every 100 grams of dry material, 2 litres of solventwere used. The taken out solvents were concentrated under reduced pressure with a rotary evaporator. The residue received was considered as crude extracts and kept in a freezer until assayed. 1g of each extract was taken and dissolved in 1ml of Di MethylSulphoxide (DMSO). Thus 500, 250 and 125mg/ml of stock were obtained as a standard concentration of extracts.

BACTERIAL STRAINS:

Three mother cultures of *S.aureus, K.pneumonia* and *E.floccosum* obtained from Adhya Bioscience laboratories are used to evaluate the increase in inhibition zones and show the synergistic effect between plant extracts and antimicrobial drugs against the respective infections.

ANTIMICROBIAL DRUGS:

Two drugs are used for the evaluation studies which include Ciprofloxacin and Amoxicillin. Desired concentrations of antibiotic drugs were prepared using water as a solvent for ciprofloxacin and amoxicillin solutions.

ANTIMICROBIAL TESTS:

Well-diffusion method is used to measure the antibacterial activity in the experiment. Three Petri plates containing 20ml of Nutrient agar media were inoculated with a 24-hour culture of Bacterial strains. 4 wells of 6mm diameter each were punched in the Petri plates containing nutrient agar media. 3 wells in each plate were filled with 125mg/ml, 250mg/ml and 500mg/ml standard concentrations of extracts/antibiotic or combination respectively and 4th well is filled with 30μl of either which is considered as a control. The Petri plates were incubated at 37°C for 24 hours. Assessment of antibacterial activity is done by measuring the bacterial inhibition zones around the well with a zone scale. The average of three replicates for each extract, antibiotic and combination was assayed.

Results and Discussion

Different mechanisms of antimicrobial drugs are seen in this experiment. Some notable synergistic interactions (Amoxicillin with *L.inermis* and *P.guajava* against *S.aureus, K.pneumonia* and *E.floccosum*) and antagonistic interactions (Ciprofloxacin with *L.inermis* and *P.guajava* against *S.aureus* and *E.floccosum*) were identified. While Ciprofloxacin showed no effect against *K.pneumonia* when combined with plant extracts. The data represented below show the potential effect of antimicrobial drugs in combination with plant extracts used against *S.aureus*, *K.pneumonia* and *E.floccosum*. [Table 1, 2, 3]

TABLE 1 - INHIBITION EFFECT AGAINST *Staphylococcus aureus*

DRUG TARGET	DRUG	*Psidium guajava*	*Lawsonia inermis*	SYNERGISM RATE [EXTRACT/DRUG]	MEAN
Nucleic acid synthesis inhibitor	Ciprofloxacin	A	A	0	0
Cell Wall Biosynthesis inhibitor	Amoxicillin	S	S	2	2
TOTAL	2	1	1	-	-

(A) – Antagonism; (S) – Synergism; (No change)

TABLE 2 - INHIBITION EFFECT AGAINST Klebsiella pneumonia

DRUG TARGET	DRUG	*Psidium guajava*	*Lawsonia inermis*	SYNERGISM RATE [EXTRACT/DRUG]	MEAN
Nucleic acid synthesis inhibitor	Ciprofloxacin	No change	No change	-	-
Cell Wall Biosynthesis inhibitor	Amoxicillin	S	S	2	2
TOTAL	2	1	1	-	-

(A) – Antagonism; (S) – Synergism; (No change)

TABLE 3 - INHIBITION EFFECT AGAINST *Epidermophyton floccosum*

DRUG TARGET	DRUG	*Psidium guajava*	*Lawsonia inermis*	SYNERGISM RATE [EXTRACT/DRUG]	MEAN
Nucleic acid synthesis inhibitor	Ciprofloxacin	A	A	0	0
Cell Wall Biosynthesis inhibitor	Amoxicillin	S	S	2	2
TOTAL	2	1	1	-	-

(A) – Antagonism; (S) – Synergism; (No change)

Respective inhibition zones assayed in this experiment are mentioned following the plant extracts taken [Table 4, 5, 6]. Assay of Inhibition is done by taking zero as negative control and positive control for a respective antibiotic used against the respective microorganism varied.

TABLE 4 – INHIBITION ZONES OF *P.guajava*

Psidium guajava (Leaf)								
MICROBIAL STRAIN	**Ciprofloxacin + Plant extract (mg/ml)**			**Ciprofloxacin (control) (30µl)**	**Amoxicillin + Plant extract (mg/ml)**			**Amoxicillin (control) (30µl)**
	125mg/ml (30µl)	**250mg/ml (30µl)**	**500mg/ml (30µl)**		**125mg/ml (30µl)**	**250mg/ml (30µl)**	**500mg/ml (30µl)**	
S.aureus	19mm	20mm	20mm	28mm	15mm	15mm	16mm	11mm
K.pneumonia	37mm	37mm	40mm	40mm	18mm	20mm	21mm	16mm
E.floccosum	33mm	31mm	28mm	40mm	16mm	18mm	22mm	19mm

TABLE 5 – INHIBITION ZONES OF *L.inermis*

Lawsonia inermis (Leaf)								
MICROBIAL STRAIN	**Ciprofloxacin + Plant extract (mg/ml)**			**Ciprofloxacin (control) (30µl)**	**Amoxicillin + Plant extract (mg/ml)**			**Amoxicillin (control) (30µl)**
	125mg/ml (30µl)	**250mg/ml (30µl)**	**500mg/ml (30µl)**		**125mg/ml (30µl)**	**250mg/ml (30µl)**	**500mg/ml (30µl)**	
S.aureus	21mm	23mm	26mm	28mm	10mm	11mm	11mm	11mm
K.pneumonia	35mm	39mm	40mm	40mm	17mm	20mm	24mm	18mm
E.floccosum	30mm	31mm	33mm	40mm	16mm	17mm	22mm	21mm

It is recognized that *S.aureus* is one of the leading causes of infections that occur in both the community and the hospital. As with many nosocomial pathogens, Multidrug-resistant Staphylococci are extremely difficult to treat because they are resistant to almost all antibiotics clinically available right now and also can cause meningitis. [5] A new approach to solving the bacterial resistance problem depends on the ability of plant extracts to act synergistically with antibiotics. [5] In this study, reference and environmental strains of pathogenic organisms were used to examine drug resistance in clinical settings often associated with these organisms. To determine the effects of combinations with antibiotics, extracts of plants are used. [6]

The therapeutic potential of leaf extracts from *L.inermis* and *P.guajava* has demonstrated the strong synergy between crude methanolic extract of the leaves and first-line antibiotics resulting in clinically useful applications against respective microbial strains. [5, 6] Bacterial infections can be treated with advantageous synergistic effects when combined antibiotic therapy is used.

This novel concept of synergism can either be beneficial (additive/synergistic interactions) or deleterious (Antagonistic/toxic interactions).[7] A study reported a synergistic effect between the plant extracts and the cell wall biosynthesis inhibitor against *S.aureus* and *K.pneumonia* while not much is known about *E.floccosum*.

In previous studies, amoxicillin was secured to synergize well with the various phytochemical compounds. However, Nucleic acid synthesis inhibitors and plant extracts had no synergistic activity. [5]

In gram-positive and gram-negative pathogens such as *Staphylococcus aureus* and *Klebsiella pneumonia*, MDP pumps, which recognized and remove a variety of compounds with unrelated structures from bacterial cells, have been identified. In vitro, it has been demonstrated that some compounds can work synergistically with antibiotics to modify the resistance phenotype in bacteria. [8] As they exhibit a low risk of causing bacterial resistance to their actions, combinations of antibiotics and plant extracts are likely to provide the basis for creating new approaches in resistance modifying agents. These extracts have a combination of different bioactive compounds, which makes microbial adaption extremely difficult compared to single component antibiotics. [5]

Our research indicates that plant extracts strengthen the antimicrobial effects when combined with drugs against clinical infections and can reduce the spread of bacteria. [7]

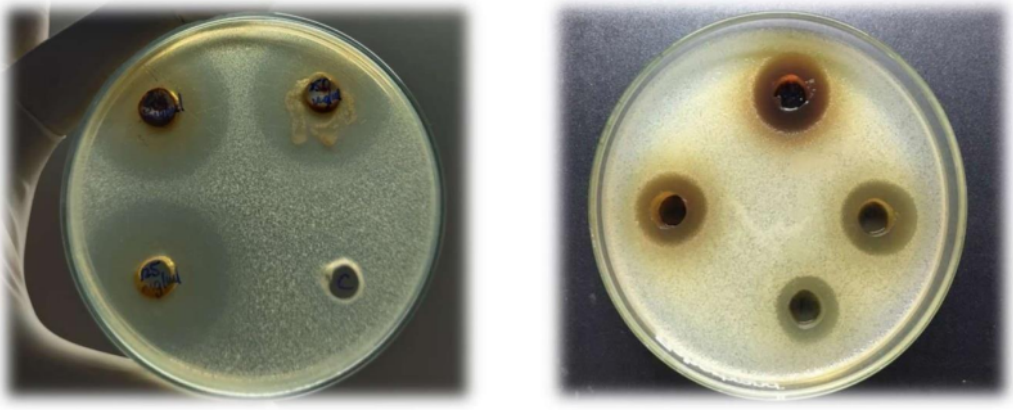

Fig: *A closer view of inhibition zones observed in the experiment.*

Conclusion

This study concluded that the extracts of *L.inermis* and *P.guajava* are found to have the capacity of increasing the susceptibility of the studied microbial strains to various antimicrobial drugs. The present study clearly states the possibility of the use of the above shown synergistic drug (amoxicillin)-plant combinations for combating infectious diseases caused by *S.aureus*, *K.pneumonia* and *E.floccosum* whereas ciprofloxacin showed a negative effect when combined with both plant extracts.

The results represented in this respective report were encouraging in correlation with amoxicillin, although clinical controlled studies are needed to define the real efficacy and possible toxic effects in vivo. This study majorly suggests the possibility of concurrent use of these antimicrobial drugs and extracts in combination in treating infections caused by respective strains used and plants may play a key future part in clinical studies.

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