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

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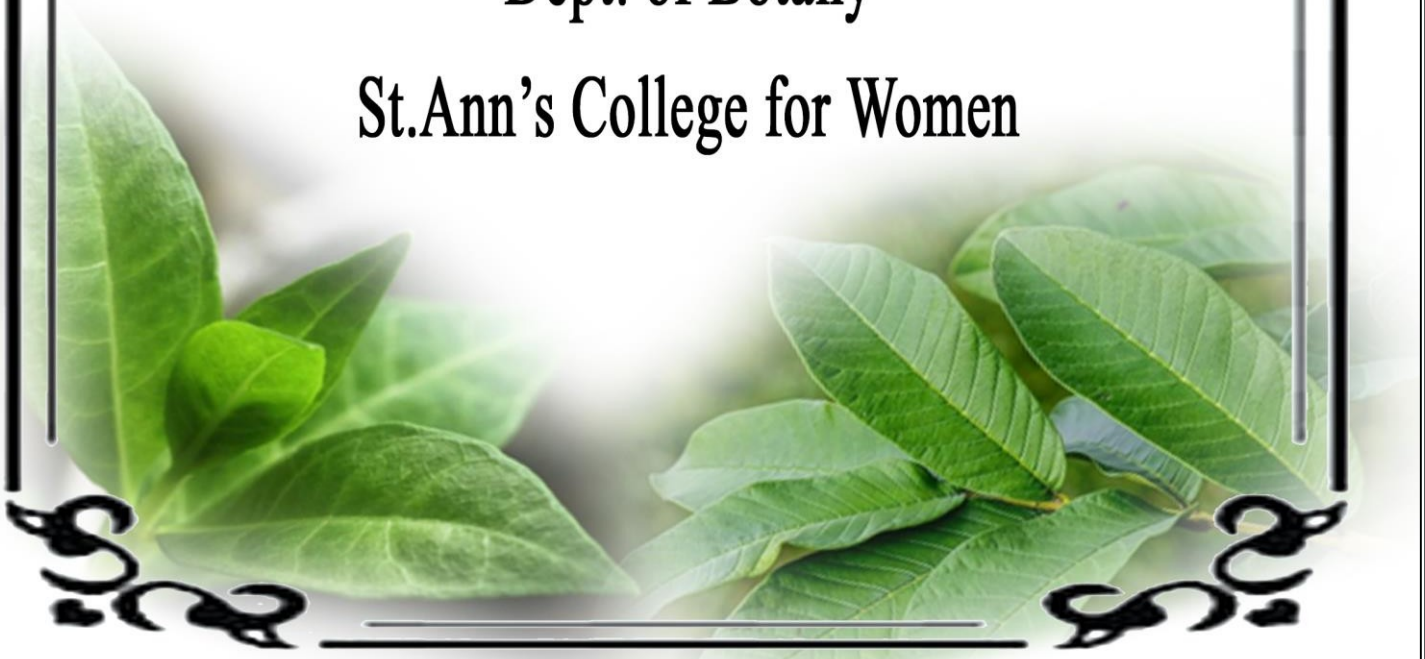


ABSTRACT

Thisisastudyconductedtoassesstheinterconnectionbetweenthemethanolicdraw out of *Lawsonia inermis* (leaf) and *Psidium guajava* (leaf) in combination withantimicrobial drugs including Ciprofloxacin and Amoxicillin against differentmicroorganismsi.e. *Staphylococcusaureus*,*Klebsiellapneumonia*and*Epidermophytonfloccosum.*

Thewelldiffusionmethodisusedtoassesstheinteractionbetween methanolicextractsandantimicrobialagents.

Theexperimentshowedthatmethanolicextractshelpincreasetheinhibitionzonesof Ciprofloxacin andAmoxicillin againstthemicrobialstrainstaken.

This study evaluates the inhibition zones and also concludes the synergisticactivityofantimicrobialdrugscombinedwithplantextractswhichmaysh owapossible wayto treat infections caused by *S.aureus*, *K.pneumonia* and*E.floccosum.*

KEYWORDS:Methanolextracts,synergism,ciprofloxacin,Amoxicillin, *Staphylococcusaureus*,*Klebsiellapneumonia*,*Epidermophytonfloccosum.*

INTRODUCTION

World's population sees deaths due to infections every single day. Infections arecaused by many known and unknown pathogens invading human systems which maycause symptoms, pain and ultimately death. Although several drugs have beendeveloped by pharmaceutical companies in past years, opposition to these drugs bybacteria has gone up over time which is now a major concern. [1] Microbialrefusal to accept to classical antibiotics and their fast increment has brought up seriousthinking in the cure of spreading diseases. Microbial spread are the reasonfor so many deaths each year worldwide. The happening of the advancement ofresistance is responsible for the available antibacterial drugs to becoming less powerful orevenineffective.Toget control oftheresistanceofprescription,manystrategieshavebeensuggested; one of them includes a mix of other molecules with imperfectantibioticswhichrestoresthe beneficialantibacterialactivity.[2]

Plantsarewidelyknownfortheirtherapeuticpropertiesandfromcenturiesofbeingused in the field of medicine can play a key role in eradicating such clinicalinfections. Plant extracts have been used in many experiments for such desirableresults as they contain many phytochemical compounds which may add strength inresistanceagainstthemicroorganisms.

Its fact,bacteriahavethegeneticpowertopass on andhaveresistancetodrugsused as therapeutic representative. *S.aureus* (Gram-positive bacterium) and *K.pneumonia*(Gram-negative bacterium) are recognized as the foremost reason of infections inpeople happening in both the community and hospitals. *S.aureus* and *K.pneumonia*are responsible for common respiratory infections while *E.floccosum* is a filamentousfungusthatisresponsibleforskin-relatedinfections.

Drugsynergismin the middle ofstudiedantimicrobialagents andbioactiveplantextractsis agreat concept that has been used widely now. [3] This study was carried out tosynthesize methanolic extract using *Lawsonia inermis* and *Psidium guajava* wereanalyzed for their antibacterial activity along with antimicrobial agents againstpathogenicbacteriain laboratoryconditions.

Synergismisanewconceptindevelopingagentsforantimicrobialactivity.Thenew

approach is a therapy or mix of synergistic therapy in opposition to resistancemicroorganismswhichmake waytonewwaysofhandlinginfectiousdiseases.[4] In present study, we reported the inhibition zones and synergistic work of themethanolic extracts of *Lawsonia inermis* (leaf) and *Psidium guajava* (leaf) in combinationwith antimicrobial drugs including Ciprofloxacin and Amoxicillin against differentmicroorganisms i.e. *Staphylococcus aureus*, *Klebsiella pneumonia* and *Epidermophytonfloccosum.*

Lawsoniainermis(L.)

Psidiumguajava(L.)

MethodsandMaterials

PLANTMATERIAL:

Driedleavesof*Lawsoniainermis*and*Psidiumguajava*are gatheredfromthe neighbourhoodof Visakhapatnam, Andhra Pradesh, India. The plant bits were rinsed and air-driedat roomtemperature.

PLANTEXTRACTPREPARATION:

The put together plant materials are cut into small pieces, shade dried andpowdered in a Willy mill. This material was put to weigh measure and extractedwith Methanol along with a soxhlet extractor for 5-6 hours at a temperature not going beyondtheboilingpointthat the solvent possess.Forevery100gramsofdrymaterial,2litresofsolventwere used. The taken out solvents were concentrated under reduced pressure with arotary evaporator. The residue received was considered as crude extracts and kept inafreezer untilassayed.1gofeachextract wastakenanddissolved in1mlofDiMethylSulphoxide (DMSO). Thus 500, 250 and 125mg/ml of stock were obtained as astandardconcentrationofextracts.

BACTERIALSTRAINS:

Threemothercultures of*S.aureus, K.pneumonia*and*E.floccosum*obtainedfromAdhyaBiosciencelaboratoriesareusedtoevaluatetheincreaseininhibitionzonesandshow the synergistic effect between plant extracts and antimicrobial drugs against therespectiveinfections.

ANTIMICROBIALDRUGS:

Two drugs are used for the evaluation studies which include Ciprofloxacin andAmoxicillin.Desiredconcentrationsofantibioticdrugswerepreparedusingwaterasasolvent forciprofloxacinandamoxicillin solutions.

ANTIMICROBIALTESTS:

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 thePetri plates containing nutrient agar media. 3 wells in each plate were filled with125mg/ml, 250mg/ml and 500mg/ml standard concentrations of extracts/antibiotic orcombinationrespectivelyand4thwellisfilledwith30µlofeitherwhichis consideredas a control. The Petri plates were incubated at 37°C for 24 hours. Assessment ofantibacterial activity is done by measuring the bacterial inhibition zones around thewell with a zone scale. The average of three replicates for each extract, antibiotic andcombinationwasassayed.

ResultsandDiscussion

Different mechanisms of antimicrobial drugs are seen in this experiment. Somenotable synergistic interactions (Amoxicillin with *L.inermis* and *P.guajava* against*S.aureus,K.pneumonia*and*E.floccosum*)andantagonisticinteractions(Ciprofloxacinwith*L.inermis*and*P.guajava*against*S.aureus*and*E.floccosum*)wereidentified. WhileCiprofloxacinshowednoeffectagainst *K.pneumonia*whencombinedwithplantextracts.Thedatarepresentedbelowshowthepotentialeffectofantimicrobialdrugs incombination with plant extracts used against *S.aureus, K.pneumonia* and *E.floccosum*.[Table1,2,3]

TABLE1-INHIBITION EFFECTAGAINST*Staphylococcusaureus*

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

(A) –Antagonism;(S)–Synergism;(Nochange)

TABLE2-INHIBITIONEFFECTAGAINSTKlebsiellapneumonia

DRUG TARGET	DRUG	*Psidium guajava*	*Lawsonia inermis*	SYNERGISM RATE[EXTRACT/ DRUG]	MEAN
Nucleic acidsynthesisinhibitor	Ciprofloxacin	Nochange	Nochange	-	-
CellWallBiosynthesisinhibitor	Amoxicillin	S	S	2	2
TOTAL	2	1	1	-	-

(A) –Antagonism;(S)–Synergism;(Nochange)

TABLE3-INHIBITIONEFFECTAGAINST*Epidermophytonfloccosum*

DRUG TARGET	DRUG	*Psidium guajava*	*Lawsonia inermis*	SYNERGISM RATE[EXTRACT/ DRUG]	MEAN
Nucleicacid synthesisinhibitor	Ciprofloxacin	A	A	0	0
CellWallBiosynthesisinhibitor	Amoxicillin	S	S	2	2
TOTAL	2	1	1	-	-

(A)–Antagonism; (S)–Synergism;(Nochange)

Respective inhibition zones assayed in this experiment are mentioned following the plantextractstaken[Table4, 5, 6].AssayofInhibitionis donebytakingzeroasnegativecontroland positive control for a respective antibiotic used against the respective microorganismvaried.

TABLE4– INHIBITION ZONES OF*P.guajava*

Psidiumguajava(Leaf)								
MICROBIAL STRAIN	Ciprofloxacin + Plant extract(mg/ml)			Ciprofloxacin (control)(30 µl)	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

TABLE5–INHIBITIONZONESOF*L.inermis*

Lawsoniainermis (Leaf)								
MICROBIAL STRAIN	**Ciprofloxacin + Plant extract(mg/ml)**			**Ciprofloxacin (control)(30 µl)**	**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

Itisrecognizedthat*S.aureus*isoneoftheleadingcausesofinfectionsthatoccurinboththe community and the hospital. As with many nosocomial pathogens, Multidrug-resistant Staphylococci are extremely difficult to treat because they are resistant toalmost all antibiotics clinically available right now and also can cause meningitis. [5] Anew approach to solving the bacterial resistance problem depends on the ability of plantextracts to act synergistically with antibiotics. [5] In this study, reference andenvironmental strains of pathogenic organisms were used to examine drug resistance inclinical settings often associated with these organisms. To determine the effects ofcombinationswithantibiotics,extracts ofplantsareused.[6]

Thetherapeutic potentialofleafextractsfrom*L.inermis*and*P.guajava*hasdemonstrated the strong synergy between crude methanolic extract of the leaves andfirst-line antibiotics resulting in clinically useful applications against respectivemicrobialstrains.[5,6]Bacterialinfectionscanbetreatedwithadvantageoussynergistic effectswhencombinedantibiotictherapy isused.

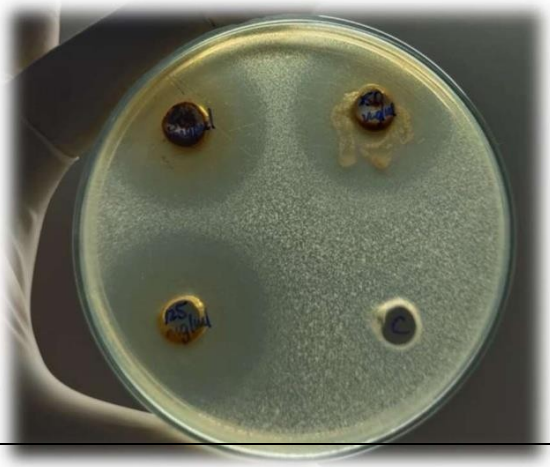
This novel concept of synergism can either be beneficial (additive/synergisticinteractions) or deleterious (Antagonistic/toxic interactions).[7] A study reported asynergisticeffectbetweentheplantextractsandthecellwallbiosynthesisinhibitoragainst*S.aureus* and*K.pneumonia*while notmuchis knownabout*E.floccosum*.

In previous studies, amoxicillin was secured to synergize well with the variousphytochemicalcompounds.However,Nucleicacidsynthesisinhibitorsandplantextractshadnosynergisticactivity.[5]

In gram-positive and gram-negative pathogens such as *Staphylococcus aureus* and*Klebsiella pneumonia*, MDP pumps, which recognized and remove a variety ofcompounds with unrelated structures from bacterial cells, have been identified. In vitro,ithasbeendemonstratedthatsomecompoundscanworksynergisticallywithantibioticsto modify the resistance phenotype in bacteria. [8] As they exhibit a low risk of causingbacterial resistance to their actions, combinations of antibiotics and plant extracts arelikelytoprovidethebasisforcreatingnewapproaches inresistancemodifyingagents.

These extracts have a combination of different bioactive compounds, which makesmicrobialadaptionextremelydifficultcomparedtosinglecomponentantibiotics.[5]

Our research indicates that plant extracts strengthen theantimicrobialeffectswhencombinedwithdrugsagainstclinicalinfectionsandcanreducethespreadofbacteria.[7]



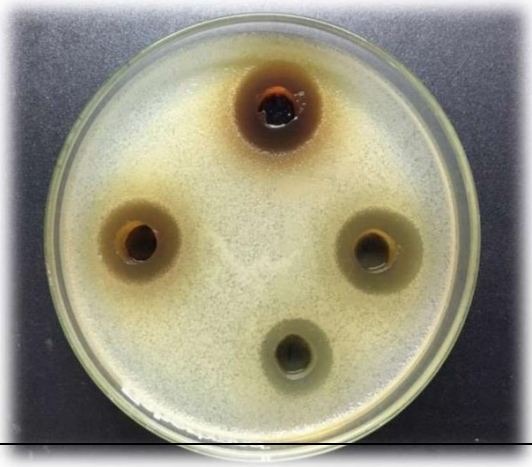


Fig:*Acloserviewofinhibitionzonesobservedintheexperiment.*

Conclusion

This study concluded that the extracts of *L.inermis* and *P.guajava* are found to have thecapacity of increasing the susceptibility of the studied microbial strains to variousantimicrobial drugs. The present study clearly states the possibility of the use of theaboveshownsynergisticdrug(amoxicillin)-plantcombinationsforcombatinginfectiousdiseases caused by *S.aureus*, *K.pneumonia* and *E.floccosum* whereas ciprofloxacinshowedanegativeeffectwhencombinedwithbothplantextracts.

The results represented in this respective report were encouraging in correlation withamoxicillin,althoughclinicalcontrolledstudies areneededtodefinetherealefficacyand possible toxic effects in vivo. This study majorly suggests the possibility ofconcurrent use of these antimicrobial drugs and extracts in combination in treatinginfections caused by respective strains used and plants may play a key future part inclinicalstudies.

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