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Phytochemical analysis and *in vitro* synergistic efficacy of leaf extracts of *Acacia polyacantha* and antibiotics against MDR *Klebsiella SPP.*

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ABSTRACT

Emerging Multidrug-resistant problem is a major concern. *Klebsiella sp.* can lead to a wide range of disease causing pathogen namely pneumonia, urinary tract infections, septicemia, meningitis, diarrhea and soft tissue infections and development of MDR against commonly used antibiotics making the case difficult to manage. Total 525 Clinical samples were screened for isolation of *Klebsiella sp*. Total 36 isolates belonging to *Klebsiella sp*. Were obtained. 29(8.06%) isolates were associated with urinary tract infection (UTI), 6(12%) with sputum and 1(1.54%) was associated with blood. Antibiogram study of these isolates revealed that all these isolates are resistant to several antibiotics out of 34 antibiotics tested. Cold and hot acacia leaf petroleum ether extract, cold and hot acacia leaf chloroform extract, cold and hot acacia leaf acetone extract, cold and hot acacia leaf methanol extract and cold and hot acacia leaf water extract were used for synergistic study. Qualitative phytochemical analysis these leaf extracts showed the prominent presence of alkaloids, Carbohydrates and glycosides, proteins and amino acids and phytosteroids. The studies on these extracts of *Acacia polycantha* and antibiotics on susceptibility of resistant *Klebsiella* isolates showed that both cold and hot solvent extracts are effective on antibiotics. The effectiveness shown by these extracts may be useful in evaluating the efficacy of combination therapy against MDR-*Klebsiella sp*.

Keywords: Phytochemical, Antibiotics, *Multidrug resistance, Klebsiella sp*.

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INTRODUCTION

Klebsiella genus is one of the most significant members of *Enterobacteriaceae*. Now days Klebsiellae are important pathogens in nosocomial infections. *Klebsiella pneumoniae* is a Gram-negative in nature ,non motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It was found in the normal flora of the mouth, skin, and intestines and causes destructive changes in human lungs if aspirated, specifically to the alveoli resulting in bloody sputum. *Klebsiella* organisms are often found resistant to multiple antibiotics. Current evidence implicates plasmids as the primary source of the resistance genes. *Klebsiella* with the ability to produce extended-spectrum beta-lactamases (ESBL) are resistant to many classes of antibiotics. The most frequent resistances include resistance to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and trimethoprim/sulfamethoxazoles. Now a day, multidrug resistance has become a major global problem throughout the world. The increased prevalence of antibiotic-resistant bacteria due to the repeated exposures and extensive use of antibiotics have made the current antimicrobial agents inefficient to control several bacterial diseases. One of the challenges infighting this problem is to have continuous search for new, safe and effective antimicrobials as alternative agents to substitute for the existing less effective ones. Plants have been conventionally used as a rich source of novel drug compounds and the herbal mixtures contribute remarkably to human health and well-being (1). Plants have unique capacity to produce variety of phytochemicals and they are generally useful in good synergistic therapy which act as multidrug resistance modifiers(2).Medicinal Plants are rich source of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones and this content plays important role in the treatment of several diseases (3). These natural products obtained from plant extracts have great potential as antimicrobial compounds against drug resistant microorganisms(4).

In the treatment of drug resistant infections, combination of antibiotics has often been used. Kazal*et al* have defined antimicrobial synergism as when two or more antimicrobial agents, in combination exert an inhibitory effect that is greater than the additive effect of the individual antibiotic (5). Use of antimicrobial drugs in future is still uncertain due to growing multidrug resistance among the bacteria. Thus it is necessary to understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural (6).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Combination of phytochemicals and antimicrobial substances against the multiresistant strains showed encouraging results. There are different

approaches to cure and control the infection caused by the multidrugs resistance (MDR) strains of bacteria. One of which is by isolation of active phytochemicals that can help to prevent the spread of infection, another method is to formulate new synergistic combinations using different commercially available antibiotics or to combine an antibiotic with active phytochemicals that have antimicrobial properties (7). Most of the studies on use of combination of Phytochemicals and antibiotic were carried out against the multiresistant*Pseudomonas*(5,7,8,9).

MATERIALS AND METHOD

Collection of clinical samples:

Clinical samples such as urine, pus, blood and sputum sample were collected from different pathology laboratories of Nagpur (MS), India.

Isolation of*Klebsiella sp*.from various clinical Samples:

A sample was immediately transferred to sterile nutrient broth for enrichment under aseptic condition and incubated at 37^0C for 48 hrs. After 48 hours, loopful of culture from enriched nutrient broth was plated on MacConkey media, so as to get well isolated colonies. Suspected colonies of *Klebsiella sp*.showing pink colour mucoid colony were picked up and were maintained on nutrient agar slant for further identification.

Identification of Isolates: Isolates were identified on the basis of morphological, cultural & biochemical characteristics and the results were compared with Bergey's Manual of Determinative Bacteriology 9th edition as well as confirmed by biochemical identification using Vitek 2 System (Figure.:1).

Preparation of inoculums:

A loopful of culture from slants was inoculated into the screw cap tube containing 5ml sterile nutrient broth and incubated at 37^0C for 24hrs. Again loopful of culture from same broth was transferred to fresh 5ml of sterile nutrient broth and incubated at 37^0C for 6-8 hrs. Turbidity was adjusted according to 0.5 McFarland standards which were then used as an inoculum which corresponds to size of 1.5×10^8 CFU/ml.

Antibiotic Susceptibility Test:

Antimicrobial susceptibility testing was performed bythe disc diffusion method with commercially availablediscs (HiMedia, Mumbai, India) of Trimethoprim TR(5mcg),Tigecycline TGC(15mcg),Ofloxacin OF(2mcg), Kanamycin K(30mcg), Cefazolin CZ(30mcg),Nalidixic acid NA(30mcg), Co-Trimoxazole COT(25mcg)Nitrofurantoin NIT(300mcg), Minocycline MI(30mcg), Tobramycin TOB(10mcg), Neomycin N(30mcg),Amoxyclav AMC(50mcg),Cefixime

CFM(5mcg),Colistin CL(10mcg), Ampicillin AMP(10mcg),Carbenicillin CB(100mcg), Tetracycline TE(30mcg), Ciprofloxacin CIP(5mcg),Imipenem IPM(10mcg),Netillin NET(30mcg), Ceftriaxone CTR(30mcg),Ceftazidime CAZ(30mcg),Meropenem MRP(10mcg),Cefotaxime CTX(30mcg), Gentamicin GEN(10mcg),Norfloxacin NX(10mcg),Amikacin AK(30mcg), Cefoperazone CPZ(75mcg), Piperacillin/Tazobactam PIT(100/10 mcg). Selected antibiotic discs placedover plates seeded with 100 µl broth culture (0.5 McFarland standards) over surface of Hi sensitivity test agar andplates were kept undisturbed in a refrigerator for 1 hour. Then plates were removed from refrigerator and shifted toincubator maintained at 37^0C for 18-24hrs. After incubationall plates were examined for zone of inhibition and zonewas noted down. Isolates were considered susceptible, intermediate, or resistant to a particular antimicrobial agent on the basis of the diameters of the inhibitory zones that matched the criteria of the manufacturer's interpretive table, which followed the recommendations ofthe performance standard for antimicrobial disk susceptibility test, CLSI (10). (formerly NCCLS)

Collection and maintenance of Plant Material:

The leaves of the plant, *Acacia polycantha* growing in the local areas of Nagpur district of Maharashtra State were collected during July 2013. The plant material was washed thoroughly, initially with tap water and then with distilled water to remove any debris or dust particles and was then allowed to dry in an oven at 40^oC. The dried plant material was ground to a fine powder and stored at room temperature in airtight containers for further use.

Preparation of plant extracts:

Hot extract (Soxhlet extract):

Over 25 g of powdered leaf was filled in Soxhlet thimble and refluxed in Soxhlet apparatus with 250 ml of desired solvent for 24 hrs. Solvent were used in a sequence with increasing order of polarity of the solvent, petroleum ether (60^0C- 80^0C), chloroform, acetone, methanol and water. Before every extraction, material inside the thimble was exposed to air to evaporate preceding solvent absorbed in material. After 24 hrs solvent is recovered till approximately55 -60ml of extract remained in round bottom flask. The solvents were then evaporated at appropriate temperature for each solvent until a very concentrated extract (less than50 ml) was obtained and final volume was made up to 50ml in volumetric flask.

Cold extract:(Maceration process)

25 g of powdered leaf was tide loosely in nylon cloth(400 mesh) and immersed in 100 ml of solvent in 250 ml conical flask with stopper and kept at 25^0C under 150 rpm in rotary B.O.D incubator for 24 hrs. Successive extraction of same powdered material was carried out in selected

series of solvents. Before every extraction ,material was exposed to air to evaporate preceding solvent absorbed in material. After 24 hrs solvent is evaporated at room temperature to obtain final volume of 50 ml.

Total 10 extracts were prepared and they were abbreviated and hereafter referred by their abbreviation. Hot Acacia Leaf Petroleum ether extract 60-80^{0}C (HALPE), Hot Acacia Leaf Chloroform extract (HALCL), Hot Acacia Leaf Acetone extract (HALAT), Hot Acacia Leaf Methanol extract (HALME), Hot Acacia Leaf Water extract (HALW). Cold Acacia Leaf Petroleum ether extract 40-60^{0}C (CALPE), Cold Acacia Leaf Chloroform extract (CALCL), Cold Acacia Leaf Acetone extract (CALAT), Cold Acacia Leaf Methanol extract (CALME), Cold Acacia Leaf Water extract (CALW). Cold and hot concentrated extracts were prepared in sufficient volume (50 ml) and preserved at 4^{0}C in sealed vials for further use to avoid batch to batch variations.

Sterility testing of Plant extracts:100 µl of hot and cold extract were spread on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) plates, and checking for growth of bacteria in 48 hrs at 37^{0}C and at25^{0}C and fungal contaminants after 1 week of incubation at room temperature (11) to ensure absence of any microbial contamination.

Antimicrobial activity by well diffusion method

20ml of sterile molten Hi sensitivity test agar medium maintained at 50-55^{0}C poured in petri dish and mixed well. The medium was allowed to solidify at room temperature. 100□l of inoculum was transferred on to the surface of agar medium with the help of micropipette under aseptic condition. The culture was spread uniformly all over the plate by using sterile cotton swab. Three equidistant wells per plate were cut with the help of sterile metal borer (diameter 5mm). 100µl from each extract was transferred to respective well with the help of micropipette with sterile micro tip. Plates were kept undisturbed in a refrigerator for 1hour for diffusion of plant extracts. After diffusion, plates were removed from refrigerator and transferred to incubator maintained at 37^{0}C for 18-24hrs. The zone of inhibition were observed in mm by zone measuring scale and the result were noted down as sensitive, intermediate or resistance using following Table 1.

Table 1: Standard pattern of zone of inhibition (12).

	Diameter of zone of inhibition (mm)
Resistant	10 or less
Intermediate	11-15
Susceptible	16 or more

Preliminary phytochemical analysis of the extracts:

To identify the chemical composition of the various extracts qualitatively, a preliminary phytochemical analysis was conducted according to the standard methods proposed by N.Raaman (13).Using these methods, the presence of several phytochemicals like alkaloids, Carbohydrates and glycosides, proteins and amino acids, phytosteroids. Saponins, flavonoids and phenolic compounds were as certained.

Testing of Synergistic activity:

100 µl of extract was transferred to sterile petri plates and15ml of sterilized molten Hi-sensitivity test agar maintained55^{0}C in constant temperature water bath was then poured in a plate, then plate is rotated for about 30 to 35 seconds to ensure even mixing of extract with the agar medium. Agar medium was then allowed to solidify. 100 µl of inoculums was added on solidified Hi-sensitivity test agar and spread over agar medium with the help of sterile disposable L-spreader. Four or five antibiotic discs were placed over it equidistantly. Plates were kept undisturbed in a refrigerator for 1 hour. Then plates were removed from refrigerator and shifted to incubator maintained at 37^{0}C for18-24hrs. After incubation all plates were examined for zone of inhibition. Zone of inhibition is then measured and classified as susceptible ,intermediate or resistant.

RESULTS AND DISCUSSION:

Total 360samples of urine, 50 of pus and 50 of sputum 65 of blood were screened for the isolation of *Klebsiella sp*. Total 36suspected isolates of *Klebsiella sp*. were identified on the basis of morphological, cultural & biochemical characteristics and all of them were found to be *Klebsiella sp*. 29 isolates were associated with urine samples, 6 with sputum and 1 was associated with blood sample. Percentage prevalence of *Klebsiella sp*. in clinical samples are graphically illustrated in Fig.: 2.*Klebsiella* sp. can lead to a wide range of diseases causing pneumonia, urinary tract infections, septicemia, meningitis, diarrhea and soft tissue infections and development of MDR against commonly used antibiotics making the case difficult to manage.

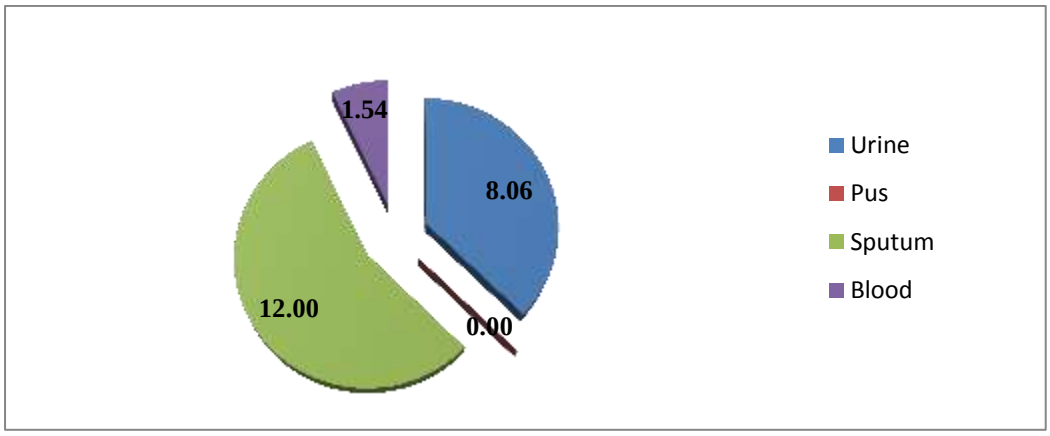

Figure 2: Prevalence of *Klebsiella* sp. with different clinical samples

More than 8.0%urine samples were found associated with*Klebsiellasp*.12 %and 1.54 % with sputum samples and blood samples respectively. *Klebsiella*sp. was not found associated with pus sample. These 36 isolates were preliminary screened for the selection of MDR using 34 different antibiotics. Antibiogram study of these isolates revealed that all these isolates are resistant to several antibiotics. Total 5 MDR isolates of *Klebsiellasp*.namelyU058, U072 ,U088,U155 andU160 were selected for further studies out of 36 isolates on the basis of their resistance pattern to more than 80% antibiotics. The % resistance of each isolate to number of antibiotics is given in Table and also illustrated graphically in Figure 3.

Table 2: The % resistance of each isolate to number of antibiotics.

Sr. No.	Isolate No.	Susceptibility pattern of Antibiotics					
		Resistant		Intermediate		Resistant + Intermediate	
		Number	Percentage	Number	Percentage	Number	Percentage
1.	B001	1	2.94%	2	5.88%	3	8.82%
2.	U019	20	58.82%	5	14.70%	25	73.52%
3.	U020	3	8.82%	0	0%	3	8.82%
4.	U021	25	73.52%	1	2.94%	26	76.46%
5.	U023	1	2.94%	2	5.88%	3	8.82%
6.	U024	2	5.88%	0	0%	2	5.88%
7.	U028	24	70.58%	1	2.94%	25	73.52%
8.	U031	6	17.64%	6	17.64%	12	35.28%
9.	U035	4	11.76%	4	11.76%	8	23.52%
10.	U037	19	55.88%	3	8.82%	21	64.70%
11.	U038	5	14.70%	4	11.76%	9	26.46%
12.	U040	10	29.41%	6	17.64%	16	47.05%
13.	U041	11	32.35%	6	17.64%	17	49.99%
14.	S001	19	55.88%	0	0%	19	55.88%
15.	S003	13	38.23%	3	8.82%	16	47.05%
16.	S007	0	0	0	0%	0	0%
17.	U160	29	85.29%	2	5.88%	31	91.17%
18.	U056	17	50%	6	17.64%	23	67.64%

19.	U058	26	76.47%	4	11.76%	30	88.23%
20.	U062	23	67.64%	4	11.76%	27	79.40%
21.	$U063I_2$	7	20.58%	7	20.58%	14	41.16%
22.	S014	22	64.70%	5	14.70%	27	79.40%
23.	U072	34	100%	0	0%	34	100%
24.	U075	13	38.23%	3	8.82%	16	47.05%
25.	U076	10	29.41%	5	14.70%	15	44.11%
26.	U088	34	100%	0	0%	34	100%
27.	U095	4	11.76%	4	11.76%	8	23.52%
28.	U100	23	67.64%	4	11.76%	27	79.40%
29.	U101	5	14.70%	0	0%	5	14.70%
30.	U115	25	73.52%	0	0%	25	73.52%
31.	U116	19	55.88%	0	0%	19	55.88%
32.	U117	16	47.05%	7	20.58%	23	67.63%
33.	U121	3	8.82%	1	2.94%	4	11.76%
34.	U125	7	20.58%	2	5.88%	9	26.46%
35.	S018	16	47.05%	3	8.82%	19	55.88%
36.	U155	31	91.17%	2	5.88%	33	97.05%

Total 10 extracts i.e. HALPE, HALCL, HALAT, HALME, HALW, CALPE, CALCL, CALAT, CALME, CALW of leaf of *Acacia polycantha* were studied for their antimicrobial activity and synergistic effect with antibiotics against MDR isolates and their results are given in Table 4.

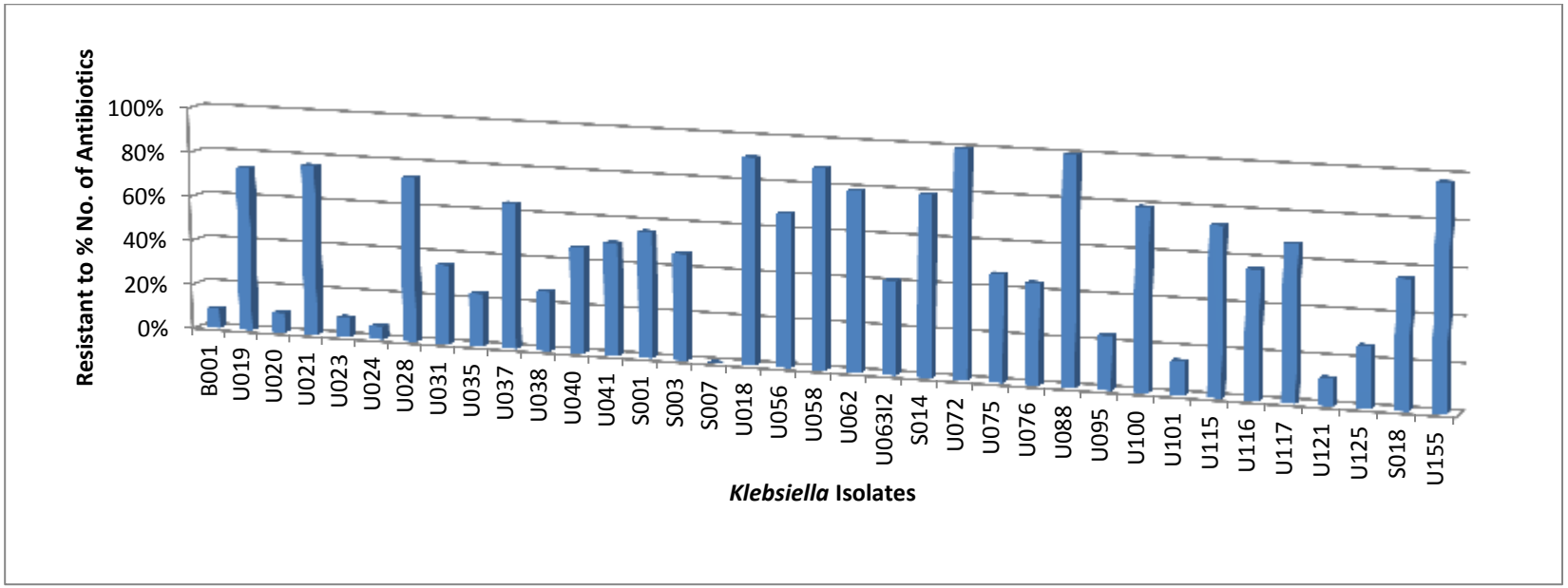

Figure. 3: Graph showing resistant of *Klebsiella* isolate to % number of antibiotics.

Table 4:Antibiogram and synergistic effect of plant extracts and antibiotics on MDR *Klebsiella sp.*

U058																																
Abbreviations	TR	OF	CTX	K	RIF	SF	CPM	CXM	C30	CZ	NA	COT	NIT	MI	TOB	N	AMC	CFM	CL	AMP	CB	CIP	NET	CTR	CAZ	MRP	CTX	GEN	NX	AK	CPZ	PIT		
Standard	R	I	R	R	R	R	R	R	R	R	I	R	R	I	R	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R		
CAL PE	R	S	I	I	R	R	R	S	I	R	S	R	R	S	R	S	I	R	R	S	R	I	S	R	S	S	I	S	I	S	I	I		
CALCL	R	I	S	I	I	R	R	S	R	R	I	R	R	S	R	S	S	R	R	S	R	S	S	R	I	S	I	S	I	I	R	R		
CAL AT	R	S	S	S	I	R	I	S	I	R	S	R	R	S	R	S	S	R	R	I	R	S	S	R	S	S	S	I	S	S	R	I		
CALME	R	S	S	S	S	R	S	S	S	R	S	R	R	S	R	S	S	R	R	S	R	S	S	R	S	S	S	S	I	S	S	S		
CALW	R	S	I	S	S	R	S	S	S	R	S	R	R	S	R	S	S	R	R	S	R	S	S	R	S	S	S	S	S	S	S	S		
HAL PE	R	S	S	I	S	R	I	I	R	R	I	R	R	I	R	S	I	R	R	I	R	I	S	R	S	S	I	R	R	I	R	R		
HALCL	R	S	S	I	R	R	I	S	I	R	I	R	R	S	R	I	I	R	R	S	R	I	S	R	R	S	R	I	R	R	R	I		
HAL AT	R	S	S	I	S	R	R	S	R	R	I	R	R	I	R	S	S	R	R	S	R	R	S	R	R	S	R	R	I	R	R	R		

HALME	R	S	S	S	S	R	I	S	I	R	S	R	R	I	R	S	I	R	R	S	R	R	I	R	R	S	R	R	R	I	R	R		
HALW	R	S	I	I	S	R	S	S	I	R	I	R	R	S	R	S	S	R	R	S	R	I	S	R	I	S	I	I	I	R	I	I		
U072																																		
Abbreviations	TR	TGC	OF	K	RIF	SF	CPM	CXM	C30	CZ	NA	COT	NIT	MI	TOB	N	AMC	CFM	CL	AMP	CB	TE	CIP	IPM	NET	CTR	CAZ	MRP	CTX	GEN	NX	AK	CPZ	PIT
Standard	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CAL PE	R	I	R	R	R	R	S	I	R	R	I	R	R	R	R	I	R	S	R	R	R	R	R	S	I	R	R	R	I	S	R	R	R	I
CALCL	I	R	I	S	R	R	R	I	R	R	R	R	R	I	R	R	R	R	S	R	R	R	R	I	R	R	R	R	I	I	R	R	R	I
CAL AT	R	R	I	R	R	R	I	R	R	R	I	R	R	I	R	I	R	R	R	R	I	R	R	I	I	R	R	R	I	I	I	R	R	R
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CALW	I	S	S	S	S	R	R	I	R	R	R	S	R	S	R	S	R	S	I	R	S	R	R	S	S	R	R	R	I	S	R	I	S	R
HAL PE	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
HALCL	R	R	I	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
HAL AT	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
HALME	I	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	I	R	R	I	R	R	I	R	R	R	R	R	I	I	R	R	R
HALW	R	R	R	I	R	R	I	R	R	R	R	R	R	R	R	R	R	I	R	R	I	R	R	I	I	R	R	R	I	I	I	R	S	R
U088																																		
Abbreviations	TR	TGC	OF	K	RIF	SF	CPM	CXM	C30	CZ	NA	COT	NIT	MI	TOB	N	AMC	CFM	CL	AMP	CB	TE	CIP	IPM	NET	CTR	CAZ	MRP	CTX	GEN	NX	AK	CPZ	PIT
Standard	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CAL PE	I	R	R	R	R	I	I	R	R	I	I	R	R	R	R	S	R	I	S	R	R	R	I	R	R	R	R	R	S	I	R	R	I	R
CALCL	I	I	R	R	R	I	R	R	R	S	R	R	I	R	R	R	R	I	I	R	R	R	I	I	R	R	R	R	R	I	R	R	R	R
CAL AT	I	R	R	R	I	I	I	R	R	S	R	R	R	R	R	R	I	I	I	R	R	R	R	I	R	R	R	R	I	R	R	R	R	R
CALME	S	S	R	R	I	I	S	R	R	S	I	R	I	R	R	I	I	S	I	R	R	R	I	S	I	R	R	R	I	I	R	R	I	I
CALW	S	S	R	R	S	S	S	R	I	S	I	R	I	R	R	S	I	S	S	R	R	R	S	S	I	R	I	R	S	I	R	R	S	I
HAL PE	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
HALCL	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
HAL AT	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R
HAL	I	R	R	R	R	R	R	R	R	I	R	R	R	R	R	I	I	I	I	R	R	R	I	I	R	R	R	R	I	R	R	R	R	R

ME																																			
HALW	I	S	R	R	I	I	R	I	R	I	R	R	I	R	R	I	I	I	I	R	R	R	I	I	I	R	I	R	I	R	R	R	I	R

U155																																		
Abbreviations	TR	OF	K	RIF	SF	CPM	CXM	C30	CZ	NA	COT	NIT	MI	TOB	N	AMC	CFM	CL	AMP	CB	TE	CIP	IPM	NET	CTR	CAZ	MRP	CTX	GEN	NX	AK	CPZ	PIT	
Standard	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	
CALPE	R	I	I	R	R	I	R	R	I	R	R	R	S	R	I	R	R	I	R	S	I	R	S	I	R	I	R	I	I	R	I	R	I	
CALCL	R	R	I	I	R	I	R	R	R	I	R	R	S	R	I	R	R	I	R	I	R	R	I	S	R	R	R	I	I	R	R	R	R	
CALAT	R	R	I	I	R	I	I	R	R	R	R	I	S	R	I	R	R	S	I	R	R	I	R	S	R	R	R	R	I	R	R	R	I	
CALME	R	S	S	S	R	S	S	R	S	I	R	S	S	R	S	R	R	S	S	S	S	I	S	S	S	I	S	S	S	I	S	R	S	
CALW	R	I	S	I	R	S	I	R	S	I	R	S	S	R	S	R	R	S	S	I	S	I	I	S	I	R	S	S	S	I	R	R	I	
HALPE	R	R	R	R	R	I	R	R	R	R	R	R	S	R	R	R	R	I	R	R	R	R	R	I	R	R	R	R	I	R	R	R	R	
HALCL	R	R	R	R	R	R	I	R	R	R	R	R	S	R	I	R	R	I	R	R	R	R	R	I	R	R	R	I	I	R	R	R	I	
HALAT	R	I	R	R	R	R	I	R	I	R	R	I	S	R	R	R	R	S	R	R	I	R	R	I	I	R	I	I	R	R	R	R	R	
HALME	R	I	I	S	R	S	R	R	S	I	R	S	S	R	S	R	R	S	S	S	I	I	S	S	I	S	S	S	S	S	S	R	S	
HALW	R	I	I	I	R	I	I	R	I	R	R	I	S	R	I	R	R	S	I	I	I	I	I	S	I	I	R	I	I	R	R	R	I	

U160																																		
Abbreviations	TR	OF	K	RIF	SF	CPM	CXM	CZ	NA	COT	NIT	MI	TOB	N	AMC	CFM	AMP	CB	TE	CIP	IPM	NET	CTR	CAZ	MRP	CTX	GEN	NX	AK	CPZ	PIT			
Standard	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R			
CALPE	I	S	R	R	I	R	R	R	I	S	R	S	R	I	R	R	R	S	I	R	I	R	R	I	R	R	I	R	I	I	R			
CALCL	R	I	R	R	R	R	R	R	R	R	R	I	R	R	R	I	R	I	I	R	R	R	R	R	R	R	I	R	I	R	R			
CAL	I	I	I	R	R	R	R	R	R	R	R	I	R	R	I	R	R	R	I	R	R	R	R	I	R	R	R	I	I	R	R			

AT																																	
CALME	I	S	I	I	I	R	S	R	I	S	I	S	R	I	I	S	R	S	S	R	I	R	R	I	S	R	S	S	S	S	S			
CALW	S	S	I	S	I	R	I	R	S	S	I	S	R	I	I	I	R	I	S	R	I	R	R	I	I	R	I	S	S	I	S			
HALPE	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R			
HALCL	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R			
HALAT	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	I	R	R	R			
HALME	I	I	R	I	R	R	I	R	S	I	R	S	R	I	R	I	R	I	S	R	I	R	R	I	I	R	S	I	I	S	I			
HALW	I	I	I	R	R	R	R	R	I	S	R	I	R	R	R	R	R	I	I	R	I	R	R	I	R	R	I	I	I	I	I			

Each isolate showed variable degree of antibiotic resistance pattern against antibiotics. Also each isolate behave differently in synergistic study with plant extract and antibiotic. None of the extracts showed any synergistic activity with the antibiotics, TR, SF, CZ, COT, NIT, TOB, CFM, CL,CB and CTR against isolate U058. Antibiotics SF,C30,CZ,NIT,TOB,AMC,AMP,TE,CIP,CTR,CAZ and MRP remained unaffected by any of the extracts against isolate U072 isolate. Similarly OF,K,COT,MI,TOB,AMP,CB,TE,CTR,MRP,NX and AK remained unaffected for U088, antibiotics TR,SF,C30,COT,TOB,AMC,CFM and CPZ for isolate U155 and for isolate U160 antibiotics CPM,CZ,TOB,AMP,CIP,NET,CTR and CTX remained unaffected by any of the extracts. Remaining antibiotics showed synergistic activity with leaf extracts of *Acacia polyacantha* and antibiotic against MDR isolates. Very promising results were obtained in present synergistic study. Isolates became susceptible to antibiotic in presence of plant extract which otherwise were resistant or intermediately susceptible to these antibiotics. Any of the individual extracts had not shown antimicrobial activity against any of the selected MDR pathogens.

Phytochemical analysis of plant extracts

Preliminary qualitative phytochemical analysis of leaf extracts (petroleum ether, chloroform, acetone, methanol and water) was carried out. The results of Phytochemical analysis is given in following table5

Table 5: Qualitative phytochemical analysis of leaf extracts

Sr. No.	**Phytochemical test**	**LEAVES**									
		HOT					COLD				
		PE	CF	AT	ME	W	PE	CF	AT	ME	W
1	Alkaloids	+	+	+	+	+	+	-	-	+	+
2	Carbohydrates and glycosides	+	+	+	+	+	+	+	+	+	+
3	Saponin	+	+	+	_	+	+	+	+	+	+
4	Proteins and Amino acids	-	-	+	+	+	+	+	+	+	+
5	Phytosteroids	-	+	+	+	+	+	+	-	-	+
6	Phenolic compounds and flavonoids	+	+	+	+	+	+	+	+	+	+
7	Gums and Mucilages	-	-	-	+	-	-	-	+	+	+

"+" – Present, "-" - Absent

Preliminary phytochemical analysis of leaf extracts (petroleum ether, chloroform, acetone, methanol and water) showed the presence of alkaloids, Carbohydrates and glycosides, proteins and amino acids, phytosteroids. The relative antimicrobial activity of leaf extracts may not be easily correlated with any individual component but may be due to mixture of compounds present in these extracts. It has also been suggested that the antimicrobial activity is mainly due to the presence of alkaloids, phytosteroids and other natural polyphenolic compounds or due to free

hydroxyl groups. Moreover, secondary metabolites such as other compounds of phenolic nature are also classified as antimicrobial compounds(1). Therefore, the presence of these phytochemicals (alkaloids, Carbohydrates and glycosides, proteins, and amino acids,phytosteroids) could to some extent justify the observed antimicrobial activity in the current study.

RNS Yadav and MuninAgarwala(2011) have analysedthe plants extracts of Bryophyllumpinnatum (Leaves), Ipomeaaquatica (Leaves), Oldenlandiacorymbosa (Whole plant),Ricinus communis (Roots), Terminaliabellerica (Leaves), Tinosporacordifolia (Leaves), Tinospora cordifolia (Stem), and Xanthium strumarium (Leaves) and found significant amount of phenolic and flavonoid.Thus their study provide the support that crude aqueous and organic solvent extracts of plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases (14).SathyabamaS.*et al* 2012 study clearly indicates that the terpenoid compound of *T. procumbens*posses antibacterial activity against both gram positive and gram negative bacteria(15). Jignaparekh and sumitra chanda 2007 have concluded that the methanol extract of *Bauhinia variegata* bark has best antibacterial activity(16).

Medicinal Plants have a traditional background that they have potentials to use as antimicrobial agents. Ekta Menghani *et al* 2011, have suggested that ethanolic extracts of traditionally used *Curculigoorchioides, Symplocosracemosa, Puerariatuberosa, Scindapsusofficinarum, Luffaacutangula and Acacia nilotica* possess good antimicrobial activity against selected test bacteria and intermediate against fungus (17). M. Thenmozhi and rajeshwarisivaraj2010 have found that antimicrobial activity of different extracts of leaves of *Polyalthialongifolia* was studied against six different bacteria. The various metabolites present in all the extracts. Among various solvent extracts studied, chloroform extract showed higher degree of inhibition followed by ethylacetate, ethanol, petroleum ether and aqueous.(18).

Study of Stephen T. *et al* 2012 have revealed that MDR Gram-negative bacteria with over expressing active efflux pump phenotypes became sensitive to methanol extracts of *Citrus medica*, the bulbs of *Allium sativum*and *Allium cepa*, the seeds of *Carica papaya*, *Cola acuminata*, *Buchholziacoriacea*, *Garcini kola*, and *Garcinialucida*, the seeds and fruits of *Picralimanitida*(19). Hariharan A.G. (2012) investigated the synergistic action of combination of four selected plants extracts namely *Terminaliaarjuna*(Combretaceae), M*oringaoleifera*(Moringaceae), *Azadirachtaindica* (Maliaceae) and *Curcuma longa* (Zingiberaceae) against bacteria and fungi and observed synergism against bacterial strains(2)..Akanwariwiak W. G. *et al*. (2012)were observed the most significant reduction of MICs with ciprofloxacin-plant extract combination (20). *Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* resistance to streptomycin,

chloramphenicol, tetracycline, amoxicillin, rifamycine became sensitive to extract of (phenol fraction and flavonoid content) *Melissa officinalis*in synergism with antibiotics (21).Jasmine R*et al* (2011)have demonstrated the presence of saponins, flavonoids and naphthoquinone in the crude extract and alcoholic extracts of *Eugenia jambolana*(EJ) seeds and *Elephantopusscaber*(ES) whole plant, inhibit the growth of vancomycin resistant *Enterococci* (VRE) (22). Fabiola F, G**,** Rodrigues *et al* (2010) have revealed that the volatile compounds of essential oil of *Zanthoxylumarticulatum* used by inhalation may suppress the growth of bacterial pathogens of respiratory infections and because of their antibacterial modifying activity could be used as adjuvants to antibiotic therapy against these pathogens(23).

CONCLUSION:

Plant extracts have great prospective as antimicrobial compounds against MDR pathogen. Thus, they provide opportunity for their use in the treatment of infectious diseases caused by resistant microbes. Moreover the synergistic effect of combination of antibiotic with plant extracts against resistant bacteria reveals new options for the treatment of infectious diseases caused by resistant bacteria. Antibiotics which are no more effective can be used by when they are combined with plant extracts. However more studies are required to isolate bioactive component of plant extracts for its use in new formulations.

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