Herbal Extracts As Beta Lactamase Inhibitors

Lakshmi Jayasri Akkiraju, M. Sowmya, Ch. Deepthi, V.V.Lakshmi.

Abstract— Bacterial resistance to antibiotics is an increasingly serious threat to the ability to routinely treat microbial infections. The emergence of bacteria resistant to several important classes of antibiotics has become a major clinical problem in the last decade. β-Lactamases secreted by bacteria confer resistance against β-lactam and they are gaining more and more prominence with increase in the number of multiresistant strains of bacteria particularly those producing Extended spectrum of β-lactamases (ESBLs). Plants have been found to have ability to synthesize a wide variety of chemical compounds that are able to inhibit pathogens. Hence herbal extracts were screened for source of β- lactamase inhibitors which can be effective in synergy with existing antibiotics in delaying the emergence of resistance.. Significant inhibition of β-lactamase activity was achieved by the herbal extracts of *Calotropis procera*, *Lawsonia inermis*, *Ocimum sanctum*, *Zingiber officinale*, *Allium sativum* against ESBL pathogens as test organisms.

Index Terms— Antibiotic resistance, β-lactam, β-lactamase inhibitors, Emergence, ESBLs, Herbal extracts, Multiresistant pathogens.

1 INTRODUCTION

The incidence of multi drug resistance bacteria has been increasingly reported currently among Gram-positive (methicillin- resistant *Staphylococcus aureus*, vancomycin resistant *Enterococci*) [1] and Gram-negative bacteria (members of *Enterobacteriaceae* producing plasmid-mediated Extended spectrum β-lactamase (ESBL) and others like *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* [2], [3]. Microbial resistance to β-lactam antibiotics is mostly due to hydrolysis by β-lactamases [4]. As current antibiotic therapy options are being limited there is an urgent need for new antimicrobial agents to combat multi drug-resistant resistant bacteria. While it is well recognized that there is an urgent need to develop new antibiotics, attempts to identify novel classes of compounds have been remarkably less productive with almost a 30 year gap before the clinical introduction of two new types of systemic antibiotics in early 2000 requiring alternate approaches.

Plants are known to produce different secondary metabolites which are naturally inhibit bacteriaand have attracted researchers worldwide [5], [6]. Plant based antimicrobial compounds have great therapeutic potential as they have lesser side effects as compared with synthetic drugs.

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The plant extracts have been to show synergistic effect with an antibiotic [7], [8]. The focus of this study was to find the phyto compounds from herbal extracts as inhibitors against β -lactamase produced by ESBL positive test cultures.Screening of crude extracts for synergistic interaction with antibiotics can provide source of bioactive compounds that can have potential in combinational therapy.

2 MATERIALS & METHODS

2.1 **Antibiotic resistance profile of test pathogenic bacteria** Different test pathogenic ESBL positive bacteria were collected from SVIMS, Tirupati, India. These pathogenic bacteria were tested for their antibiotic sensitivity by disc diffusion method [9]. Antibiotic discs were purchased from Hi-Media Labortory Ltd., Mumbai (India). These test bacteria were confirmed for the production of β-lactamase [10].

2.2 **Detection of β-lactamase:** Penicillin starch paper strips were prepared by taking a 7cm x 4cm strips of whatman no.1 filter and dipped in 2% starch and air dried. The strip of the starch paper was soaked in benzyl penicillin (1, 00,000 iu/ml) for 10min to prepare the test strip. Overnight test bacterial culture was centrifuged pellet was collected. This was transferred to over an area of 2-3mm on the the test paper spread in a petri-dish. Control cultures were simultaneously maintained. The samples were incubated at 37° C for 2 hours after which the paper was flooded with iodine solution and drained immediately. The zone of decolourisation was observed. Zone around the bacteria confirmed the production of β-lactamase.

2.3 **Collection of Plants and preparation of extracts:** Plants were selected based on literature and therapeutic significance. The plants selected for investigation were *Calotropis procera*, *Lawsonia inermis*, *Ocimum sanctum*, *Zingiber officinale*, *Allium sativum*. These plants were collected from in and around Tirupati. 5 grams of fresh leaves/bulb/rhizome were weighed and macerated with 20ml of ethyl acetate in the ratio of 1:4.

The samples were filtered through muslin cloth and then with whattman no. 1 filter paper. These extracts were allowed to air dry at room temperature and dry weights of plant extracts were determined. The samples were stored in a refrigerator for further use. The MIC for each plant extract was determined with the test stains. Control and solvent control was maintained.

2.5 **Screening of herbal extracts for inhibition of β- lactamase:** The sub inhibitory concentration of the herbal extracts of test pathogens from the MIC were used for study of inhibition of β- lactamase activity by starch filter paper method as described above.

3 RESULT

The antibiotic sensitivity of the test pathogens (Table 1) showed that these test pathogens are resistant to Amoxicillin with clavulanic acid, Ampicillin, Chloramphenicol, Cefonicid, Cotrimoxazole, Cefotaxime and Benzyl penicillin.

TABLE 1
ANTIBIOTIC SENSITIVITY OF THE TEST PATHOGENS

S.No	Test Pathogen	Resistance pattern
1	*E. coli*	P, AMC, AMP, CID, COT
2	Pseudomonas	AMP, P, C, AMP
3	Staphylococcus	P, OX, COT
4	Klebsiella	P, COT, AMP, CTX, AMC,

AMC- Amoxicillin with clavulanic acid, AMP- Ampicillin, C- Chloramphenicol, CID- Cefonicid, COT- Cotrimoxazole, CTX- Cefotaxime, P- Penicillin, OX- Oxacillin.

Among the plant extracts tested, the ethyl acetate extracts of *Calotropis procera* and *Allium sativum* (Table 2) showed the anti bacterial activity of significant zone of inhibition against test strains (*E. coli, Pseudomonas, Staphylococcus and Klebsiella*).

TABLE 2
ANTIBACTERIAL ACTIVITY OF CRUDE PLANT EXTRACTS (ETHYL ACETATE) AGAINST ESBL STRAINS

S. No.	Name of the plant	Part used	Zone of Inhibition (diameter in mm)			
			E. coli	*Pseudomonas*	*Staphylococcus*	*Klebsiella*
1.	*Calotropis procera*	leaves	14	12	13	15
2.	*Lawsonia inermis*	leaves	10	08	10	11
3.	*Ocimum sanctum*	leaves	12	09	11	09
4.	*Zingiber officinale*	rhizome	09	11	10	11
5.	*Allium sativum*	bulb	12	13	11	14

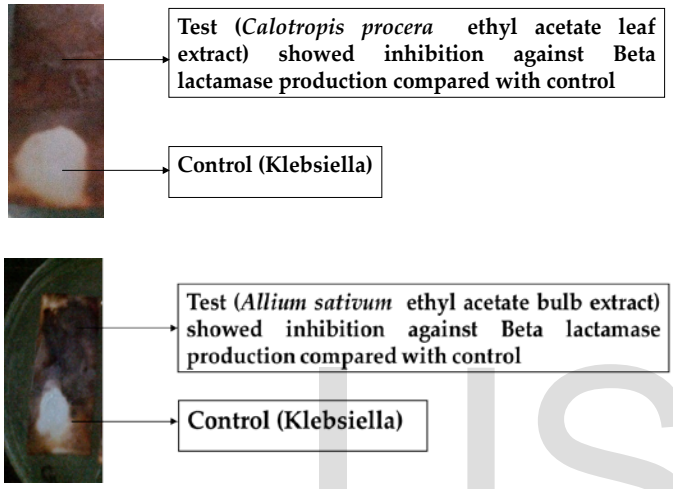
The growth was observed upto 0.8mg/ml concentration of *Allium sativum extract* , 1 mg/ml concentration for *Calotropis procera* and *Lawsonia inermis*, 1.2 mg/ml concentration of *Zingiber officinale*, 1.5 mg/ml concentration of *Ocimum sanctum* when tested against all the 4 test strains and hence the cells grown at this concentratioin were used to examine for β-lactamase inhibtion(Fig:1).

TABLE 3
INHIBITION OF B-LACTAMASE PRODUCTION BY ETHYL ACETATE EXTRACTS OF PLANTS

S. No.	Name of the plant	Inhibition of β-lactamase production			
		E. coli	*Pseudomonas*	*Staphylococcus*	*Klebsiella*
1.	*Calotropis procera*	complete	complete	complete	complete
2.	*Lawsonia inermis*	partial	No	No	partial
3.	*Ocimum sanctum*	No	No	No	No
4.	*Zingiber officinale*	partial	No	No	No
5.	*Allium sativum*	complete	complete	complete	complete

Complete inhibition of β-lactamase production was observed with ethyl acetate extracts *of Calotropis procera* and *Allium sativum* with all the 4 test strains where as partial inhibition of β-lactamase production was observed with ethyl acetate extracts of *Lawsonia inermis* with *E.coli* and *Klebsiella* but no inhibition was observed with *Pseudomonas* and *Klebsiella*sp. *Zingiber officinale* showed partial Inhibition of β-lactamase production only with *E.coli* (Table 3).

Fig. 1 Inhibition of β- lactamase by plant extract (a) *Calotropis procera (b) Allium sativum*



Novel antibacterial action of plant extracts against antibiotic resistant bacteria have been reported [11], [12]. The ethyl acetate extracts of *Calotropis procera* and *Allium sativum* showed the most promising β- lactamase inhibitory activity. It shows that the herbal extracts contain substance(s) that can inhibit the β- lactamase activity.The combination of antibiotics and bioactive compounds from natural sources like plant is emerging as a potential strategy for combating for infections caused by multidrug resistant specially ESBL producing bacteria. The active compound exhibiting inhibition activity is being further investigated.

4 Conclusion

The ethyl acetate extracts of *Calotropis procera* and *Allium sativum* have been shown to inhibit β-lactamase activity from ESBL positive isolates. Further purification and extraction of the active compounds from the herbal extracts is under investigation. The results are of significance in view of the challenge faced due to the wide spread prevalence of ESBL positive bacteria.

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