

## STUDY OF ANTIMICROBIAL ACTIVITY OF BETEL LEAVES EXTRACTS AGAINST ESBL AND MBL PRODUCING PATHOGENES

\***Kalpana Tilak and Tulika Mishra**

Department of Biotechnology, University Institute of Biotechnology Chandigarh University,  
Gharaun, Mohali, India.

Article Received on  
19 June 2017,

Revised on 09 July 2017,  
Accepted on 30 July 2017

DOI: 10.20959/wjpr20178-9159

### \*Corresponding Author

**Kalpana Tilak**

Department of  
Biotechnology, University  
Institute of Biotechnology  
Chandigarh University,  
Gharaun, Mohali, India.

### ABSTRACT

Bacterial resistance to antibiotics is an emerging problem of today's world. Studies on herbal and Ayurvedic extracts are done in order to treat the infections caused by uropathogens. The aim of the study was to assess the anti microbial activity of piper betle leaf extract on ESBL and MBL producing uropathogens under in-vitro conditions. Piper betle is a plant which exhibits medicinal properties. The active components of piper betle were extracted for 12hours at 60°C with the help of Soxhlet apparatus using ethanol, methanol, water and acetone as solvents. The above solvents extracts were concentrated at 40°C on water both to obtain a semi-solid mass which was used to carry out qualitative and quantitative analysis. The antimicrobial activity of

ethanol, methanol, acetone and water extracts were tested against ESBL and MBL producing uropathogens i.e. 41 escherichia coli, 10 proteus, 9 Citro, 12 Pseudomonias and 17 Klebsiella pneumonia. The ethanol and methanol extract showed inhibition on all these cultures by agar well diffusion method and the zone size ranged from 15-25mm. the minimum bactericidal concentration (MBC) for ethanol extract was found to be 10mg/ml. the significance of the study was conducted to investigate the in – vitro anti microbial activity of Piper betle plant against antibiotic resistant uropathogens.

**KEYWORDS:** Betel Leaves, Piper Betel Plant, Uropathogenes.

### INTRODUCTION

UTI is characterized by the presence of organisms in the urinary tract, which is usually sterile. Approximately 150 million people universal are diagnosed with Urinary tract infection (UTIs) each year.<sup>[1]</sup> They are common among the female population. And its

frequency about 1% in school-aged girls and 4% in women through child-bearing years. The most commonly encountered gram negative uropathogens are *E.coli*, *K.pneumoniae*, *Citrobacter spp*, *P.aeruginosa* and *Proteus spp*.<sup>[2]</sup> Antibiotic resistance is a effect of evolution through natural selection. The whole disaster is a result of many years of sustained selective pressure due to the human application of antibiotics, via underuse, overuse and misuse.<sup>[3]</sup> Extended-spectrum-beta-lactamase (ESBLs) are enzymes that can be produced by bacteria making them resistant to third generation Cephalosporins e.g. Cefuroxime, Cefotaxime and Ceftazidime, which are the most widely used antibiotics in many hospitals.<sup>[4]</sup> Gram-negative pathogens were collected from local pathological laboratories and hospitals situated in Mumbai and characterized for ESBL and MBL production in our laboratory in our previous studies.<sup>[5,6]</sup>

## MATERIALS AND METHOD

### 1. Plant Material

Piper betle leaves were obtained from locality and also purchased from shop. The leaves were shade dried, crushed into powder and used for removing extracts with the help of Soxhlet apparatus.



### 2. Test organism

41 *Escherichia Coli*, 10 *Proteus Mirabilis*, 9 *Citrobacter*, 12 *Pseudomonas Aeruginosa* and 17 *Klebsiella Pneumoniae* were tested.

### 3. Preparation of plant extract

100g of shade dried and powdered leaves of piper betle were extracted with 200ml of different solvents using Soxhlet apparatus for a period of 8 hours.

#### 4. Sterility testing

In this method the extract were checked for sterility. The extracts were streaked on sterile nutrient agar plates; the plates were then incubated at 37°C for 24 hours and checked for any growth.

#### 5. Agar well diffusion method

This bioassay method is used to determine the effect of antibiotics on pathogen. Here leaves extract will be used for testing the activity against the culture. Sterile nutrient agar molten cooled butts were seeded with 0.4ml of 0.5 McFarland's test cultures and poured into a sterile Petri plate. Later wells were bored onto solidified plates and the extracts were added into the wells. The plates were incubated at 37°C for 24hours and checked for inhibition.<sup>[7]</sup>

#### 6. Determination of MBC of piper betle

The agar dilution method was used to determine the MBC (minimum Bactericidal concentration) of piper betle leaves extract. Different concentration of various solvents extracts of piper betle (5-50mg/ml) were supplemented into molten NA butts cooled to around 40°C. After solidification of the medium 5µL of test isolates were spot inoculated on the plates and incubated at 37°C for 24 hours.

### RESULTS

#### 1. Sterility testing

The extracts tested were sterile as they did not show any growth on nutrient agar plate.

#### 2. Agar well diffusion method

The ethanol and methanol extract showed inhibition if 15-25mm on almost all organism. But methanol extract showed the highest activity.



### 3. Determination of MBC of piper betle

The MBC of extract was found out to be 10mg/ml.

Cultures	Zones of inhibition in mm of piper betle	
	Methanol Extract	Ethanol Extract
<b>Esbl Procedures</b>		
Escherichia coli	15-25	15-20
klebsiella pneumoniae	10-25	13-22
pseudomonas aeruginosa	10-14	10-14
Citrobacter	14-26	14-25
proteus mirabilis	15-22	15-20
<b>Mbl Procedures</b>		
Escherichia coli	15-20	15-19
klebsiella pneumoniae	11-22	10-20
pseudomonas aeruginosa	9-12	9-12
Citrobacter	13-23	12-20
proteus mirabilis	14-22	14-22

#### Signification

The growing resistances of micro organisms to antibiotics have built the consequences of finding new and improved methods for acting against these pathogens. Ayurvedic and herbal extracts are being studied on a large scale which can act against these organisms and also do not exert any side effects on these are the natural sources. Hence the active components of plant can be studied for anti-bacterial activity, which can be boon to clinical field.

#### DISCUSSION

The piper betle extract shows the presence of certain bioactive components which has inhibitory effects against ESBL and MBL producing uropathogens. The zones of inhibition observed in bioassay technique proved antimicrobial activity of betel extracts prepared. On future this extract obtained can be used in combination with Ampicilin.

#### CONCLUSION

The study will be beneficial and a useful for establishing new guidelines for treatment of infections caused by ESBL and MBL pathogens. As herbal and ayurvedic medicines have less side effects they can be widely used in treating pathogenic infection. As betel leaves are cost effective and readily available of medicines made from they can bring a lot of changes into medical field.

**ACKNOWLEDGEMENTS**

The author thankful to the team worker at Chandigarh university, Faculty of Biotechnology, department of biotechnology, for providing necessary facilities and cooperation during this research work.

**REFERENCE**

1. Goyal P, Verma S, Sharma A, et al. (Pharmacological and phytochemical Aspects of Lichen *Parmelia perlata*: A review). *Int. J. Res. Ayurveda pharm*, 2016; 7(1): 102-107.
2. Truls, E, Bjerklund, J, Mete, C., Kurt, G, Naber, Leonid, S., Peter, T, Hospital acquired urinary tract infections in urology departments: pathogens, susceptibility and use of antibiotics, *International Journal of Antimicrobial Agents*, 2006; 28: 91-107.
3. Powers, J.H, Phoenix, J.A, Zuckerman, D.M, *Antibiotic Uses and Challenges: A Comprehensive Review from NRCWF*, 2010.
4. Davies, J.D, Davies, D, *Origin and Evolution of Antibiotics Resistance. Microbiology and Molecular Biology Reviews*, 2010; 3.
5. Mishra, S.K, Acharya, J, Kattel, H, Pokhrel, B.M, Rijal, B.P, Extended-spectrum beta-lactamase and metallo-beta-lactamase-producing bacterial strains among the patients attending a tertiary care center in Nepal, *15th ICID Abstracts / International Journal of Infectious Diseases*, 2012; 1.
6. Aruna K, Mobashshera T. Prevalance of extended spectrum beta-lactamase production among uropathogens in south Mumbai and its antibiogram pattern. *EXCLI Journal*, 2012; 11.
7. Tariq M, Aruna K. Phenotypic and Molecular characterization of MBL genes among uropathogens isolated in Mumbai city. *British Microbiology Research Journal*, 2015; 4.
8. Khan J A and Chaurasia V., *In Vitro Evaluation of Antimicrobial Properties of Psidium guajava J. Pharma and Biomed. Sci*, 2011; 10.