

ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS AGAINST EXTENDED SPECTRUM BETA LACTAMASE PRODUCING BACTERIA CAUSING URINARY TRACT INFECTION

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ABSTRACT

The aqueous leaf extract of *Euphorbia hirta*, *Erythrophleum suaveolens* and methanolic leaf extract of *Thevetia peruviana* showed antibacterial activity against extended spectrum beta lactamase (ESBL) producing bacteria *E.coli*, *Pseudomonas*, *Klebsiella*, *MRSA (methicillin-resistant staphylococcus aureus)*, *Salmonella*, and *Proteus*. Methanolic leaf extract of *Thevetia peruviana* showed highest antibacterial activity against *Klebsiella*, *E.coli* (15mm, 14mm) respectively and significant against other. While *Euphorbia hirta*, *Erythrophleum suaveolens* showed least antibacterial activity against all these bacteria. The main purpose of the study is to eradicate the urinary tract infection problem across the world by using medicinal plants.

Keywords: Antibacterial, ESBL, Medicinal Plants, Aqueous extract.

INTRODUCTION

Urinary tract infections are serious health problem affecting millions of people each year. Urinary tract infection is defined as the proliferation of active microorganisms inside the urinary channel which are harmful to their environment. Bacteriuria is the isolation of bacteria in the urine specimen. Presence of 1×10^5 or more colony forming units (CFUs) of the same active microorganism per milliliter in two consecutive urine specimens has been a historically threshold value for the diagnosis of bacteriuria (Stauffer *et al.*, 2004). The incidence of UTI is higher among females, in whom it commonly occurs in an anatomically normal urinary tract. Conversely, in males and children, UTI generally reveals a urinary tract lesion that must be identified by imaging and must be

treated to suppress the cause of infection and prevent recurrence. UTI can be restricted to the bladder (essentially in females) with only superficial mucosal involvement, or it can involve a solid organ (the kidneys in both genders, the prostate in males). Multiple drug resistance has significantly increased in recent years. The existence of enzymes of Extended-Spectrum β - Lactamase (ESBLs) producing organism that are resistant to virtually all β -lactam antibiotics have been reported (Philippon *et al.*, 1989). *Escherichia coli*, *Klebsiella*, *Streptococcus pyogenes*, *S. faecalis*, *Pseudomonas vulgaris*, *P. faecalis* *Proteus vulgaris* *MRSA* are the bacteria responsible for Urinary tract infection. Bacterial resistance to antibiotics has been a great problem for many years. The threats that bacterial resistance

present today are greater than they were in the past. The first bacteria that were detected to be resistant to several antibiotics were reported in Japan during 1950s (Schlegel & Schmidt, 1985). Main purpose of the study is to make suitable drug against UTI with the help of medicinal plant as number of antibiotics has become resistant to ESBL producing bacteria cause urinary tract infection.

MATERIALS AND METHODS

Collection of ESBL producing bacterial strain sample

Bacterial sample of *MRSA*, *E coli*, *Pseudomonas*, *Klebsiella*, *Salmonella* and *Proteus* were Collected from civil hospital Paonta sahib (H.P.) Identified by gram staining and biochemical characterization.

Collection of plants

Euphorbia hirta, *Erythrophleum suaveolens* and *Thevetia peruviana* were collected from local area of Paonta Sahib and identified by FRI Dehradun.

Preparation of extract

For this purpose, dried powdered of leaves were used for extraction with aqueous solvent by sonicator and methnolic solvent by Soxhlet apparatus. Then water bath the solvent at 70-80⁰ C in china dish to get semisolid crude extract.

Determination of the antibacterial activity of medicinal plants

From the crude extract, the 500mg/ml dilution of plant paste was prepared for antibacterial assay. The modified agar well diffusion method was employed to determine the antibacterial activity of plant extracts, 200µl of the extract (500mg/ml) were poured in to the well. All the plates were incubated at 37⁰C for 24 hrs and zone of inhibition were observed in the form of mm (mili meter).

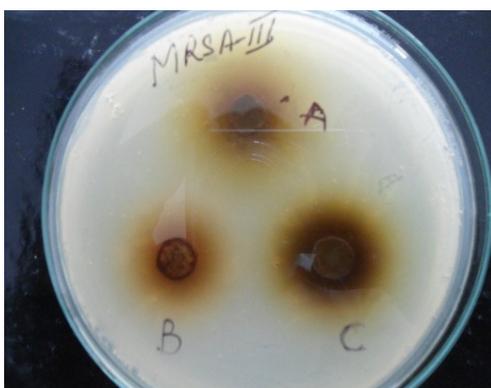
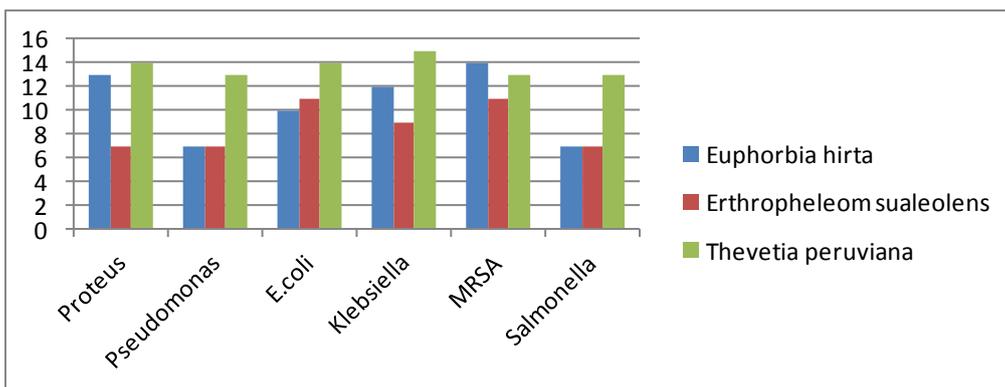
RESULTS

Euphorbia hirta, *Erythrophleum suaveolens* and *Thevetia peruviana* showed significant antibacterial activity against ESBL producing bacteria.

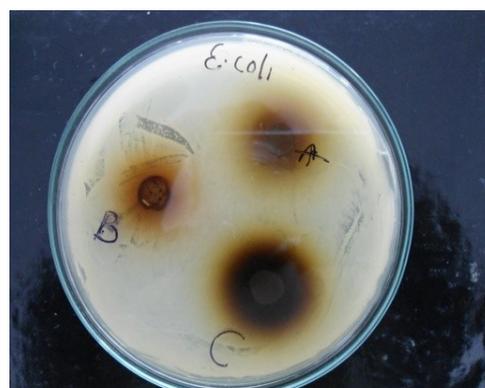
Table 1: Antibacterial Activity of Medicinal plant

Sample No.	<i>Euphorbia hirta</i> (Aqueous)	<i>Erythrophleum suaveolens</i> (Aqueous)	<i>Thevetia peruviana</i> (Methanolic)
	Zone of inhibition	Zone of inhibition	Zone of inhibition
<i>Proteus</i>	13	7	14
<i>Pseudomonas</i>	7	7	13
<i>E.coli</i>	10	11	14
<i>Klebsiella</i>	12	9	15
<i>MRSA</i>	14	11	13
<i>Salmonella</i>	7	7	13

Table 2: Graph showing different antibacterial activity in millimeter



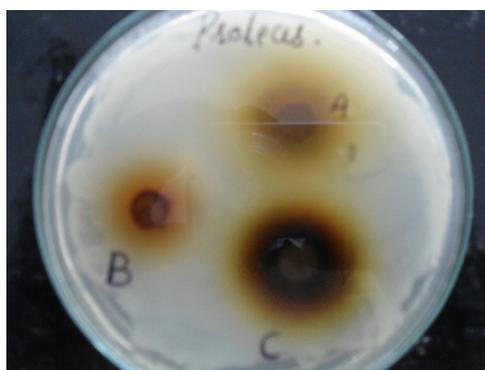
MRSA



E. coli



Salmonella



Proteus



Klebsiella



Pseudomonas

Figure1: Zone of inhibition showed by aqueous leaf extract of *Euphorbia hirta* (A), *Erthropheleom sualeolens* (B) and methanolic leaf extract of *Thevetia peruviana* (C) against ESBL producing bacteria

DISCUSSION

Out of these three the maximum zone of inhibition was observed in the methanolic extract of *Thevetia peruviana* against *Klebsiella* (15mm), *Proteus* & *E.coli* (14mm, 14mm), *MRSA* (14mm) and *Pseudomonas* (13mm) respectively. While work done by Anjana Sharma (2009) using aqueous extract of plant *Terminalia chebula* and *Zinziber officinale* showed the zone of inhibition against *Proteus* (1mm, 0mm), *E.coli* (9mm, 0mm), *Pseudomonas aeruginosa* (5mm, 4mm) and *Klebsiella pneumoniae* (5mm, 6mm) respectively, thus our studied plant is showing more effective results and can be used to prepare drug against disease caused by ESBL producing bacteria. On the other hand work done by Aliyu et al (2008) by using aqueous extract of plants *Vernonia blumeoides* and *Phyllanthus amarus* against *MRSA* observed the maximum zone of inhibition is 11mm and 13mm respectively which is also less effective than our studied plants.

Urinary tract infection (UTI) is among the most common infections ranking next to upper respiratory infection with an increasing resistance to antimicrobial agents. These ailments affect patients in all age of groups and sexes. Majority of UTIs are not life threatening and do not cause any irreversible damage. Multiple antimicrobial resistances among gram negative organisms have been a long term and well recognized problem with urinary tract infection. Resistance has been observed in multiple genera including *E.coli*, *Enterobacter*, *Klebsiella*, *Proteus*, *Salmonella*, *Serrata*, and *Pseudomonas*. *Escherichia coli* has been documented as the most important pathogen associated with urinary tract infection in many countries. These bacteria normally live in the intestine, but they sometime get into the urinary tract. The incidence of UTI is greater in women than men who may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factor. The ESBL (Extended Spectrum of Beta-Lactamase) producing bacteria has been identified in members of Enterobacteriaceae, are increasingly causing UTI. Some of the major

contributing factor for high level of urinary tract infection (UTIs) in predominantly areas is poor sanitary conditions and lack of proper hygiene. One of the most common UTIs is leucorrhoea among women, characterized by whitish discharge from female genitalia.

CONCLUSION

From our study we concluded that the prevalence of ESBL producing bacteria in UTI patients are increasing day by day and antibiotics are failed to cure. So, the medicinal plants are the best alternate for treating such ESBL producing bacteria because antibiotics are expensive to use, in our study out of three plants methanolic *Thevetia peruviana* showed significant antibacterial activity against all six tested bacteria (*E.coli*, *Klebsiella*, *Proteus*, *Salmonella*, *MRSA* and *Pseudomonas*). So we can use these medicinal plants extracts individually as well as in combination for better and cheaper treatment, because of high medicinal value of these plants.

ACKNOWLEDGEMENT

I extremely very thankful to Director of Himachal Institute of Life Science Dr. Gaurav Gupta for providing good research lab, equipment and faculty. Last but not least I would like to thank my all colleague for their valuable support as and when require.

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