

## Exploring Antibacterial Activities of *Euphorbia* spp of Kashmir Against Bovine mastitis

Saleem Mushtaq<sup>1</sup>, Qazi Parvaiz Hassan<sup>1</sup> and Md.Niamat Ali<sup>2</sup>

<sup>1</sup>Indian Institute of Integrative Medicine, IIM, Sanatnagar, J&K

<sup>2</sup>Corresponding author: Centre of Research for Development, COR, University of Kashmir

Email: mdniamat@hotmail.com

### ABSTRACT

Bovine mastitis is the most significant economic drain on the worldwide dairy industry. Concerns regarding poor cure rates, emergence of bacterial resistance, and residues in milk necessitate development of alternative therapeutic approaches to conventional antibiotics for treatment of mastitis. New antibiotics and new therapeutic strategies are needed to address this challenge. Advances in identifying new sources of antibiotic natural products and expanding antibiotic chemical diversity are providing chemical leads for new drugs. Plants have traditionally provided a source of new chemical entities and numerous clinical studies have proved the therapeutic value of molecules of plant origin. The present study was conducted with the objective to evaluate the antibacterial activity of the hexane, methanol and aqueous extracts of selected medicinal plants against the microbes responsible for causing bovine mastitis. The hexane, methanol and aqueous extracts were obtained by extraction in cold maceration using hexane, methanol and water as solvents, respectively. The extracts were assessed for their antibacterial activity against *S.aureus* and *E.coli*, the two common pathogens responsible for causing mastitis. Out of the different extracts prepared only the methanolic and aqueous extracts of *Euphorbia* sp. were effective against *S.aureus*, the most important pathogen of bovine mastitis. The minimum inhibitory concentration (MIC) for the methanolic and aqueous extracts was found to be about 128 ug/ml and 256 ug/ml, respectively. The extracts of several other species of *Euphorbia* are under process.

**Keywords:** Bovine mastitis, antibacterial activity, mastitis pathogens, minimum inhibitor concentration (MIC), cold maceration, *Euphorbia* sp.

## **INTRODUCTION**

Mastitis is an inflammation of the mammary glands of dairy cows that can be caused by physical or chemical agents, with the majority of cases caused by bacterial infection. Mastitis is the most common and expensive disease affecting the dairy industry worldwide (Harmon, 1994; Quinn *et al.*, 1994; Oussaoui *et al.*, 2004). The economic losses due to mastitis in the United States and worldwide have been estimated at US \$2 billion (Ott, 1999) and \$35 billion (Wellenberg *et al.*, 2002), respectively. In India, it is associated with an annual loss of about 7165.51 crores (Bansal and Gupta, 2009). The most common treatment method available for treating mastitis is the intramammary infusion of antibiotics. However, the cure rates obtained with antibiotics are generally poor and vary for different mastitis pathogens (Dingwell *et al.*, 2003). Further, the use of antibiotics may potentially lead to the emergence of antibiotic resistant strains of bacteria (Berghash *et al.*, 1983; White, 1999) Moreover, the use of antibiotics to treat bovine mastitis has been implicated as a common source of drug residues in milk (Erskine, 1996).. In light of the aforementioned problems and concerns, there is a need for alternative approaches for controlling mastitis in dairy cows.

Plants have traditionally provided a source of new chemical entities and numerous clinical studies have proved the therapeutic value of molecules of plant origin (Gibbons, 2004). Indeed, higher plant-derived products represent approximately 25% of drugs in current clinical use (Phillipson 2007). Of the more than 350 000 species of higher plants currently recognized, only 510% have been investigated and considering that each plant species may contain 500800 different secondary metabolites, the potential for the discovery of new therapeutic products in this largely untapped resource is considerable (Sibanda, *et al.*, 2007).The present study was undertaken to study the antibacterial effects of different extracts of *Euphorbia* sp. against important bovine mastitis pathogens.

## MATERIALS AND METHODS

Plant collection. The plant material was collected from Gurez area of Kashmir Valley in the month of September 2013. It was properly identified with the help of experts.

**Preparation of extracts:** The collected plant material was separated into root and shoots and properly washed with tap water, air dried and pulverized into fine powder. Three different types of extracts namely hexane, methanol and water were prepared using the cold maceration technique. Briefly, the plant material was submerged in the respective solvents for 72 hours at room temperature with regular shaking and filtered through Whatman No 1 filter paper. The residue was macerated twice with the same solvent overnight and filtered. The filtrates obtained from each extraction were mixed and concentrated under vacuum. The extracts obtained were kept at 4 for further use.

**Bacterial strains:** The in vitro antimicrobial activities of the extracts of *Euphorbia sp.* were tested against *Staphylococcus aureus* and *Escherichia coli*, the two most common and important pathogens causing bovine mastitis in Kashmir Valley.

**Antibacterial assay:** Antimicrobial activities of the crude extracts were first screened for their inhibitory zone by the agar disc-diffusion method. Briefly, crude extracts were prepared at a concentration of 100mg/ml with dimethyl sulphoxide (DMSO) as solvent. The Mueller Hinton Agar (MHA) medium was used for disc diffusion assay and Mueller Hinton broth was used for the minimal inhibition concentration (MIC) determination. One hundred microliters (100 ul) of cell suspension with approximately  $10^6$ - $10^8$  bacteria per milliliter was placed in petri dishes and dispersed over agar. In the following, a sterile paper disc (6 mm in diameter) impregnated with 10 ul of the plant extracts at the concentration of 100mg/ml and allowed to dry at 37 °C for 24 h was placed on to the agar and incubated overnight.

**Minimum inhibitory concentration (MIC) determination:** The minimum inhibitory concentration (MIC), which is considered as the lowest concentration of the sample

which inhibits the visible growth of a microbe was determined by the micro-broth dilution method. The MIC method was performed on extracts that showed their high efficacy against microorganisms by the disc diffusion method (inhibition zone higher than 11mm).

**Table.1. Primary Screening of the samples against bacterial pathogens by disc diffusion assay.**

S.No.	Tested Sample	Zone of inhibition (in mm)	
		S.aureus	E.coli
1	ELH	Inactive	Inactive
2	ELM	Active	Inactive
3	ELW	Active	Inactive
4	ERH	Inactive	Inactive
5	ERM	Active	Inactive
6	ERW	Active	Inactive
7	Ciprofloxacin	0.125	0.007

**Table.2. MIC of the active sample by microdilution method**

S.no	Compound	<i>S.aureus</i>
1	ERM	128
2	ERW	256
3	ELM	128
4	ELW	256
5	Ciprofloxacin	0.125

## RESULTS AND DISCUSSION

In the present study, the in vitro antimicrobial activity of six extracts against two important microbial strains and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values. The zones of inhibition greater than 6mm were considered as positive. According to the

results given in Tables 12, the methanolic and aqueous extracts of the investigated plant species (i.e., *Euphorbia* sp.) showed in vitro antimicrobial activities against *S.aureus*, the most common and important pathogen causing bovine mastitis.

The results of this study provide a guide for further study of specific compounds in this plant particularly the methanol extract. Future work involves the purification and characterization of these compounds, determination of their antibacterial activity (MIC) against *S aureus* and to study their possible mechanism of action. The extracts of several other species of *Euphorbia* are under process.

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