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Journal of Dairy, Veterinary & Animal Research

Research Article

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In vitro antibacterial activity and minimum inhibitory concentration of *ocimum sanctum* leaves against common bovine mastitis pathogens

Abstract

Mastitis, the inflammation of udder, is caused by a wide range of contagious and environmental microbial pathogens. In the present study, hydro-alcoholic extract of *Ocimum sanctum* leaves was evaluated, *in vitro,* for its antibacterial activity and minimum inhibitory concentration (MIC) against common bovine mastitis pathogens by agar cup and tube dilution method, respectively. In general, extract of *O. sanctum* revealed higher antibacterial activity against gram negative as compared to gram positive mastitis pathogens with inhibitory zones of 17.38±0.92 mm against *Escherichia coli* and 10.88±0.58 mm for *Streptococcus* spp. The corresponding MIC values were 125 mg/ml and 62.5 mg/ml respectively. The evaluation of antibacterial activity of herb in terms of enrofloxacin revealed 72.95% and 58.71% inhibition of *E. coli* and *Staphylococcus aureus* respectively. Overall, the study revealed a good antibacterial activity of the extract against common bovine mastitis pathogens which could be exploited as therapy for treating mastitis in lactating dairy cows.

Keywords: antibacterial, hydro-alcoholic extract, mastitis, mic, *ocimum sanctum*

Volume 7 Issue 6 - 2018

Tawheed Ahmad Shafi, Baljinder Kumar Bansal, Dhiraj Kumar Gupta

Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University, India

Correspondence: Tawheed Ahmad Shafi, PhD Scholar, Department of Veterinary Medicine, ADVASU, Ludhiana- 141004, Punjab, India, Tel 9420139548, Email tawheed78@gmail.com

Received: February 26, 2018 | **Published:** December 31, 2018

Introduction

Mastitis is one of the most costly diseases of dairy cattle resulting in the reduction of milk yield and quality.[1] The principle incriminate of mastitis is bacterial infection. Appropriate clearance of the pathogens from the bovine udder requires both the effectiveness of the drug and optimum functioning of the immune cells[2] which depends largely, till date, on the use of antibiotics. The use of antimicrobials has, overtime, increased the number of antimicrobial-resistant microbe's globally.[3] Further, the antibiotics used for the treatment of mastitis depress the activity of the polymorphonuclear cells (PMNs) that are considered primary cellular defences of the mammary gland;[4] are moderately efficacious requiring prolonged milk withdrawal due to residues in milk and development of hypersensitivity syndromes in human beings and effect on the manufacture of dairy products. For this reason, now-a-days, the concept of using non-antibiotic strategies for controlling mastitis is gaining more attention, based on, enhancement of the animal's natural defence mechanism by use of non-specific immuno-modulators such as plant materials, which constitute a major source of alternative medicine. These being organic in nature, possess less toxicity, lesser side effects, and generally does not pollute the milk and hence there is no milk withdrawal loss, a major problem with antibiotic use. Keeping above facts in view, the present study was planned to evaluate *in vitro* antibacterial activity of *Ocimum sanctum* (*Tulsi)* for exploitation of this herb in the therapy of bovine mastitis. The *O. sanctum* is a valuable herbal medicine being used in wide spectrum of animal diseases. The key constituents of *O. sanctum,* were volatile oil (Eugenol 80%), flavonoids and triterpine[5] and the herbal extract possessed immuno-modulatory and anti-inflammatory properties.[6] Singh et al.,[7] observed that *O. sanctum* fixed oil has good antibacterial activity against *Bacillus pumilus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and concluded that higher content of linolenic acid in *O. sanctum* fixed oil could contribute towards its antibacterial activity. Prakash et al.,[8] found eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum*, to be largely responsible for its therapeutic potentials.

Materials and methods

Collection of plant materials

Ocimum sanctum leaves were collected locally. The materials were shade dried in the laboratory and further dried in the incubator at 40°C to remove any excess moisture. The dried materials were then ground in a Willey Grinder (Arthur H. Thomas type) at room temperature. The powder thus obtained was stored in clean and air tight polythene bags till further use.

Preparation of extract

For preparation of the hydro-alcoholic extract, 100g powder of herb was weighed and soaked separately in ethanol (absolute) and distilled water in a ratio of 1:1 for 48hrs with continuous stirring at 37°C. After 48 hrs, the mixture was filtered through several layers of muslin cloth, then Whatman's filter paper using a funnel. The final extract was obtained after drying the filtrate in incubator with fan at 40°C and stored at 4°C till further use. The percent recovery of extract was recorded on dry weight basis (w/w).

Microorganisms

Common bovine mastitis pathogens such as *S. aureus,* coagulase negative *Staphylococcus* (CNS), *Streptococcus* spp., *Escherichia coli, Klebsiella* spp., and *Corynebacteriam* spp., were isolated as per criteria of National Mastitis Council.[9] For the preparation of fresh working inoculums a loop full of isolated bacteria was taken and streaked radially on to the sterile agar plates in such a manner that individual colony could develop. After incubation, five colonies of the test bacteria were taken and emulsified in 5ml of sterilized nutrient broth and incubated overnight at 37°C to get the required concentration of the pathogens.

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Antibacterial activity and assessment of minimum inhibitory concentrations (MIC)

The antibacterial activity of the extract was screened by agar cup method as described by Cruickshank et al.[10] On sterile agar plates, wells of 8 mm diameter were punched with a sterile gel cutter and wells sealed with molten nutrient agar to prevent escape of extracts through bottom. Stock inoculum of test bacterium was swept over the agar plate and subsequently 100µl (50mg) of herbal extract was poured into the wells, along with selected antibiotics (amoxycillin-clavulanate-30/15mcg, ceftriaxone-10mcg, enrofloxacin-10mcg, gentamicin-10mcg and penicillin-2 units) were placed equidistantly on the plate. Plates were incubated at 37°C for 24hr when zones of inhibition were measured. Eight replicates for each bacterial isolate were made and results were taken as mean ± S.E. Enrofloxacin showed good antibacterial activities against all the tested pathogens and was selected for comparing the antibacterial activity of herbal extract. MIC of the extract was evaluated by tube dilution method as described by Robert et al.,[11] with slight modifications. For it, serial dilutions of the extracts were prepared from 500 mg/ml dilution with the help of tween-20.

Results and discussion

The final yield (g/100g powder) of the hydro-alcoholic extract of *O. sanctum* leaves was recorded to be 9.6%. Inhibitory zones of variable diameters were formed by extract of *O. sanctum* were different for different bacteria tested, with the highest (17.38±0.92mm) value against *E. coli* and lowest (10.88±0.58) for *Streptococcus* spp. during the course of the present investigation. The zone of inhibition shown by *O. sanctum* against *S. aureus was* found to be 14.27±0.43mm. The hydro-alcoholic extract of *O. sanctum* leaves revealed highest antibacterial activity against gram negative pathogens as compared to gram positive pathogens during the present investigation (Table 1). The MIC of hydro-alcoholic extract of *O. sanctum* leaf for *Streptococcus* spp. and *Corynebacterial* spp. was observed to be lower as compared to other pathogens during the present course of study. Ali et al.,[12] observed the zones of inhibition for flavanoids of *O. sanctum* in concentration of 400 mg/ml as 20.12, 20.75, 20.95, 19.55 and 20.1mm against *E. coli*, *Proteus*, *S. aureus*, *Staphylococcus cohni* and *Klebsialla pneumonia,* respectively. Also Mukherjee[13] recorded zones of inhibition ranging 12.5-18.3mm for aqueous extract of *O. sanctum* leaves against *S aureus*, *Streptococcus agalactiae* and *E. coli* and herb was found to have inhibitory effect on *Aspergillus ochraceous* mycelial growth and ochratoxin production as well.[14] Shokeen et al.[15] isolated and characterized the components of *O. sanctum* with activity against *Neisseria gonorrhoea*. They found H12c as the active compound which was characterized as eugenol, with a MIC of 85-256mg/ml. Singh et al.[7] observed that *O. sanctum* fixed oil has good antibacterial activity against *B. pumilus*, *P. aeruginosa* and *S. aureus* and concluded that higher content of linolenic acid in *O. sanctum* fixed oil could contribute towards its antibacterial activity.

The antibacterial activity of *O. sanctum* could be attributed to its key constituents, like volatile oil (Eugenol), linolenic acid, flavonoids and triterpine (Ursolic acid). Preliminary studies revealed that the hydro-alcoholic (1:1) extract, of *O. sanctum* leaves showed greater zones of growth inhibition against common mastitis pathogens and this antibacterial potential of the herb could be used in, *in vivo* therapeutic trials to validate its beneficial role for prevention of mastitis.

Table 1 Antibacterial activity and assessment of MIC against common mastitis pathogens

Pathogen	Zones of inhibition in mm (Mean±SE)		% activity	MIC (mg/ml)
	O. sanctum	Enrofloxacin		
Staphylococcus aureus	14.27±0.43	24.30±0.32	58.71	125
CNS	13.88±0.97	27.70±0.34	50.09	125
Streptococcus spp.	10.88±0.58	27.40±0.37	39.69	62.5
Escherichia coli	17.38±0.92	23.82±0.27	72.95	125
Corynebacterial spp.	11.00±0.53	26.90±0.33	40.89	62.5
Pseudomonas spp.	11.75±0.45	24.60±0.24	47.76	125
Klebsiella spp.	17.13±0.48	21.70±0.21	78.92	125

Acknowledgments

The authors are highly thankful to the Director of Research, GADVASU, Ludhiana for providing necessary facilities to carry out this work.

Conflicts of interest

The author declares that there are no conflicts of interest.

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Citation: Shafi TA, Bansal BK, Gupta DK. *In vitro* antibacterial activity and minimum inhibitory concentration of *ocimum sanctum* leaves against common bovine mastitis pathogens. *J Dairy Vet Anim Res*. 2018;7(6):322–324. DOI: 10.15406/jdvar.2018.07.00233