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Evaluation of antibacterial activity of *Kedrostis foetidissima* (Jacq.) Cogn. plant extracts against pathogens causing bovine mastitis

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Abstract

Mastitis is an infectious, inflammatory disease of mammary gland caused by various invading pathogenic microorganisms. Occurrence of this disease in bovine causes low yield of milk and huge economic loss to the dairy farming community. Antimicrobials are commonly used to treat mastitis. But, disadvantages like antimicrobial resistance, food residues, environmental pollution and high cost of antimicrobials prompted the search of alternative therapeutics. In this study, the aqueous and alcoholic extracts of *Kedrostis foetidissima* (Jacq.) Cogn. herb at different concentrations was evaluated for its antibacterial activity using disc diffusion method and compared with standard antibiotics. A total number of 125 milk samples were collected from mastitis cows and organisms like *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumonia* were isolated and used for the demonstration of antibacterial activity by estimating the zone of inhibition. The results of present study revealed the antibacterial potential of *Kedrostis foetidissima* (Jacq.) Cogn. herb extracts against mastitis causing pathogens. At higher concentration both extracts produced therapeutic level of zone of inhibition against pathogens, which is almost similar, that of gentamicin and tetracycline. Since the herbal extracts have proved for their antimicrobial properties they may become an alternative source for the treatment of mastitis in future with further detailed studies.

Keywords: *Kedrostis foetidissima* (Jacq.) Cogn., antibacterial activity, zone of inhibition, antibiotic sensitivity test

1. Introduction

In any country, mastitis is recognised as one of the most costly diseases affecting the dairy industry with severe economic loss ^[1]. Mastitis is an infectious disease caused by pathogenic microorganisms, out of which *Staphylococcus aureus* is a major pathogen causing 50% of incidence. Antibiotics have long been considered the only effective way to fight against such infectious pathogens causing mastitis. However they are very expensive and pose serious disadvantages like resistance, food residues and environmental pollution. So the focus of research has turned back to traditional medicines as an alternative for the treatment and control of infectious diseases like mastitis.

Kedrostis foetidissima (Jacq.) Cogn. is one of the promising medicinal plants from Cucurbitaceae family used widely in Paliyan tribes of Sirumalai Hills of South India ^[2] and tribes and non-tribes of Siruvani Hills, Coimbatore, Tamilnadu. This herb was extensively studied for treating urinary tract infections ^[3], skin diseases ^[4], cough in children^[2], fungal infections ^[5] and for bloat in cattle ^[6]. Phytochemical screening of this plant revealed the presence of alkaloids, flavonoids, steroids, phenols, triterpenoids, glycosides and saponins ^[7]. Factors such as rich source of various phytochemicals, current usage in many communities and previous studies on beneficial effects against fungi and bacteria ^[8] gives an interest and importance to study the antibacterial effect of *Kedrostis foetidissima* (Jacq.) Cogn. against pathogens causing mastitis. Hence the study was designed to evaluate the antibacterial activity of extracts of *Kedrostis foetidissima* (Jacq.) Cogn. against mastitis causing microorganisms and to compare the antibacterial effect of its extracts with standard antibacterial drugs.

Materials and Methods**a. Collection and identification of *Kedrostis foetidissima* (Jacq.) Cogn. plant.**

Whole plants of *Kedrostis foetidissima* (Jacq.) Cogn. were collected from Coimbatore, Tamilnadu and authenticated by the Department of Botany, Aringar Anna Govt. Arts and Science College, Laddivadi, Namakkal, Tamila Ndu, India.

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b. Preparation of aqueous and alcoholic extracts from *Kedrostis foetidissima* (Jacq.) Cogn.

The collected plants were washed with distilled water and blotted gently on filter paper sheets and shade dried. The plant material was powdered after complete drying using a mechanical mixer / grinder, sieved and stored in a sterile container under refrigeration.

Aqueous and alcoholic extracts were prepared separately from the powdered plant material (100 g each) by using 400 ml of sterile distilled water and 400 ml of 95% ethanol respectively. Both extracts were kept in an orbital shaker for 24 hours at room temperature. Then the extracts were filtered by using Whatman filter paper No. 1 to remove the extractable substances and then evaporated at 37 °C in hot air oven and the dried extracts were stored at 4 °C in a sterile container for further study.

c. Collection of milk samples from bovines having clinical / subclinical mastitis

125 milk samples from bovines having clinical and sub-clinical mastitis by using standard sampling procedures [9]. Relevant information regarding animal having clinical condition was recorded. Sterile vials of 15 ml capacity were used for the collection of milk samples and stored in a cooler with ice packs. Then the samples were processed for identification of microorganisms.

d. Isolation and identification of microorganism

Isolation and identification of bacterial organisms from milk samples was done by using standard microbiological procedures at Dept. of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal. The samples were cultured on Nutrient agar, Blood agar and MacConkey agar plates, supporting the growth of various types of bacteria for their study and isolation. The plates then incubated at 37 °C for 24-48 hours. The isolated bacteria were identified on the basis of their cultural and morphological characteristics [10]. The pure cultures of bacterial isolates were obtained by sub culturing on differential and selective media. The bacterial isolates further subjected to biochemical tests for confirmation and tested *in-vitro* for their antimicrobial susceptibility by agar disc diffusion method. Six samples of common pathogens causing mastitis were selected and used further for antibacterial studies.

e. Demonstration of antibacterial activity

Antibacterial activity was demonstrated using a modification of the method originally described by [11] which is widely used for the antibacterial susceptibility testing.

f. Comparison of antibacterial activity of the plant extracts with standard antibiotics

The antibacterial activity of the plant extracts was compared with standard antibiotics by Antibiotic Sensitivity Testing (ABST) method which is similar to disc diffusion method. In this procedure different broad spectrum antibacterial discs (Standard discs of ciprofloxacin, cefotaxime, gentamicin, ampicillin & cloxacillin and tetracycline) were used.

g. Statistical analysis

All statistical analysis was performed using SPSS 2013 software. Comparison of zone of inhibition between standard antibacterial agents and plant extracts was performed using ANOVA. Inhibition zone are expressed as Mean \pm SD.

Results

The different concentration of aqueous and alcoholic extracts of *Kedrostis foetidissima* (Jacq.) Cogn. and standard antibiotics like ciprofloxacin, gentamicin, cefotaxime and tetracycline produced zone of inhibition against *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumonia* were depicted in table 1. Compared to this group, its level of zone of inhibition in the ciprofloxacin and cefotaxime were comparably and significantly higher.

There was no significant difference between different concentrations of aqueous and alcoholic extracts of *Kedrostis foetidissima* (Jacq.) Cogn. produced zone of inhibition against *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumonia*, except 100% of both the extract against *Klebsiella pneumonia*. Further, the 100% of the both extract produced higher level of zone of inhibition was evidenced against all the tested pathogens.

Among the standard antibiotics, ciprofloxacin and cefotaxime only showed the significant difference in the zone of inhibition as compared to different concentration of both the extracts and other standard antibiotics. However, there was no appreciable difference between the different concentration of both the extracts and gentamicin or tetracycline. Moreover, the 100% of the both aqueous and alcoholic extract produced therapeutic level of zone of inhibition against *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumonia*, which is almost similar, that of gentamicin and tetracycline. Also the combination of ampicillin and cloxacillin had showed no results against any of the microorganisms tested in this study.

Table 1: Zone of inhibition produced by herbal extracts at different concentrations and some standard antibacterial agents

S. No.	Drug	Zone of Inhibition (mm)		
		<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Klebsiella pneumonia</i>
1	Aqueous 25%	18.02 \pm 2.00 ^{ab}	16.92 \pm 0.87 ^a	19.82 \pm 0.25 ^a
2	Aqueous 50%	20.23 \pm 1.69 ^{ab}	18.10 \pm 0.82 ^{ab}	21.71 \pm 0.30 ^{ab}
3	Aqueous 100%	21.65 \pm 1.39 ^{ab}	19.41 \pm 0.77 ^{ab}	22.87 \pm 0.33 ^{bc}
4	Alcoholic 25%	18.15 \pm 2.53 ^{ab}	18.33 \pm 1.01 ^{ab}	21.54 \pm 0.53 ^{ab}
5	Alcoholic 50%	21.58 \pm 1.21 ^{ab}	19.99 \pm 1.12 ^{ab}	22.67 \pm 0.68 ^{bc}
6	Alcoholic 100%	23.20 \pm 1.10 ^b	21.50 \pm 1.00 ^{ab}	24.01 \pm 0.31 ^c
7	Ciprofloxacin	32.47 \pm 2.75 ^c	23.68 \pm 1.79 ^b	33.32 \pm 0.26 ^f
8	Gentamicin	22.32 \pm 1.38 ^{ab}	17.056 \pm 1.22 ^a	19.98 \pm 0.43 ^a
9	Cefotaxime	33.53 \pm 0.35 ^c	30.33 \pm 0.39 ^c	30.76 \pm 0.44 ^e
10	Tetracycline	14.72 \pm 2.18 ^a	15.82 \pm 2.40 ^a	27.30 \pm 0.52 ^d

Discussion

In dairy cows, mastitis is an economically devastating disease and considered as a serious problem since it causes an immense economic loss in dairy cows and health hazard to human worldwide especially in developing countries [12]. According to the World Health Organization, herbs are considered as best source for the development of therapeutic active components [13]. This study evaluated the antibacterial activity of *Kedrostis foetidissima* (Jacq.) Cogn. herbal extracts against microorganisms isolated from mastitis milk. Previous studies on *Kedrostis foetidissima* (Jacq.) Cogn. herb and its extracts proved its antibacterial properties against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* [14] *Enterobacter aerogenes*, *Proteus vulgaris*, *E. coli* and *Serratia marcescens* [15], *Vibrio cholera*, *Lactobacillus brevis*, *Bacillus sp.*, *Micrococcus luteus* and *Proteus vulgaris* [16].

In this study, both aqueous and alcoholic extracts of *Kedrostis foetidissima* (Jacq.) Cogn. herb demonstrated an antimicrobial activity against mastitis causing *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* organisms. The zone of inhibition against the microorganisms tested has increased with increasing concentrations of herbal extracts and proved the extent of effectiveness on increasing concentration [17].

The qualitative and quantitative phytochemical analysis of *Kedrostis foetidissima* (Jacq.) Cogn. herb showed an enriched level of phytochemicals like alkaloids, tannins, phenols, carbohydrates, lipids, cardiac glycosides, flavonoids, saponins and proteins [16] and they are suggested to be responsible for their biological activities [7]. Among the extracts, the ethanolic extract showed better antibacterial potency than aqueous extract and it is proven that, different solvents extract different compounds [18] and such compounds could be the reason for their better therapeutic properties.

Microorganisms isolated from the milk samples of bovines having mastitis were proved for their increased drug resistance by many studies [19]. Antibiotic sensitivity testing is an important method, commonly followed to identify a suitable antibacterial agent for the treatment of bacterial infection and to prevent the occurrence of antibiotic resistance, which is considered as potential threat to the mankind. In this study, drugs like ciprofloxacin and cefotaxime were proved for having significant antibacterial effects and drugs like gentamicin and tetracycline were found to be less effective against all microorganisms tested. Interestingly, all the organisms tested in this study have not showed any sensitivity against amoxicillin and cloxacillin drug combination proved the development of resistance against this group.

Conclusion

The results of present study revealed the antibacterial potential of *Kedrostis foetidissima* (Jacq.) Cogn. against major mastitis causing pathogens in bovines. The zone of inhibition produced by various concentrations of aqueous and alcoholic extracts showed significant difference with antibacterial agents and all organisms showed resistance towards combination of amoxicillin and cloxacillin. Since the plants used in this study have proved to possess antimicrobial properties, and are locally available, they may become alternative sources of antimicrobial drugs and further studies on *Kedrostis foetidissima* (Jacq.) Cogn. may lead toward discovery of novel antimicrobial compounds in future.

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