



Evaluation of antibacterial activity of methanol extract of leaves of *Adhatoda vasica* on mastitis pathogens

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Abstract

Plan: The present study was undertaken to evaluate the antimicrobial activity of aqueous and methanol extracts of *Adhatoda vasica* against mastitis pathogens.

Methodology: Milk samples were collected from mastitis cows and five bacteria were isolated. Based on Grams staining and various biochemical tests, they were identified as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Klebsiella pneumoniae*, *Streptococcus dysgalactiae* and *Escherichia coli*. Leaves of *Adhatoda vasica* was subjected to aqueous and methanol extraction. Antibacterial activity of the extracts was evaluated by disc diffusion method and zone of inhibition was measured.

Outcome: The methanol extract was found to be having significant antibacterial activity against *Staphylococcus aureus*, *Streptococcus agalactiae*, *Klebsiella pneumoniae*, *Streptococcus dysgalactiae* and *Escherichia coli* with zone of inhibition 21.7 ± 0.58 mm, 18.3 ± 0.58 mm, 21.3 ± 0.58 , 18.3 ± 0.58 and 28.3 ± 0.58 mm respectively at 200 mg/ml concentration.

Keywords: Mastitis, *Adhatoda vasica*, antibacterial activity, methanol extract, disc diffusion

1. Introduction

Mastitis, an inflammation of udder is the most common disease of dairy cattle. At present mastitis is treated with antibiotics and most of the antibiotics are ineffective. The presence of antibiotic residues in milk causes health hazard to human and also results in the development of drug resistant microorganisms that are difficult to treat. Bacterial resistance to antibiotics is a serious threat to animal and human. Alternate approaches to the treatment are gaining importance to reduce the human health hazards. Alternate approaches include medicinal herbs, enzymes and cytokines. Antiviral, antifungal, antibacterial and anti-inflammatory effects of many plants are reported^{1, 2, 3, 4, 5}.

Adhatoda vasica plant extract has been used to relieve cough and breathlessness⁶. Antibacterial, wound healing and hypoglycemic activity of *Adhatoda vasica* has been reported⁷.



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Many animal diseases such as abscesses, anthrax, throat diseases, asthma, tuberculosis, jaundice, scabies, urticaria, rheumatism, pneumonia, hematuria and contagious abortion were treated with *Adhatoda* in veterinary medicine⁸. The present study was undertaken to study the effect of aqueous and methanol leaf extract of *Adhatoda vasica* on mastitis pathogens.

2. Materials and Methods

Mastitis milk samples were collected from Madras Veterinary College and Chengalpattu Veterinary Hospital. Teats were washed with sterile water and dried. Teat ends were cleaned with cotton and the first milk was discarded and collected into a sterile vial. After serial dilution, the pure cultures were isolated from streak plates. Blood agar was used as a selective culture medium for mastitis pathogens. The individual colony characteristics were observed. The cultures were stained using Gram's staining procedure. The isolates were identified using motility and biochemical characteristics⁹.

Fresh plant leaves of *Adhatoda vasica* were collected from Maduranthakam in Kancheepuram district, Tamilnadu. The leaves were washed with 70% ethanol and then rinsed with sterile distilled water, air dried and stored in air tight bottles. Clean dry leaves were ground to fine powder with the help of mortar and pestle. These powdered materials were used for the aqueous and methanol extracts. Fifty gram of powdered materials were mixed with 10 ml of sterile distilled water and kept on a rotary shaker for 12 hours at 30° C. Thereafter, it was filtered with the help of Whatman no.1 filter paper. The filtrate was then centrifuged at 2000 rpm for 10 minutes. Then the supernatant was collected and stored at 4° C for further use.

100 gm of powdered materials were soaked in 200 ml of methanol and kept at 30° C for 24 hours in a rotary shaker. After that it was filtered through Whatman No.1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant was collected and allowed to evaporate until completely dried. The dried extract was resuspended in sterile distilled water in different concentrations of 100 and 200 mg/ml.

Table 1 Zone of inhibition (in mm) produced by methanol extract of *A. vasica* against mastitis pathogens

Organism	Zone of inhibition in mm by methanol extract				Amicacin 30 µg/disc (Control) Mean ±SD
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	50mg/ml	100mg/ml	150mg/ml	200mg/ml	
<i>Staphylococcus aureus</i>	14.3±0.58	16.8±0.58	17.7±0.58	21.7±0.58	20.3±0.58
<i>Streptococcus agalactiae</i>	10.7±0.58	13.7±0.58	15.3±0.58	18.3±0.58	21.3±0.58
<i>Escherichia coli</i>	10.3±0.58	12.3±0.58	20.7±0.58	28.3±0.58	22.3±0.58
<i>Klebsiella pneumoniae</i>	8.7±0.58	14.3±0.58	16±1.0	21.3±0.58	20.7±0.58
<i>Streptococcus dysgalactiae</i>	12.7±0.58	16.7±0.58	16.7±0.58	18.3±0.58	20.7±0.58

Values are mean of 3 experiments ± S.D

Antibacterial activity was assessed using standard disc diffusion method. 10µL (50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml) of extract was added to sterile discs and used and Amicacin (30 µg/disc) obtained from Himedia laboratories, Mumbai was used as positive control.

3. Results and Discussion

Based on the Gram's staining and biochemical tests, the five isolates were identified as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Klebsiella pneumoniae* and *Escherichia coli*. The aqueous extract of *A.vasica* was not found to be active against all organisms tested. The methanol extract showed significant antibacterial activity at 150 mg/ml and 200 mg/ml concentration (Table-1). Methanol extract of *Adhatoda vasica* showed antibacterial activity against *Streptococcus agalactiae*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Klebsiella pneumoniae* and *Escherichia coli* isolated from mastitis milk. Johnson et al. 2011 reported the antibacterial activity of alcohol extract of leaves of *Calotrophis procera* against *E.coli* and *S.aureus*¹⁰.

The study of Doss et al. 2012 showed significant antibacterial activity of *Astercantha longifolia*, *Rhynchosia capitata* and *Cenchrus ciliaris* against bovine mastitis pathogens¹¹. In the present study at 200 mg/ml concentration of methanol extract of *A.vasica*, the maximum zone of inhibition observed was 21.7±0.58 mm against *S.aureus*, 21.3±0.58 mm against *Klebsiella pneumoniae* and 28.3±0.58 mm against *E.coli* at 200 mg/ml concentration. Mubarack et al 2012 reported some of the ethno-veterinary herbs against mastitis pathogens in which *Dactyloctenium indicum* was reported to have antibacterial activity against *E.coli* with 22 mm. Mubarack et al 2012 reported that *Asteractantia longifolia* extract showed zone of inhibition of 25 mm against *S.aureus*¹². *Combretum molle*, used in Ethiopian traditional medicine, was reported to have antibacterial activity against mastitis pathogens¹³.

4. Conclusion:

Methanol extract of *Adhatoda vasica* was found to be having significant antibacterial activity against mastitis pathogens. Further analysis of extract, compound isolation and *in vivo* animal studies may help in treatment of mastitis.

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