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In-Vitro Antimicrobial Effects of Some Selected Plants against Bovine Mastitis Pathogens

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Abstract

Bovine mastitis continues to be the most costly disease to the dairy farmers. It dominates in Tamilnadu as one of the most prevalent diseases in dairy cattle among the dairy farms. Mastitis treatment with antibiotics leads to the development of antibiotic resistant strains and consumer health problem. The present study is an *in vitro* antibacterial activity of three medicinal plants against bovine udder isolated bacterial pathogens. Aqueous and methanol extracts of three plants were investigated by agar disc diffusion method. Methanol extracts of *Asteracantha longifolia* and *Dactyloctenium indicum* showed significant activity against *Staphylococcus aureus* (25mm) and *E.coli* (22mm) respectively.

***Key words*:** Bovine mastitis pathogens, Methanol, Disc diffusion method, *Asteracantha longifolia*, *Dactyloctenium indicum* and *Trichodesma indicum*

1. Introduction

Mastitis continues to be among the costliest diseases to the dairy industry, and annual economic losses attributed to this disease in the United States are estimated to approach $2 billion. Among cattle diseases, bovine mastitis is a serious problem which affects the basic income of the farmers depleting their dairy sources. Worldwide, mastitis is associated with economic losses of $35 billion annually. It adversely affects milk production whereby losses due to subclinical mastitis are more severe than those due to clinical cases. Controlling subclinical mastitis can reduce the losses in milk production substantially. Decreased milk production and quality, as well as veterinary expenses, all contributes to these economic losses [1].

Clinical and subclinical cases of mastitis are routinely treated with antimicrobials both intramammarily and parenterally. The use of antimicrobials over long periods has triggered the development of multidrug resistant strains, which has resulted in the use of increasing doses of antimicrobials, causing the danger of increasing amounts of drug residues in milk, a potential biohazard [2].

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Medicinal plants have been used for ages in developing countries as alternative treatment to health problems. India has a diverse flora and a rich tradition in the use of medicinal plants for antimicrobial applications. In India specifically in Tamil Nadu ethnoveterinary practices are very common in villages. Most of the approaches of the farmers are based on empiric knowledge with significant results in cattle. A short survey prior to this study was undertaken among known farmers about their interest in ethnobotany and treatment of their cattle sources. Most of them expressed a desire to learn more about the proper use and application of ethnoveterinary practices as these were economically, socially and culturally more acceptable for marginalized communities.

The present study was undertaken to investigate the effects of aqueous and methanolic extracts of *Asteracantha longifolia*, *Dactyloctenium indicum* and *Trichodesma indicum*. To our knowledge, no reports or studies exist relating to *in vitro* application of *A. longifolia, D. indicum* and *T. indicum* extracts in bovine mastitis studies. This is the first report on *A. longifolia, D. indicum* and *T. indicum* antibacterial action against bovine mastitis isolated contagious pathogens.

2. **Materials and Methods**

2.1. *Plant collection*

Fresh plant parts of *Asteracantha longifolia*, *Dactyloctenium indicum* and *Trichodesma indicum* were collected randomly from the gardens and villages of Coimbatore district, Tamilnadu, India. The taxonomic identities of plants were confirmed by Dr.V.Sampath Kumar, Scientist, Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu, India and the voucher specimen of the plant was preserved in RVS College Microbiology Laboratory. The collected plants were washed with running tap water, air dried, homogenized to a fine powder and stored in air-tight bottles at 4^{o}C.

2.2**.** *Preparation of Crude Extracts*

Solvent extraction

100 grams of dried plant material was extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume [3]. It was stored at 4^{o}C in airtight bottles for further studies.

Aqueous extraction

100 grams of dried plant material was extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume [3].

2.3. *Bacterial strains*

Bacterial strains used in this study were the isolated pathogens isolated from clinical cases of bovine mastitis such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Streptococcus agalactiae*. All the strains were confirmed by cultural and biochemical characteristics [4] and maintained in slants for further use.

2.4. *Antibacterial activity*

The antibacterial assay of aqueous and methanolic extracts was performed by Bauer *et al.* [5]. The Mueller Hinton Agar media, along with the inoculum (10^6 cfu/ml) was poured into the petridishes. For the agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 mg/ml of the test compound, allowed to dry and then placed on the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) surrounding bacterial growth. For each bacterial strain, controls were included that comprised pure solvents instead of the extract [6].

3. **Results and Discussion**

The traditional ethno-veterinary medicinal practices are being followed by the rural folk through which a number of veterinary diseases are managed in the developing countries. The use of antibiotics and other chemical products are banned for animal healthcare in a number of countries because of human healthcare. The World Health Organization (WHO) states that 74% of the medicines derived from plant resources have a modern indication that correlates with their traditional, cultural (and sometimes ancient) uses [6].

Results obtained in the present study revealed that the tested three plant extracts posses' potential antibacterial activity against *S.aureus, E.coli*, S. *agalactiae* and *K.pneumoniae* (Table 1). Each plant extract of the three plant species were tested at two different concentrations (100 & 200 mg/ml) to see their inhibitory effects against bovine mastitis isolated pathogens. Of the three candidate plants in this study, *A.longifolia* showed significant antibacterial activity against all the tested bacteria and the remaining plants showed moderate activity after alcoholic extraction. None of the extracts showed activity against *K.pneumoniae*.

The most pronounced activity with inhibition zones of more than 14.0 mm was shown by methanol extract (inhibition zone 25 mm against *S.aureus* at concentration 200mg /ml) and aqueous extract (inhibition zone 16 mm against *E.coli* at concentration 200mg/ml) of *A. longifolia*. The methanol extract of *D.indicum also* showed significant antimicrobial activity against *Staphylococcus aureus* and *E.coli* with inhibition zones 22 and 20 mm respectively at concentration 200 mg /ml while the aqueous extract showed inhibition against *E.coli* with 18mm inhibition zones at concentration 200 mg/ml. When the concentration of the extracts were decreased from 200-100 mg/ml slight decrease in inhibition zones were observed.

Wynn [7] describes today's traditional medicine, as undoubtedly the oldest form of medicine and probably had evolved simultaneously with the evolution of human beings. EVM has been a mainstay of developing countries that lack access to conventional medicines for veterinary health care, often the only unaffordable means to poor farmers. The Ethno veterinary medicine (EVM) practices could be an effective approach for tackling problems like mastitis, bovine viral diarrhea and many deficiency disorders. With the traditional knowledge in the background potential plants can be prospected to reach the active fraction or molecule(s), which can be further formulated. Besides, the dried plant material itself could be utilized, by premixing it with the fodder of cattle feed thereby utilizing the pure molecule indirectly as a marker to maintain the product quality control. Further studies may be necessary to elucidate the specific phytoactive compounds in the leaf extract of the plant *A. longifolia* and *D. indicum*

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Table 1: Antibacterial activity of ethno veterinary medicinal plants

Medicinal Plants	**Extracts**	**Conc. (mg/ml)**	**Zone of Inhibition (mm)**			
			S.aureus	*E.coli*	*S. agalactiae*	*K. pneumoniae*
A. longifolia	Methanol	100	18.7 ± 0.3	14.1 ± 0.1	-	-
		200	24.2* ± 0.52	16.7 ± 0.25	9.0 ± 0.1	-
	Water	100	11.6 ± 0.55	12.0 ± 0.05	-	-
		200	13.7 ± 0.25	15.7 ± 0.26	-	-
D. indicum	Methanol	100	19.8 ± 0.28	16.8 ± 0.15	10.0 ± 0.05	-
		200	21.7* ± 0.25	20.0* ± 0.05	10.7 ± 0.25	-
	Water	100	10.9 ± 0.1	13.8 ± 0.32	-	-
		200	14.5 ± 0.5	17.8 ± 0.20	9.7 ± 0.251	-
T.indicum	Methanol	100	-	10.7 ± 0.26	-	-
		200	14.8 ± 0.28	14.9 ± 0.11	15.8 ± 0.152	-
	Water	100	-	-	-	-
		200	-	10.8 ± 0.28	-	-
Standard (Ciprofloxacin)		10µg	29±0.1*	30±0.15*	22±0.1*	20±0.15*

n=3, student ‘t’ test, * p value < 0.01, ** p value < 0.01

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