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Activity of Some Selected Medicinal Plant Extracts Against Bovine Mastitis Pathogens

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Abstract: The study was conducted with the objective to evaluate the antibacterial activity of the aqueous and alcoholic extracts of some selected medicinal plants against the microbes responsible for causing diseases in mastitis. The aqueous and alcoholic extracts of aerial parts of selected medicinal plants were obtained by extraction in cold maceration using water and methanol (95%) as solvents, respectively. Both the extracts were assessed for their antibacterial activity against *Streptococcus agalactiae*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The extracts were effective against the bacteria tested with zone of inhibition ranging from 9.0-24.0 mm. The Minimum Inhibitory Concentration (MIC) values for the extracts ranged from 0.125-2.00 mg mL^{-1}.

Key words: Antibacterial activity, mastitis pathogens, disc diffusion method, Minimum Inhibitory Concentration (MIC), solvent, India

INTRODUCTION

India stands first in milk production in the world (Lahoti and Chole, 2009). Infection of the cow's udder (bovine mastitis) has remained one of the major constraints in growth of dairy industry in India and abroad (Sasidhar *et al*., 2002; Osteras and Solverod, 2009). Amongst 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.

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 (Dhanabalan *et al*., 2008).

Indians have been traditional users of plant derived medicines both directly and as an integral constituent of plethora of packages and practices of indigenous medicine. These plants and their extracts are being used in the pharmaceutical preparations of modern medicine, veterinary and in agriculture (Virmani *et al*., 2010). 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. The antimicrobials obtained from plants are of much therapeutic potential and are effective in treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Kokoska *et al*., 2002).

The present study was undertaken to investigate the effects of aqueous and methanolic extracts of *Brachiaria* sp. *Cenchrus ciliaris*, *Abutilon indicum* and *Coccinia grandis*. To the knowledge, no reports or studies exist relating to *in vitro* application of *Brachiaria* sp. and *Cenchrus ciliaris* extracts in bovine mastitis studies. This is the 1st report on *Brachiaria* sp. and *Cenchrus ciliaris* antibacterial action against bovine mastitis isolated contagious pathogens.

MATERIALS AND METHODS

Plant collection: Fresh plant parts of *Brachiaria* sp. *Cenchrus ciliaris*, *Abutilon indicum* and *Coccinia grandis* 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

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voucher specimen of the plants have been preserved at 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°C.

Preparation of crude extracts

Solvent extraction: About 100 g of dried plant material was extracted with 200 mL of methanol kept on a rotary shaker for 24 h. There after, 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 1-5th of the original volume (Sasikumar *et al*., 2005). It was stored at 4°C in airtight bottles for further studies.

Aqueous extraction: About 100 g 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 1-5th of the original volume (Sasikumar *et al*., 2005).

Bacterial strains: Bacterial strains used in this study were the 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 (Klastrup, 1975) and maintained in slants for further use.

Antibacterial activity: An inoculum of each of the bacterial strains (single colony) was suspended in 5 mL of broth (nutrient broth) and incubated at 37°C for 18 h. The antibacterial activity was tested by the disc diffusion assay (Bauer *et al*., 1966). About 0.1 mL of inoculum (10^5 CFU mL^{-1}) was spread on sterile Mueller Hinton plates and sterile study discs were placed on the inoculated surface.

The discs were impregnated with 15 μL of each of the extract at 2 different concentration (100 and 200 mg mL^{-1}), kept at room temperature for absorption of extract in the medium and then incubated at 37°C in the incubator for 24 h.

The antibacterial activity was evaluated by measuring the diameter of inhibition zone as per the procedure described by Kim *et al*. (1995). Ciprofloxacin was used simultaneously as control.

Minimum Inhibitory Concentration (MIC): For determination of MIC, 1 mL of broth medium was taken into 10 test tubes for each bacteria. Different concentrations of plant extracts ranging from 0.125-8 mg mL^{-1} concentration were incorporated into the broth and the tubes were then inoculated with 0.1 mL of inoculum of respective bacteria (10^5 CFU mL^{-1}) and kept at 37°C for 24 h. The test tube containing the lowest concentration of extract which showed reduction in turbidity when compared with control was regarded as MIC of that extract.

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 (Das Ayyappa and Doss, 2009).

Results obtained in the present study revealed that the tested 4 plant extracts posses potential antibacterial activity against *S. aureus*, *E. coli*, *S. agalactiae* and *K. pneumoniae* (Table 1). The plant extracts of the 4 plant species were separately tested at 2 different concentrations (100 and 200 mg mL^{-1}) to see their inhibitory effects against bovine mastitis isolated pathogens.

Of the four candidate plants in this study *C. ciliaris* and *C. grandis* 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 >24.0 mm was shown by methanol extract (inhibition zone of 24 mm against *S. aureus* at concentration 200 mg mL^{-1}) and aqueous extract (inhibition zone of 16 mm against *E. coli* at concentration 200 mg mL^{-1}) of *C. ciliaris*. The methanol extract of *C. grandis* also showed significant antimicrobial activity against *Staphylococcus aureus* and *E. coli* with inhibition zones 21 and 12 mm, respectively at concentration 200 mg mL^{-1} while the aqueous extract showed inhibition against *S. aureus* with 13 mm inhibition zones at concentration 200 mg mL^{-1}.

When the concentration of the extracts were decreased from 200-100 mg mL^{-1} slight decrease in inhibition zones were observed. Minimum Inhibitory

Table 1: Antibacterial activity of ethnoveterinary medicinal plants

Medicinal plants	Extracts	Conc. (mg mL^{-1})	Zone of inhibition (mm)			
			S. aureus	*E. coli*	*S. agalactiae*	*K. pneumoniae*
C. ciliaris	Methanol	100	16	10	-	9
		200	24	16	10	11
	Water	100	10	-	-	-
		200	12	9	9	-
C. grandis	Methanol	100	15	9	12	9
		200	21	12	11	10
	Water	100	10	9	-	-
		200	13	11	-	-
Brachiaria sp.	Methanol	100	10	-	-	-
		200	11	10	-	-
	Water	100	-	-	-	-
		200	9	-	-	-
A. indicum	Methanol	100	10	8	9	-
		200	12	11	10	-
	Water	100	-	-	-	-
Standard (ciprofloxacin)		200	9	-	-	-
		-	29	30	22	20

Table 2: Minimum inhibitory concentration of ethnoveterinary medicinal plants

Medicinal plants	Extracts	Minimum inhibitory concentrations (mg mL^{-1})			
		S. aureus	*E. coli*	*S. agalactiae*	*K. pneumoniae*
C. ciliaris	Methanol	0.125	250.0	2	2
	Water	0.500	-	-	-
C. grandis	Methanol	0.125	0.5	2	2
	Water	0.500	0.5	-	-
Brachiaria sp.	Methanol	0.500	1.0	-	-
	Water	-	-	-	-
A. indicum	Methanol	0.500	2.0	-	-
	Water	-	-	-	-

Concentration (MIC) of the active extracts are shown in Table 2. *C. ciliaris* and *C. grandis* showed the strongest antibacterial activity with MIC values of 0.125 mg mL^{-1}, followed by *Brachiaria* sp. and *A. indicum* (MIC of 0.5 mg mL^{-1}). Available literature results indicate a strong activity when MIC values are between 0.05-0.50 mg mL^{-1}, moderate activity in values between 0.6-1.50 mg mL^{-1} and weak activity above 1.50 mg mL^{-1} (Diaz *et al*., 2009). In conformity to the existing trend, *C. ciliaris* and *C. grandis* showed strong activity while *Brachiaria* sp. and *A. indicum* displayed moderate activity.

CONCLUSION

Wynn describes today's traditional medicine, as undoubtedly the oldest form of medicine and probably had evolved simultaneously with the evolution of human beings. Ethno Veterinary Medicine (EVM) has been a mainstay of developing countries that lack access to conventional medicines for veterinary health care, often being 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 extracts of the plant *C. ciliaris* and *C. grandis*

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