Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 2, 2012 ISSN - 0974-2441

Research Article

IN-VITRO ANTIBACTERIAL ACTIVITY OF CERTAIN WILD MEDICINAL PLANTS AGAINST BOVINE MASTITIS ISOLATED CONTAGIOUS PATHOGENS

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Received:20 January 2012, Revised and Accepted:14 April 2012

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 8.0 to 25.0 mm. The Minimum inhibitory concentration (MIC) values for the extracts ranged from 0.125 to 2.00 mg/ml.

Keywords: antibacterial activity, mastitis pathogens, disc diffusion method, Minimum Inhibitory Concentration (MIC)

INTRODUCTION

Milk is a very nutritional food that is rich in carbohydrate, proteins, fats, vitamins and minerals. The increase in human population, accessibility to technology input, high demand for animal products and purchasing power in urban center had helped the urban and per urban dairy farm in the country to flourish[1]. There are so many diseases which affect the health of animal reduce their production and have great economic importance. 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[2].

Bovine mastitis, an inflammatory response in cow's udder, is the main infecto-contagious disease affecting dairy cattle and is considered a limiting factor in many dairy properties[3]. The use of antibiotics to treat bovine mastitis produces antibiotic residues in milk and decreased quality of dairy products. Resistance of pathogens to common veterinary antibiotics hampers mastitis treatment and motivates the discovery of new antimicrobials. 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[4]. 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[5]. 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[6]. 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 some wild medicinal plants against bovine mastitis isolated contagious pathogens.

MATERIALS AND METHODS

Collection of Plants

Fresh plant parts were collected randomly from the gardens and villages of Coimbatore district, Tamilnadu, India. (*Dactyloctenium indicum* L., *Argemone Mexicana* L., *Calotropis gigantea* (L.) W.T.Aiton, *Trigonella foenum-graecum* L., *Acacia leucophloea* (Roxb.) Willd., *Acacia nilotica* (L.) Delile, *Achyranthes aspera* L., *Acalypha indica* L., *Coccinia grandis* (L.) J.Voigt *Asteracantha longifolia* (L.) Nees, Abutilon *indicum* (L.) Sweet., *Cenchrus ciliaris* L., *Brachiaria* sp. (Rendle) Schweick, *Trichodesma indicum* (L.) R. Br., *Melinis repens* (Willd) Zizka, *Barleria lupulina* Lindl., *Chloris inlata* (L.) Sw., *Medicago sativa* L., *Spermacoce hispida* L., *Rhynchosia capitata* (Heyne ex Roth) DC). (Table 1). 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 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.

Table 1: list of ethnoveteriny medicinal plants

S.NO.	BOTANICAL NAME	FAMILY	PARTS USED
1	*Cenchrus ciliaris*	Poaceae	Whole Plant
2	*Brachiaria* sp	Poaceae	Whole Plant
3	*Abutilon indicum*	Malvaceae	Leaves
4	*Coccinia grandis*	Cucurbitaceae	Leaves
5	*Asteracantha longifolia*	Acanthaceae	Leaves
6	*Trichodesma indicum*	Boraginaceae	Whole Plant
7	*Barleria lupulina*	Acanthaceae	Leaves
8	*Melinis repens*	Poaceae	Whole Plant
9	*Chloris inlata*	Poaceae	Whole Plant
10	*Medicago sativa*	Poaceae	Whole Plant
11	*Spermacoce hispida*	Rubiaceae	Whole Plant
12	*Rhynchosia capitata*	Fabaceae	Whole Plant
13	*Trigonella foenum-graecum*	Leguminoseae	Whole Plant
14	*Calotropis gigantea*	Apocyanaceae	Leaves
15	*Argemone mexicana*	Papaveraceae	Leaves
16	*Dactyloctenium indicum*	Poaceae	Whole Plant
17	*Acacia leucophloea*	Mimosaceae	Leaves
18	*Acacia nilotica*	Mimosaceae	Leaves
19	*Achyranthes aspera*	Amaranthaceae	Leaves
20	*Acalypha indica*	Euphorbiaceae	Whole Plant

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. It was stored at 4°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.

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 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 hr. The antibacterial activity was tested by the disc diffusion assay[7]. 0.1 ml of inoculum (10^5 CFU/ml) was spread on sterile Mueller Hinton plates and sterile paper discs were placed on the inoculated surface. The discs were impregnated with 15µl of each of the extract at two different concentration (100 & 200mg/ml), kept at room temperature for absorption of extract in the medium and then incubated at 37°C in the incubator for 24 hr. The antibacterial activity was evaluated by measuring the diameter of inhibition zone as per the procedure described by Kim *et al.*[8]. 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 to 8 mg/ml 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) and kept at 37°C for 24 hr. 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[2].

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[9].

Results obtained in the present study revealed that the tested four plants extract posses' potential antibacterial activity against *S.aureus, E.coli*, S. *agalactiae* and *K.pneumoniae* (Table 2). The plant extracts of the twenty plant species were separately tested at two different concentrations (100 & 200 mg/ml) to see their inhibitory effects against bovine mastitis isolated pathogens. Of the twenty candidate plants in this study, *Asteracantha longifolia, Rhynchosia capitata* and *Cenchrus ciliaris* 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 24.0 mm was shown by methanol extract (inhibition zone of 25 mm against *S.aureus* at concentration 200mg /ml) of *A. longifolia* & *R. capitata* and aqueous extract (inhibition zone of 24 mm against *S.aureus* at concentration 200mg/ml) of *R. capitata*. The methanol extract of *D. indicum* and *C.grandis* also showed significant antimicrobial activity against *Staphylococcus aureus* with inhibition zones 22 and 21 mm respectively at concentration 200 mg /ml while the aqueous extract showed against *S.aureus* with inhibition zones 15 & 13 mm respectively 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.

Table no. 2: antibacterial activity of ethnoveterinary medicinal plants against bovine mastitis isolated pathogens.

medicinal plants	extracts	conc.	zone of inhibition (mm)			
		(mg/ml)	*S.aureus*	*E.coli*	*Streptococcus sp.*	*K.pneumoniae*
A. longifolia	Methanol	100	19	14	-	-
		200	25	17	9	-
	Water	100	12	12	-	-
		200	14	16	-	-
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	-	-	-
D. indicum	Methanol	100	20	17	10	-
		200	22	20	11	8
	Water	100	11	14	-	-
		200	15	18	10	-
T.indicum	Methanol	100	-	11	-	-
		200	15	15	16	-
	Water	100	-	-	-	-
		200	-	11	-	-
C. ciliaris	Methanol	100	16	10	-	9
		200	24	16	10	11
	Water	100	10	-	-	-
		200	12	9	9	-
A. indicum	Methanol	100	10	8	9	-
		200	12	11	10	
	Water	100	-	-	-	-
		200	9	-	-	-
S. hispida	Methanol	100	10	10	-	-
		200	19	18	8	-
	Water	100	-	-	-	-
		200	15	-	-	-

R. capitata	Methanol	100	14	-	-	-
		200	25	8	10	-
	Water	100	12	-	-	-
		200	24	10	-	9
C. inlata	Methanol	100	16	12	-	-
		200	24	20	-	-
	Water	100	-	-	-	-
		200	-	10	-	-
M. repens	Methanol	100	-	-	8	-
		200	16	-	-	10
	Water	100	-	-	-	-
		200	13	-	10	-
B. lupulina	Methanol	100	10	-	-	-
		200	12	10	-	9
	Water	100	8	-	-	-
		200	10	9	-	-
M. sativa	Methanol	100	10	-	-	8
		200	13	10	10	10
	Water	100	9	-	-	-
		200	10.5		-	-
A.mexicana	Methanol	100	10	-	10	-
		200	15	10	12	12
	Water	100	-	-	-	-
		200	12	-	9	-
C. gigantea	Methanol	100	15	-	-	-
		200	18	-	12	-
	Water	100	10	-	-	-
		200	13	-	-	-
A. aspera	Methanol	100	9	-	-	-
		200	11	-	10	9
	Water	100	-	-	-	-
		200	-	-	-	-
A. indica	Methanol	100	8	-	-	-
		200	10	-	-	-
	Water	100	-	-	-	-
		200	8	-	-	-
A. leucophloea	Methanol	100	10	-	-	-
		200	12	-	12	-
	Water	100	-	-	-	-
		200	9	-	-	-
A. nilotica	Methanol	100	9	-	9	-
		200	11	-	13	-
	Water	100	8	-	-	-
		200	10	-	-	-
T.graecum	Methanol	100	10	8	-	-
		200	12	10	-	8
	Water	100	9	-	-	-
		200	11	9	-	-

Minimum inhibitory concentrations (MIC) of the active extracts are shown in Table 3. *C. ciliaris, C.grandis , D.indicum , A. longifolia* and *R. capitata* showed the strongest antibacterial activity with MIC values of 0.125 mg/ml, followed by all the candidate plants, (MIC of 0.125 - 0.5 mg/ml). Available literature results indicate a strong activity when MIC values are between 0.05-0.50 mg/ml, moderate activity in values between 0.6-1.50 mg/ ml and weak activity above 1.50 mg/ml[2]. In conformity to the existing trend, *C. ciliaris* and *C.grandis* showed strong activity, while *Brachiaria sp* and *A. indicum* displayed moderate activity.

Table no. 3: minimum inhibitory concentrations of ethnoveterinary medicinal plants against bovine mastitis isolated pathogens

medicinal plants	extracts	minimum inhibitory concentrations (mg/ml)			
		s.aureus	*e.coli*	*s. agalactiae*	*k. pneumoniae*
C. ciliaris	Methanol	0.125	250	2	2
	Water	0.5	-	-	-
C.grandis	Methanol	0.125	0.5	2	2
	Water	0.5	0.5	-	-
Brachiaria sp	Methanol	0.5	1	-	-
	Water	-	-	-	-
A. indicum	Methanol	0.5	2	-	-
	Water	-	-	-	-
T.indicum	Methanol	0.250	0.250	0.250	-
	Water	-	-	-	-
D.indicum	Methanol	0.125	0.250	0.5	-
	Water	0.250	0.125	0.5	-
A. longifolia	Methanol	0.125	0.5	-	-
	Water	0.250	0.250	-	-
S. hispida	Methanol	0.5	-	-	-
	Water	0.5	-	-	-

R. capitata	Methanol	0.125	-	-	-
	Water	0.250	0.5	-	-
C. inlata	Methanol	-	0.5	-	-
	Water	-	-	-	-
M. repens	Methanol	0.250	-	-	-
	Water	0.5	0.5	0.5	0.5
B. lupulina	Methanol	0.5	-	-	-
	Water	-	-	-	-
M. sativa	Methanol	0.5	-	-	-
	Water	-	-	-	-
A. leucophloea	Methanol	0.5	-	0.5	-
	Water	-	-	-	-
A.nilotica	Methanol	0.5	-	0.5	-
	Water	0.5	-	-	-
A.indica	Methanol	0.5	-	0.5	-
	Water	0.5	-	-	-
A.aspera	Methanol	0.5	-	0.5	-
	Water	-	-	-	-
A. mexicana	Methanol	0.250	-	0.5	-
	Water	2	-	-	-
C. gigantea	Methanol	0.250	-	0.50	-
	Water	0.5	-	-	-
T.graecum	Methanol	0.5	-	-	-
	Water	0.5	-	-	-

Wynn[10] 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 *A. longifolia, R. capitata* and *C. ciliaris*

ACKNOWLEDGEMENT

The first and second authors are grateful to University Grant Commission (UGC) for the financial support given to the present study under the Major Research Project programme entitled "A Study of Ethno-veterinary Medicinal Plants and *in-vitro* antimicrobial activities against Bovine Mastitis isolated bacterial pathogens" [Sanction No. F. No. 35-121 / 2008 (SR) dt.20 March 2009]. The authors are thankful to the management of RVS Educational Trust for their encouragement and support.

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