



ISSN: 0975-833X

RESEARCH ARTICLE

ANTIMICROBIAL ANALYSIS OF SOME PLANTS TRADITIONALLY USED TO TREAT MASTITIS

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ARTICLE INFO

Article History:

Received 20th April, 2014

Received in revised form

07th May, 2014

Accepted 18th June, 2014

Published online 20th July, 2014

Key words:

Antimicrobial, Herbal Plants,
Mastitis, Traditional Use,
Wayanad.

ABSTRACT

The present paper describes the antibacterial activity of six plants traditionally used for the treatment of mastitis by Kattunaikka tribe of Wayanad district of Kerala. The antibacterial activity was tested by the disc diffusion assay. All the test plants showed antibacterial activity against all the tested microorganisms. Among these plants *Elephantopus scaber* and *Annona reticulata* showed promising activity against many of the tested microorganisms associated with bovine mastitis. *Tinospora cordifolia* showed low activity against all the tested microorganisms and in lowest concentration of plant extract no activity was observed for *Klebsiella pneumonia* and *Staphylococcus aureus*. Among the various concentrations of aqueous extracts tested for the antimicrobial studies 100% found to be highly effective followed by 50% and 25%. The study reveals the importance of plants in the control of dreadful disease like mastitis. Potential herbal drugs will be developed from these plants that can reduce the burden of antibiotics and the resultant emergence of antibiotic resistant microorganisms.

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INTRODUCTION

Mastitis continues to be the most costly disease while the worldwide dairy industry and research efforts continue towards the development of improved methods for the control and elimination of this disease from dairy herds (Salmon *et al.*, 1998). Since ancient times plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings as well as livestock. In India, plant remedies are still the most important and sometimes the only sources of therapeutics for human and livestock. Despite their vital role in catering for the health of human and livestock population, large part of the knowledge of ethno medicinal plants is on the verge of irreversible loss and declining to deterioration due the oral passage of herbal heritage from generation to generation rather than in writings. In India, livestock diseases remain among the principal causes of poor performance, leading to an ever increasing gap between the supply and the demand of livestock products. Conventional veterinary services, despite its overriding role, have limited coverage in developing countries and development of antimicrobial resistance is another woe. If at all, the usefulness of modern pharmacotherapy is still limited by the cost of treatment. Due to these reason livestock keepers particularly in rural areas frequently visit traditional healers to get solutions for their ill-health animals including clinical cases of skin,

udder, teats and gastrointestinal tract infections. Developing a socially acceptable and effective remedy from inexpensive resources that can complement modern medicine would be an attractive option. However, in most traditional healers the units of measurements to determine dosage are not standardized and there are variations in the unit of measurement, duration and time at which remedies are taken and prescribed by healers for the same kind of health problems. The precision, standardization and their toxic effect were not studied in the country which is as one drawback for the traditional health care system. Also 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.

To the best of our knowledge, in vitro antimicrobial trial on plants used in ethnoveterinary practices in and Wayanad region is scanty. In the present study, the plants having traditional claims for the treatment of Mastitis were investigated for their antimicrobial activities on Gram positive and Gram negative bacteria. Prior to the in-vitro antimicrobial trial, inventory of ethnoveterinary data on the use of these plants was obtained directly from a primitive tribal community Kattunaikka of Wayanad district of Kerala, South India.

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MATERIALS AND METHODS

Community profile

The Kattunaikkas are referred to primitive tribal community predominantly distributed in the Wayanad district of Kerala. They speak their own dialect which is closer to the Dravidian language, Kannada. They are considered as semi-nomadic community in earlier days and eat a diverse variety of meat. Rice and Millets are their staple cereals supplemented by roots and tubers. Food gathering, hunting, fishing and trapping of birds and animals are the traditional occupations of the *Kattunaikka*, which a few of them continue to pursue to the day. Presently a few of them are land-owning cultivators and engaged in settled agriculture and cattle rearing for ensure their livelihood security.

Field survey and preparation of plant material

A survey was conducted to gather information on the traditional usage of plants in the health care system of livestock. Information of medicinal plants was obtained through direct interview with livestock farmers, local and religious healers. The interview was done based on a standard feedback questionnaire intended to address details on the types and characteristics of plant and their traditional preparation, method of application and other plants or substance used together. More than 50 individuals were involved in the interview. Specimens of plants that were mentioned by the traditional herbalists and farmers for treatment of livestock ailments were collected. Based on the resources available, six plant species which were most widely used in the treatment of bovine mastitis were selected for in-vitro antimicrobial screening. Plant species recognized as ethnoveterinary medicinal plants, their uses and properties are summarized in Table: 1. Leaves of the selected plants were collected from their natural habitats and washed with tap water to remove unnecessary particles and shade dried at room temperature. Shade dried leaves were then separately grounded using a commercial blender at the Microbiology Laboratory of M.S. Swaminathan Research Foundation, Wayanad.

Preparation of extracts

Ten gm of homogenized powder of each plant material were extracted in 100 ml of hot distilled water separately and left overnight under refrigerator (4°C) until further use. After 24 h, the extracts kept in rotary shaker at 100 rpm for 1 h, filtered through 8 layered muslin cloth. The extracts were freeze dried and stored at 4°C until further use. Various concentrations (25%, 50%, 100%) were prepared in Dimethyl Sulfoxide (DMSO) for determining antimicrobial activity (Mahdi Khodaei Motlagh *et al.*, 2013).

Isolation of Bacteria

Milk samples were collected from mastitis tainted cows. Total ten samples were collected directly from dairy farmers of Wayanad District, Kerala. Milk samples collected in sterile vials after cleaning the teat surface with 70% ethanol and after discarding a few streams of milk. The isolation and

identification of mastitis causative organisms were carried out in selective media. All the strains were identified by biochemical characteristics and were routinely cultured on brain heart infusion agar at 37°C for 16h. Stock cultures were maintained in brain heart infusion agar medium containing 25% glycerol at 80°C (Ciro Cesar Rossi *et al.*, 2011; Ujjwal Kumar De and Reena Mukherjee 2009; Al Laham Sit and Al Fadel 2013). Bacterial strains isolated from clinical cases of mastitis such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Streptococcus agalactiae* were maintained in Nutrient agar slants for further research (Bauer *et al.*, 1996).

Antibacterial activity

Antimicrobial activity of each plant extracts were carried out using modified method of Kirby Bauer (Doss *et al.*, 2012). Inoculums of each of the bacterial strain was suspended in 5 ml of broth (nutrient broth) and incubated at 37°C for 24 h. The antibacterial activity was tested by the disc diffusion assay. 0.1 ml of inoculums 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 three different concentrations (25%, 50%, and 100%), kept at room temperature for half an hour 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. Ciprofloxacin was used as control (Rajesh Kowti *et al.*, 2010).

RESULTS AND DISCUSSION

The results of antimicrobial screening of the extracts of test plants are shown in Table 2. All the test plants showed antibacterial activity against all the test microorganisms. Among these plants *Elephantopus scaber* and *Annona reticulata* showed promising activity against common microorganisms of veterinary importance. There was a dose dependent inhibition on the tested micro-organisms. *Tinospora cordifolia* showed low activity against the test microorganisms and in low concentration (25 %) of plant extract no activity was observed for *K.pneumonia* and *S.aureus*. The extracts of *Elephantopus scaber* exhibited the highest growth inhibition zone against *E.coli* (63.4), *K. pneumonia* (82.2), *S. aureus* (61.2) and *S. agalactiae* (92.3). Available evidence from indigenous practices in different areas is in agreement with the present in-vitro study. The plant has been documented as a remedy for a wide range of human and livestock diseases such as an antipyretic, cardiotoxic and diuretic (Nadakarni Indian Materia Medica 1954). Decoction of roots and leaves is used as emollient and given in dysuria, diarrhea, dysentery and in stomachic pain (Kiritkar and Basu 1991). The aqueous extract of leaves is applied externally to treat eczema and ulcers (Chopra *et al.*, 1956).

Annona reticulata extracts also showed an impressive antibacterial activity against all test microorganisms. Traditionally juices of the leaves are topically applied in the treatment of mastitis and various skin diseases, such as dermatophilosis, dandruff etc. *Azadirachta indica*, *Asparagus racemosus* and *Tinospora cordifolia* showed potent activity

Table 1. List of plants used to treat bovine mastitis by *Kattunaika* tribe of Wayanad District

S.No.	Scientific Name	Vernacular name	Brief Method of Administration
1.	<i>Asparagus racemosus</i> Willd.	Chathavari	Rhizome paste applied on the infected teats twice in a day up to cure
2.	<i>Annona reticulata</i> L.	Aatha	Leaf paste applied on teats and the pastes wrapped in cotton cloth which is tied on neck.
3.	<i>Elephantopus scaber</i> L.	Anachovadi	Entire plant paste applied on teats
4.	<i>Aloe vera</i> (L.) Burm. f.	Katturvazha	Sap applied on the infected teats twice in a day up to cure
5.	<i>Tinospora cordifolia</i> (Willd.) Miers.	Amurthuvalli	Pasted leaf and stems applied on the infected teats twice in a day up to cure
6.	<i>Azadirachta indica</i> A. Juss.	Veppu	Leaf paste applied on the infected teats twice in a day up to cure

Table 2. Percentage of inhibition against different bacterial strains by plants extracts

Plant species selected	Concentration in (%)	Microorganisms and percentage of inhibition			
		<i>E.coli</i>	<i>K. pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus agalactiae</i>
<i>Asparagus racemosus</i>	25	24.5	19.2	17.2	15.4
	50	29.9	27.7	20.1	26.1
	100	56.9	45.1	33.6	39.2
<i>Annona reticulata</i>	25	22.3	36.0	28.0	18.9
	50	39.4	51.3	33.6	39.0
	100	56.9	60.9	45.2	59.1
<i>Elephantopus scaber</i>	25	39.1	29.1	37.8	43.1
	50	51.3	40.0	45.0	78.0
	100	63.4	82.2	61.2	92.3
<i>Aloe vera</i>	25	19.3	40.3	12.3	06.0
	50	29.1	51.3	25.4	12.0
	100	44.3	69.0	36.9	18.1
<i>Tinospora cordifolia</i>	25	19.0	0	0	10.1
	50	21.3	03	05.3	15.6
	100	33.0	07.9	09.0	18.3
<i>Azadirachta indica</i>	25	42.3	11.2	13.2	21.0
	50	56.3	13.0	25.4	25.9
	100	63.0	29.3	36.1	33.2

against *E. coli* and *Aloe vera* to *K. pneumoniae*. These plants have been documented as a remedy for a wide range of human and livestock diseases. The antibacterial potential of *Azadirachta indica* against the mastitis causing pathogens was previously studied (Hunter *et al.*, 1997 and pointed out that the total bacterial count in disease was drastically reduced. The presence of triterpene in the extract was the responsible agent for antibacterial activity and anti inflammatory properties (Bouchard *et al.*, 1999). The sensitivity of many of the *S. aureus* and *E. coli* were tested against spice plants and found to be effective and found to be effective (Omer Ertuk 2006). Antibiotics are used widely against mastitis disease and however the success rate is very low and it cannot prevent the inflammatory reaction driven by host leukocytes against bacterial intruders (Bouchard *et al.*, 1999). The use of antibiotics is not recommending because of the tendency of widespread resistance to antibiotics (Omer Ertuk 2006). Herbal formulations and phytochemicals extracted from natural sources will have great potential in the control of dreadful disease like bovine mastitis. The bio active principle from the studied plants especially *Elephantopus scaber* and *Annona reticulata* has to be extracted and identified and to be used against the bovine mastitis.

The present study was conducted by extracting of the crude substance of the herbal medicines used by a primitive tribal community *Kattunaikka* of Wayanad district of Kerala and the result indicated that all the test plants possess antibacterial activity. There was a dose reliant inhibition on the tested microorganisms signifying towards the necessity of exhaustive study of these medicinal plants. The results of the study throws

light into the vast knowledge treasure of the tribal communities especially the *Kattunaika* tribe and of the importance of documenting and preserving the traditional knowledge. Moreover, a continuous and comprehensive research needs to be conducted to prove the biological ingredients and test the safety, efficiency and to determine the types of compounds responsible for the antibacterial effects of these medicinal plants.

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