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Herbal Plants: A potential agent to cure infectious mastitis in bovine animals

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Abstract

The medicinal plants are used in traditional treatments in order to cure variety of diseases since prehistoric period. Bovine mastitis is an inflammation of mammary gland and it is the most serious and economically important disease in the dairy milk production worldwide. The main aim of this study was to identify such plants with antimicrobial efficacy against some mastitis pathogens. For this purpose the locally available plants viz., Gymnemasylvestre, Holarrhenaantidysenterica, VernoniaanthelminticaEnicostemmalittorale, Momordicacharantia,Swertiachirata,Azadirachtaindica,Caesalpiniabonducellawere selected for the study. These plants were screened against 18 bacteria that were isolated from clinical and sub clinical milk samples of buffaloes. The plants were extracted using methanol and showed the inhibitory effect against gram- positive and gram-negative bacteria. But gram-positive bacteria are more sensitive than gram-negative bacteria. The largest zone of inhibition [24mm in diameter] was recorded against corynebacteriumxerosis with the leaf extract of Azadirchtaindica. It was concluded that these plant extract can be used as a drug against the mastitis causing pathogens.

Keywords: Antibacterial activity,Clinical mastitis, Medicinal Plants, Subclinical mastitis.

Introduction

There are about two and a half lakh species of flowering plants that belongs to 10,500 genera and about 300 families are known. Recently it has been estimated that over 9,000 plants have known medicinal applications in various cultures and countries, and this is without having conducted comprehensive research amongst several indigenous and other communities [1].Medicinal plants are widely used at the household level by women taking care of their families at the village level by medicine men or tribal people, and by the practitioners of classical traditional systems of medicine such as Ayurveda, Chinese medicine, or the Japanese kampo system. According to the world Health Organization, over 80% of the world's population or 4.3 billion people rely upon such traditional plant based systems of medicine to provide them with primary health care [2].

In India, animal diseases remain among the principal causes of poor livestock performance, leading to an ever increasing gap between the supply of, and the demand for, livestock products. Conventional veterinary services, despite its paramount role, have limited coverage in developing countries and development of antimicrobial resistance is another problem [3, 4]. If at all, the usefulness of modern pharmacotherapy is still limited by the cost of treatment.

Due to this 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.

In light of the recent emergence of the bacteria that are resistant to multiple antimicrobial drugs posing a challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such micro-organisms becomes pertinent. Resistant bacteria representing a challenge in the treatments of various well-known infections necessitated the need to find new substances with antimicrobial properties to be used in the combat against these micro- organisms [5].

Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases [6]. Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and selected on the basis of their ethno-medicinal use [7]. The antimicrobial compounds produced by plants are active against plant and pathogenic microorganisms [8]. There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation to yield active principles [9-

14]. Inspite of the antimicrobial properties of these medicinal plants a very less documentation seen in the utilization of these plants in treating mastitis of inhibiting mastitis causing pathogens. The present study was aimed to evaluate the potentiality of methanolic extracts of 8 Indian medicinal plants against some microbes isolated from clinical and subclinical mastitis milk samples buffaloes.

Current treatment to this disease mainly relies on administering antibiotics to the cattle. However, the biggest threat with antimicrobials is resistance development amongst the microbial community [15, 16].Antibiotics have been used for many years to eliminate bacterial pathogens causing disease in the case of mastitis it is important to note that antibiotic therapy cannot be relied upon to reduce the incidence of mastitis as a standalone anti mastitis action [17]. The ultimate goal thus would be to reduce the use of antibiotics and search for a better and effective alternative.

Material and Methods

Plant materials

The Plants Gymnemasylvestre, Holarrhenaantidysenterica, Vernoniaanthelmintica, Enicostemmalittorale, Momordicacharantia,Swertiachirata,Azadirachtaindica,Caesalpinia bonducellawere collected from the Godhra and Amritsar, and several other places of Gujarat, India in February, 2013 which were used for the treatment of mastitis. The plant materials were oven-dried at 40ºC and then ground into coarse powder.

Table 1 Plants details collected with the name of locality

	Names of plant	Part of plant used	Family
1	*Gymnemasylvestre*	Leaf	Asclepiadaceae
2	*Holarrhenaantidysenterica*	Bark	Apocynaceae
3	*Vernoniaanthelmintica*	seeds	Asteraceae
4	*Enicostemmalittorale*	leaf	Gentianaceae
5	*Momordicacharantia*	Fruit	Cucurbitaceae
6	*Swertiachirata*	leaf	Gentianaceae
7	*Azadirachtaindica*	leaf	Meliaceae
8	*Caesalpiniabonducella*	leaf	Caesalpiniaceae

Extraction

The coarse powder of the Gymnemasylvestre(10 g),Holarrhenaantidysenterica(10),Vernoniaanthelmintica(10 g),Enicostemmalittorale(10 g),Momordicacharantia(10 g),Swertiachirata(10 g),Azadirachtaindica(10 g),Caesalpiniabonducella(10 g) were extracted with 100ml methanol for two days at room temperature. The extracts were then filtered off through Whatman filter paper number-1. For the solvent removal the plates containing solvent were kept in open overnight and the solvent was allowed to evaporate.On the next day the extract was dissolved in 1ml Dimethyl sulfoxide (DMSO). The extracts were stored at 4ºC in eppendrof tubes for further studies.

Antibacterial activity against test microorganisms

The bacteria used includesLactobacillus spp., Corynebacteriumxerosis, Micrococcus varians, Serratiamarcescens, Bacillus alcalophilus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus arlettae, Staphylococcus epidermis, Serratiafonticola, Streptococcus spp., Streptococcus agalactiae, klebsiellapneumonae, Micrococcus luteus, and Enterococcus spp.. These bacteria were isolated from the clinical and subclinical mastitis milk samples of buffaloes. Bacterial cultures were maintained on Nutrient agar media. All cultures were subcultured monthly and subsequently stored at 10 C.

Screening for Antimicrobial Activities

Nutrient agar plates were prepared. The nutrient agar plates were then heavily inoculated with the young bacterial culture (16-20hrs) by means of sterile spreader to ensure efficient growth of the organism. Four wells were made in to each plate using cork borer of 6mm. 0.1ml of various extracts were added to the wells of the plate which is pre spreaded with the culture to observe the antagonistic effect of various microorganisms. The plates were incubated for 24 to 48 hours. The zone of inhibition was calculated by measuring the diameter of inhibition zone around the well (mm) excluding the well diameter. The readings were taken in three fixed directions and the average was tabulated.

Results and Discussion

In the present Study, an attempt has been made to explore antimicrobial properties, of Methanolic extracts of Gymnemasylvestre, Holarrhenaantidysenterica, Vernoniaanthelmintica,Enicostemmalittorale, Momordicacharantia, Swertiachirata,Azadirachtaiindica and Caesalpiniabonducellawhich help in the development of new, novel drugs to cure mastits in dairy animals. The extracts were studied against 18 different microorganisms, isolated from Clinical and subclinical Mastitis milk samples of Buffaloes.

During this study, eight plants were selected which were used for the treatment of infectious diseases by tribal people. The results

are summarized in Table 2 and Table 3. From primary Screening, it was found that all 8 plants exhibited more or less antibacterial activity against all mastitis pathogens.

Table 2: Antimicrobial activity and zone of inhibition in mm

Plants \ Bacteria	Enterococcus spp	Lactobacillus spp	Corynebacteriumxerosis	Escherichia coli	Micrococcus varians	Serratiamarcescens	Bacillus alcalophilus	Bacillus subtilis	Staphylococcus saprophyticus
S. chirata	11	14	18	5	10	8	16	[-]	[-]
V. anthelmintica	13	17	15	7	16	10	21	[-]	[-]
E. littorale	10	12	13	8	5	13	6	[-]	[-]
M. charantia	1	22	19	3	14	1	12	17	10
H. antidysenterica	9	2	17	4	9	[-]	10	16	9
C. bonducella	5	12	16	5	[-]	[-]	8	7	6
G. sylvestre	3	13	18	[-]	8	4	7	18	14
A. indica	6	17	24	[-]	10	12	6	[-]	[-]

Table 3: Antimicrobial activity and zone of inhibition in mm

Plants \ Bacteria	Staphylococcus arlettae	Staphylococcus epidermis	Micrococcus luteus	Streptococcus spp	Pseudomonas aeruginosa	Serratiafonticola	klebsiellapneumonae	Streptococcus agalactiae	Staphylococcus aureus
S. chirata	16	9	(-)	4	8	6	8	5	10
V. anthelmintica	20	16	(-)	10	16	12	16	14	15
E. littorale	14	10	(-)	7	12	1	5	9	6
M. charantia	12	7	6	4	15	14	10	13	12
H. antidysenterica	13	4	(-)	6	8	5	(-)	(-)	6
C. bonducella	17	9	(-)	5	7	9	1	5	7
G. sylvestre	16	12	(-)	13	17	16	7	(-)	6
A. indica	15	14	3	10	9	14	8	12	12

(-) indicates no Zone of inhibition

S.chirata showed moderate (4 - 18 mm in diameter zone of inhibition) antibacterial activity against all organisms except isolates *B.subtilis, S.saprophyticus*and *M.luteus*. *V.anthelmintica*showed moderate to good (7mm - 21 mm in diameter zone of inhibition) antibacterial activity against all organisms except isolates *B.subtilis, S.saprophyticus*and*M.luteus*. *E.littorale* showed moderate activity (5mm- 14mm in diameter zone of inhibition) antibacterial activity against all organisms except isolates *B.subtilis, S.saprophyticus*and*M.luteus*. However, only 1mm zone of inhibition is obtained against isolate *Lactobacillus spp, S.marcescens*and*S.fonticola*which showed poor activity against them. *M.charantia* showed good to moderate activity (5mm - 22mm in diameter zone of inhibition) antibacterial activity against all organisms. However, very less zone of inhibition is obtained against isolate *Enterococcus spp, Lactobacillus spp, S.marcescens*and*S. fonticola*which showed poor activity against them. *H.antidysenterica* showed moderate activity (4mm- 17mm in diameter zone of inhibition) antibacterial activity against all organisms except isolates *S.marcescens, M. luteus, K.pneumonae*, *Strep.agalactiae*. *C. bonducella* showed moderate activity (5mm- 17mm in diameter zone of inhibition) antibacterial activity against all organisms except isolates *M.varians, S.marcescens*and*M.luteus*. However, very less zone of inhibition is obtained against isolate *K.pneumonae* which showed poor activity against it. *Gymnemasylvestre* showed good to moderate activity (4mm- 18mm in diameter zone of inhibition) antibacterial activity against all organisms except isolates *E. coli, M.luteus,* and *K. pneumonae*. However, very less zone of inhibition is obtained against isolate Enterococcus spp. which showed poor activity against it. *Azadirachtaindica* showed good to moderate activity (3 mm- 24 mm in diameter zone of inhibition) antibacterial activity against all organisms except isolates *E. coli, S.saprophyticus*. However, very less zone of inhibition is obtained against isolate *M.luteus*which showed poor activity against it.

The largest zone of inhibition (24mm in diameter) was recorded against isolate*Corynebacteriumxerosis*with the leaf of *Azadirachtaindica,* followed by zone of inhibition (22mm in diameter) was recorded against *Lactobacillus spp.* with the fruit of *M.charantia*. Similar antibacterial activity of other plant extracts has been reported previously [18,19].The most susceptible bacterium wasS. aureus and methanol extract of Woodfordiafruticosa showed the best antibacterial activity. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties [20]. Concerning the plant Adiantumcuneatum, results have confirmed and justified the popular use of this plant for the treatment of dolorous processes [21]. In this study, we have used A. capillus-veneris that do not have valuable antibacterial activity. The extract of I. emodi a plant of Bignoniaceae family, shows good activity against the Staphylococcus and Proteus. A study by Rasadahand Houghton[22] revealed that crude extract of all species of Bignoniaceae family have antibacterial activity against Gram positive and negative bacteria and yeast. Tabebuiaspectabilis, a plant of Bignoniaceae family is the most active against the Gram positive bacteria.

Conclusion

The present investigation ensures that the crude extracts of all the 8 plants used contain antibacterial properties to greater or lesser extent. During the study it was observed that gram-positive bacteria are more sensitive than gram negative bacteria. From our results, it appeared that the crude extracts of some traditional medicinal plants has good inhibitory effect against selected bacterial strains. Among the medicinal plants tested herein, *Azadirachtaindica*showed most promising antibacterial properties indicating the potential for discovery of antibacterial agent to cure mastitis in Bovine Animals

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