Antibacterial activity of some herbal extracts against *Staphylococcus aureus* isolated from Bovine Mastitis

H. Muhamed Mubarack\(^1\), A. Doss\(^1\), M. Vijayasanthi and R. Venkataswamy\(^2\)

\(^1\)Department of Microbiology, RVS College of Arts and Science, Coimbatore, Tamilnadu India.
\(^2\)Department of Pharmacognosy, Sri Ramakrishna College of Pharmaceutical Sciences, Coimbatore, Tamilnadu India.

Received on:08-11-2011; Revised on: 21-12-2011; Accepted on:28-01-2012

**ABSTRACT**

*Staphylococcus aureus* is the main causative agent of bovine mastitis. The activity of aqueous and methanol extracts from four medicinal plants was investigated against fifteen strains of *Staphylococcus aureus* isolated from animals with mastitis manifestation by the disc diffusion method. The interference of the extracts on cell in the form of adherent colonies was also evaluated. Results revealed the potential of extracts of *Asteracantha longifolia*, *Brachiaria sp.*, *Trichodesma indicum* and *Abutilon indicum* as antibacterial agents against *S. aureus* strains isolated from bovine mastitis. Using microbroth dilution method, *A. longifolia* extract was able to inhibit the tested bacterial pathogens in this study at minimum inhibitory concentration (MIC) between (0.125 – 0.500 mg/ml). The present study supports the possible use of these phytotherapeutic agents in the clinical management of bovine mastitis.

**Key words:** Bovine mastitis, methanol, medicinal plants, disc diffusion

**INTRODUCTION**

Mastitis is an infectious disease that is associated with massive financial losses in the dairy sector. It is incriminated as an important disease constraint in dairy cow and is responsible for reduction in quality and quantity of milk and milk products mainly due to microorganisms (Radostits et al., 2007). Majority of microorganisms that are responsible for mastitis and spoilage of milk could be *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*, *Mycoplasta* species, *Streptococcus uberis* (Erskine, 2001), coliforms (*Escherichia coli*, *Klebsiella* species and *Enterobacter aerogenes*), *Serratia*, *Pseudomonas*, *Proteus* species environmental *Streptococci*, *Enterobacter* species. Among the various causative agents, *Staphylococcus aureus* is one of the most prevalent and contagious pathogens of intra-mammary infections in dairy cattle globally. The evolution of antibiotic resistance in *S. aureus* strains is a serious cause of concern in dairy animals (Wang et al. 2008). The continuous use of antibiotics for a long period may lead to multi drug resistance in causative organisms which has resulted in the use of high doses of antibiotics and leads to the danger of increasing amounts of antibiotics residues in milk, a potential hazard (Gopinath et al., 2011). The conventional drugs used for the treatment of mastitis are limited in types, in the developing countries in general and India in particular. Due to this and other factors, causal agents have showed variable degrees of resistance.

Medicinal plants have been used for ages in developing countries as alternative treatment to health problems. India has a diverse flora and a rich traditional knowledge 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 empirical knowledge with significant results in cattle (Mubarack et al., 2011). The efficiency of some of these plants/herbs has been tested against a range of causative agents of mastitis. Marisa et al. (2009) has screened ten herbal preparations; namely, *Artemisia absinthium*, *Cymbopogon nardus*, *Symphytum officinale*, *Baccharis dracunculifolia*, *Solanum asperolanatum*, *Salvia officinalis*, *Bauhinia forficata*, *Calendula officinalis*, *Chenopodium ambrosioides* and *Senna macranthera* on major isolates of bovine mastitis and also Mubarack et al. (2011) have conducted in-vitro tests of *Acacia nilotica* and *Acranythus aspera* on *Staphylococcus aureus* isolate and observed encouraging results.

**Hygrophila auriculata** (Schum) Heine. (synonym: *Asteracantha longifolia Nees*, *Barleria auriculata schum*, *Barleria longifolia linn*, family: *Acanthaceae*) is described in the ayurvedic literature as **Ikshura**, **Ikshagandha** and **Kokilasha**, having eyes like kokila or Indian cuckoo. The plant is widely distributed throughout India, Sri Lanka, Burma, Malaysia, and Nepal. *Hygrophila auriculata* is a traditional folk medicine widely used in the treatment of urinary infection, gout, hepatic obstruction and as a diuretic (Sarfaraj Hussain et al., 2009). *Abutilon indicum* (Malvaceae) is found throughout tropical and sub tropical regions of India, is known as Atibal in Sanskrit. Traditionally various parts of the plant *Abutilon indicum* Linn. have been used in treating various human ailments. The roots are useful in treating uterine haemorrhagic discharges. Similarly, seeds are used in the treatment of bronchitis, gonorrhoea and piles. Leaves are useful in toothache, lumbago, piles and all kinds of inflammation. Bark is used as antiinflammatory, diuretic and alexeteric (Lakshmaya et al., 2003). *Trichodesma indicum* is found as a weed throughout the greater part of India. The decoction of the root is used for diarrhea, dysentery, and fever in Indian traditional medicine (Perianayagam and Sharma, 2005). Therefore the main objectives of the present study were to determine and compare the *in-vitro* antimicrobial effects of seven phytopreparations; namely *Asteracantha longifolia*, *Brachiaria sp.*, *Trichodesma indicum* and *Abutilon indicum* on *Staphylococcus aureus* isolated from bovine clinical mastitis.

**MATERIALS AND METHODS**

Aerial parts of the medicinal plants *Asteracantha longifolia*, *Brachiaria sp.*, *Trichodesma indicum* and *Abutilon indicum* were collected from Western Ghats, Coimbatore region, South India and authenticated by Dr. V.Sampath Kumar, Scientist - C, Botanical Survey of India (Southern Circle), Coimbatore, South India. The collected plants were dried under shade in open air to reduce deterioration of the plant drug material.

**Preparation of 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 (Sasikumar et al., 2005). 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 (Sasikumar et al., 2005).

**Bacterial strains**

Bacterial strains used in this study were the pathogen isolated from clinical cases of bovine mastitis such as *Staphylococcus aureus*. The strain was confirmed by cultural and biochemical characteristics and maintained in slants for further use.

**Antibacterial activity**

An inoculum of each of the bacterial strain (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., 1996). 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 h. The antibacterial activity was evaluated by measuring the diameter of inhibition zone as per the procedure described by Kim et al., 1995. Ciproflaxacin was used simultaneously as control.

**Minimum Inhibitory Concentration (MIC)**

For determination of MIC, 1 ml of broth medium was taken into 10 test tubes. 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 test strains (10^5 CFU/ml) 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.

**Examination of Mode of Action**

The crude plant extracts were added to 4.9 ml of bacterial cultures (10^5 CFU/ml). After incubation at optimal temperature for 24 h, 100 µl of the mixtures were inoculated into 4.9 ml of fresh culturing broth. As a control, 100 µl of untreated cultures of bacteria at a concentration of 10^5 CFU/ml were transferred to 4.9 ml of fresh culturing broth. The optical density at a wave length of 600 nm (OD 600nm) of the tested and control cultures were determined at the time of inoculation and after incubation for 24h (Doss et al., 2011).

**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 plants derived medicines have a modern indication that correlates with their traditional, cultural (and sometimes ancient) uses. Each plant extract of the four plant species were tested at two different concentrations (100 & 200 mg/ml) to see their inhibitory effects against *Staphylococcus aureus*. Of the three candidate plants in this study, *A. longifolia* showed antibacterial activity against the tested bacteria and the remaining plants showed moderate activity after alcoholic extraction (Table 1). The inhibiting zone increased with increasing concentrations of the extracts for the three plant species (*Asteracantha longifolia*, *Brachiaria* sp. *Trichodesma indicum* and *Abutilon indicum*). A significant difference was observed against *Staphylococcus aureus* inhibition by the aerial parts of all the medicinal plants at higher concentrations (Table 1). Minimum inhibitory concentrations (MIC) of the active extracts are shown in Table 2. *A. longifolia* showed the strongest antistaphylococcal activity with MIC values of 0.125 mg/ml, followed by *T. indicum*, *Brachiaria* sp. and *A. indicum*, (MIC of 0.250 – 0.500 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 (Marisa A. N. Diaz et al., 2009). In conformity to the existing trend, *A. longifolia* showed strong activity, while other plants displayed moderate activity.

**Table 2: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of some selected Ethnoveterinary medicinal plants against Staphylococcus aureus**

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>MIC (mg/ml)</th>
<th>Water (mg/ml)</th>
<th>MBC Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Asteracantha longifolia</em></td>
<td>0.125</td>
<td>0.500</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Brachiaria</em> sp.</td>
<td>1.0</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Trichodesma indicum</em></td>
<td>0.250</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Abutilon indicum</em></td>
<td>0.250</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In order to determine whether the crude extracts had bacterial or bacteriostatic mode of action on the sensitive bacteria, the abilities of *A. longifolia* inhibited bacteria to resume their growth in flesh culturing broth was observed there results suggested that the *A. longifolia* had bacteriostatic mode of action on the bacteria (Table 3). The use of antimicrobial substances with bacteriostatic mode of action may have fewer side effects than those with bactericidal mode of action. The latter ones tend to kill all of the bacteria in the body including normal flora whereas the former ones just retard the growth of the bacteria which are further killed by the immune response of the body.

**Table 3: Recovery ability of the crude plant extracts inhibited bacteria**

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th><em>Optical density 620 nm</em></th>
<th>Water (0°)</th>
<th>Water (24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Asteracantha longifolia</em></td>
<td>0.71 ± 0.01</td>
<td>0.54 ± 0.05</td>
<td></td>
</tr>
<tr>
<td><em>Brachiaria</em> sp.</td>
<td>0.52 ± 0.05</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Trichodesma indicum</em></td>
<td>0.62 ± 0.01</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Abutilon indicum</em></td>
<td>0.60 ± 0.01</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*OD 600nm (Optical Density at a wave length of 600nm). Values are the mean of three replicates.
 Time after inoculation of the crude extracts bacteria into fresh broth (h)
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 author is thankful to the management of RVS Educational Trust for their encouragement and support.

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Source of support: Nil, Conflict of interest: None Declared