IN VITRO ANTIMICROBIAL ACTIVITY OF AQUEOUS AND METHANOLIC ROOT EXTRACT OF GLYCYRRHIZA GLABRA AGAINST PATHOGENIC MICROORGANISM ISOLATED FROM BOVINE MASTITIS

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ABSTRACT
The present study aimed to evaluate the antibacterial activity of aqueous and methanolic root extracts of Glycyrrhiza glabra against Staphylococcus aureus (S. aureus) and Streptococcus agalactiae (St. agalactiae) the major pathogens isolated from the cases of clinical and sub clinical bovine mastitis. Phytochemical screening of the herbal extract revealed the presence of alkaloids, glycosides, saponins, tannins, steroids and flavonoids. The antibacterial sensitivity test (ABST) of the aqueous extract and methanolic extract ranged between 10 mm to 22 mm against Staphylococcus aureus, Streptococcus agalactiae and E. coli at different concentrations. The methanolic extract was more effective than aqueous extract against Staphylococcus aureus (20 mm), Streptococcus agalactiae (22 mm) and E. coli (17 mm) at the concentration of 8 mg/disc. Minimum Inhibitory concentration (MIC) was determined by the tube dilution method. The MIC and MBC (Minimum bactericidal concentration) value of methanolic extract was 3.125 mg/ml for S. aureus, 1.56 mg/ml for St. agalactiae and 12.5 mg/ml. Whereas the aqueous extract having higher MIC and MBC values i.e. 6.25 mg/ml for S. aureus, 3.125 mg/ml for St. agalactiae and the result was negative for E. coli. The results of the present study showed that the methanolic root extract of G glabra at different concentrations depicted better antimicrobial activity compared to aqueous extract.

Key words: Glycyrrhiza glabra, Bovine mastitis pathogens, Antimicrobial activity, MIC.
1. INTRODUCTION
Ayurveda is the most ancient successfully medical practice in rural India. Herbs with medicinal properties are useful and effective source of treatment for various diseases. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary healthcare systems for man and animal (Farnsworth, 1993; Houghton, 1995). Herbs are widely exploited in the traditional medicine and their curative potentials are well documented (Dubey et al, 2004). The researches on the antimicrobial activities of medicinal plants are being reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Shaikh et al, 1994). Glycyrrhiza glabra (Liquorice) L. belongs to Family: Fabaceae, commonly known as Yashti-madhuh or Mulhati. The roots are extensively used for medicinal purposes, roots are long, cylindrical, thick and multi-branched with red or lemon color outside and yellowish or pale inside (Wealth of India, 1985). Liquorice has been used in medicine for more than 4000 years as anti inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities (Asl and Hosseinzadeh, 2008; Chopra, 2002). Free radical scavenging and antibacterial properties of the root extract have been studied in vitro (Sharma et al, 2013). Roots have great antimicrobial and anxiolytic activities (Ambawade et al., 2001). The medicinal properties of the root is due to its phytoconstituents like triterpenes (glycyrrhizin, glycyrrhetinic acid and liquiritic acid) and flavonoids (liquiritin and formononetin) compounds of Glycyrrhiza glabra (Farag et al., 2012). Some researchers have studied the antioxidant and antimicrobial constituents of licorice (Demizu et al., 1988).

Mastitis generally refers to an inflammation of the mammary gland which is characterized by physical and chemical changes of the milk and pathological changes in the udder tissues (Radostits et al., 2000). Staphylococcus aureus and Streptococcus agalactiae are the most common pathogens mainly associated with contagious mastitis (Monecke, 2007). E. coli bacteria infect the udder through teat canal if environment is contaminated with faeces (Nemeth et al., 1994). Multidrug resistance in causative organisms occur due to repeated use of high doses of antibiotics to treat mastitis, antibiotics in milk and milk products lead to potential health hazard (Annapoorani, 2007). Therefore the present study was undertaken to evaluate the antimicrobial activity of Glycyrrhiza glabra root extract against major pathogens like Staphylococcus aureus, Streptococcus agalactiae and Escherichia coli isolated from the clinical cases of bovine mastitis.
2. MATERIALS AND METHODS

2.1 Collection of plant material
Roots of licorice were collected from West Singhbhum district, Jharkhand, were washed thoroughly with water and dried at 40°C and grounded into coarse powder for extract preparation. The plant material was authenticated from Botany department of Bareilly College, Bareilly (Voucher Specimen No. 2014064199a).

2.2 Test microorganisms
Microorganisms were isolated from mastitis milk sample as per the standard procedure (Griffin et al., 1977). Microorganisms were initially identified on the basis of colony morphology and odour on 5% blood agar as per Cruikshank (1962) and later by gram staining and growth on selective media, later identified by standard biochemical kits (HiStaph, HiStrep and Hi E. coli identification kit HiMedia, Mumbai).

2.3 Preparation of extracts
2.3.1 Aqueous extract
The powdered root material 50 gram was extracted with distilled water in soxhlet apparatus upto 4 cycles (Peaech et al., 1956), filtered using whatmann filter paper no. 1 and extract was evaporated in hot air oven at 40°C. The dried powder was weighed and reconstituted in sterile phosphate buffer saline (PBS, pH 7.4, 0.01M) and the final yield was 9 gram. Finally the filtrate was filtered through membrane filter (pore size 0.45 µm) and stored in airtight vials at 4°C for subsequent use. Extract is then used for further antimicrobial assay.

2.3.2 Methanolic extract
The powdered material was macerated in methanol at the ratio of 50 gm of plants per 250 ml of methanol in a clean flat-bottomed glass container and percolated with methanol. The supernatants were collected after keeping the plant materials at 14 days in room temperature, and then filter the extract using whatmann filter paper no. 1. The extract was evaporated in hot air oven at 40°C. The dried powder was weighed and reconstituted in sterile phosphate buffer saline (PBS, pH 7.4, 0.01M). The yield was 8 gm for methanolic extract. Finally the filtrate was filtered through membrane filter (pore size 0.45 µm) and stored in airtight vials at 4°C for subsequent use. Extract was then used for further antimicrobial assay.
2.4 Phytochemical screening: Phytochemical screening of the plant material was performed following the method of Khandelwal (2005). The metabolites to be determined were: alkaloids, glycosides, saponins, tannins, steroids and flavonoids.

2.5 Antimicrobial assay: The Staphylococcus aureus, Streptococcus agalactiae and E. coli organism were isolated from milk, 3-4 colonies were suspended in Nutrient broth. The organism was thoroughly mixed in solution; thereafter the turbidity of the inoculum was matched with 0.5 of the McFarland tube standards which was equivalent to $1.5 \times 10^8$ cfu/ml. A sterile swab was dipped in this solution and smeared over Mueller-Hinton (MH) agar plate and were dried for 15 minutes. Sterile blank discs were impregnated in 25 µl of the prepared herbal extracts (with concentration of 2mg, 4mg, 6mg and 8mg respectively) and standard antibiotic disc of ciprofloxacin (5µg/disc) was used as positive control (NCCLS, 1997). The discs were then dried and placed on the plates. The diameter of the zone of inhibition was measured in millimeters. All the tests were done in duplicate to minimize the test error.

2.6 Determination of minimum inhibitory concentration (MIC)
MIC used as a research tool to determine the in-vitro activity of new antimicrobials. Determination of MIC was carried out using the tube dilution method (Oyeleke et al, 2008). A series of two fold serial dilution of each extract ranging from 100 mg/ml to 0.78 mg/ml was made in Mueller Hinton broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1998). 1000 µl of standard inoculum of the bacterial strains matched to 0.5 McFarland standards was seeded into each dilution. Two control tubes were maintained for each test batch, negative control tube containing extract and growth media without inoculum and positive control tube containing the growth medium and the inoculum. The tubes were incubated at 37°C for 24 hours and observed for turbidity. MIC was determined as the highest dilution (that is, lowest concentration) of the extract that prevents visible growth of bacteria.

2.7 Minimum Bactericidal Concentration (MBC)
MBC is the lowest concentration of antimicrobial that will prevent the growth of an organism after sub-culture on to antibiotic free media. Tube showing no growth during MIC determination was selected for MBC determination, a loop full from each tube was sub cultured on to Muller Hinton agar plates and incubated for further 24 hours at 37°C. The least concentration, at which no growth was observed, was denoted as the MBC.
3. RESULTS AND DISCUSSION

Phytochemical screening of the herbal extract revealed the presence of alkaloids, glycosides, saponins, tannins, steroids and flavanoides (Table 1). The antibacterial activity (ABST) of the extracts at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter (Table 2). The ABST of the aqueous extract ranged between 9 mm to 22 mm against *Staphylococcus aureus*, *Streptococcus agalactiae* and *E. coli* at different concentration. However the higher zone of inhibition was depicted by methanolic extract which ranged between 13 mm to 22 mm against *Staphylococcus aureus*, *Streptococcus agalactiae* and *E. coli* at different concentration. The methanolic extract was more effective than aqueous extract against *Staphylococcus aureus* (20 mm), *Streptococcus agalactiae* (22 mm) and *E. coli* (17 mm) at the concentration of 8 mg/disc.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined for the extracts. The MIC and MBC values of methanolic extract were found to be lower as compared to aqueous extract. The methanolic extract depicted lower MIC and MBC values i.e. 3.125 mg/ml for *S. aureus* 1.56 mg/ml for *St. agalactiae* and 12.5 mg/ml for *E. coli*. Whereas the aqueous extract having higher MIC and MBC values i.e. 6.25 mg/ml for *S. aureus* and 3.125 mg/ml for *St. agalactiae*. For *E. coli*. MIC and MBC value of aqueous extract was negative The MIC and MBC of the aqueous extract was was higher to an extent of 50.01 % for *S. aureus*. Similarly the MIC and MBC of the aqueous extract was higher to an extent of 50 % for *St. agalactiae*. The lower MIC and MBC value is an indication of high effectiveness of the extract whereas higher MIC and MBC indicates the less effectiveness of the extract.

Mastitis is the inflammatory changes in the mammary parenchyma and affecting milk and milk quality (Viguier, 2009). For the dairy industry mastitis continues to be recognized as a most serious disease problem. The present study was to evaluate the antibacterial activity of aqueous and methanolic root extracts of *Glycyrrhiza glabra* against major mastitis causing organisms. In the present study we have recorded the zone of inhibition of the *Glycyrrhiza glabra* extract which ranged between10 mm to 22 mm against pathogenic *S. aureus*, *St. agalactiae* and *E. coli* . The phytochemical analysis revealed presence of glycosides, saponins tannins and flavanoids, the antibacterial activity of the extract of the licorice could be due the presence of these phytoconstituents. The findings of this present study agreed with earlier studies for its antibacterial activity (Awandkar et al., 2012; Nitalikar et al., 2010;
Sultana et al., 2010). Licorice root contains triterpenoid saponins mostly glycyrrhizin, a mixture of potassium and calcium salts of glycyrrhizic acid showing antibacterial properties (Williamson, 2003). Soulef (2014), reported glycoside of *Glycyrrhiza glabra* has a very large and diverse antibacterial activity. High flavonoid content has also been reported to exhibit antibacterial activity (Rauha, 2000). Tannins act as antimicrobial agents by preventing the development of microorganism by precipitating microbial proteins (Jain, 2011).

Table 1: Phytochemical analysis of the roots of *Glycyrrhiza glabra* extract

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Aqueous extract</th>
<th>Methanolic extract</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Present: (+), Absent: (-)

Fig 1: Antibacterial activity of aqueous root extract of *Glycyrrhiza glabra*

Table 2: Antimicrobial activity of *Glycyrrhiza glabra* showing zone of inhibition against microorganisms

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent</th>
<th>Concentration</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Streptococcus agalactiae</em></th>
<th><em>E. coli</em></th>
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<tbody>
<tr>
<td>1</td>
<td>Aqueous</td>
<td>2 mg</td>
<td>10 mm</td>
<td>12 mm</td>
<td>Negative</td>
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<tr>
<td></td>
<td></td>
<td>4 mg</td>
<td>12 mm</td>
<td>14 mm</td>
<td>Negative</td>
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<tr>
<td></td>
<td></td>
<td>6 mg</td>
<td>14 mm</td>
<td>16 mm</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 mg</td>
<td>17 mm</td>
<td>18 mm</td>
<td>Negative</td>
</tr>
<tr>
<td>#</td>
<td>Compound</td>
<td>Dose (mg)</td>
<td>Zone of Inhibition (mm)</td>
<td></td>
<td></td>
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<td>----</td>
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<tr>
<td>2</td>
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<td>8</td>
<td>20</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>Ciprofloxacin</td>
<td>5</td>
<td>25</td>
<td></td>
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<td></td>
<td></td>
<td>8</td>
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<td></td>
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<td>10</td>
<td>21</td>
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</table>

**Fig 2: Antibacterial activity of methanolic root extract of *Glycyrrhiza glabra***

**4. CONCLUSION**

It is concluded that both the aqueous and methanolic root extract of *Glycyrrhiza glabra* possesses potent antimicrobial activity against gram positive (*S. aureus* and *St. agalactiae*) and gram negative (*E. coli*) organisms. The extract is having great antimicrobial potential and can be used as raw materials for herbal therapy for bovine mammary gland infection.

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**REFERENCES**


