The Effect of Mentha piperita, Peppermint and Matricaria chamomilla extracts on Agents Causing Bovine Mastitis in Vitro Condition

Akbar Pirestani¹,*, Gholam Galamkari¹, Shahin Eghbalsaied¹, Mehrdad Jafarpour², Nafiseh Ghasemi Por³

1. Department of Animal Science, Khorasgan (Esfahan) Branch, Islamic Azad University, Esfahan, Iran
2. Department of Horticulture Science, Khorasgan (Esfahan) Branch, Islamic Azad University, Esfahan, Iran
3. Laboratory of Microbiology, Khorasgan (Esfahan) Branch, Islamic Azad University, Esfahan, Iran

*Corresponding author email: a.pirestani@khuisf.ac.ir

ABSTRACT: The aim of this study was to compare the antibacterial effect of plants extract on bacterial caused bovine mastitis in vitro condition. In this study, extracts from Mentha piperita, Peppermint and Matricaria chamomilla were used as anti-microbial agents on Staphylococcus aurus, Escherichia coli and Streptocococcus agalactie with agar well diffusion method. Evaluation of minimal inhibitory concentration (MIC) was on base of diameters of the inhibition zones as non-inhibition zone: resistance, moderate-inhibition zone: semi sensitive and large-inhibition zone: sensitive. Results indicated that Matricaria chamomilla extract was affected on 10 to 50 µl Staphylococcus aurus and 50 µl Escherichia coli. It was concluded that Matricaria chamomilla extract could be used as teat disinfection and had antibacterial activity on some of the bovine mastitis agents.

Keywords: Bactria, bovine mastitis, dairy cattle, In vitro, medical plant extract

INTRODUCTION

Bovine mastitis is mammary gland infection in dairy cattle. Mastitis occurs when white blood cells (leukocytes), are released into the mammary gland, usually in response to an invasion of bacteria of the teat canal (National Mastitis Council, 2001). It is also the most costly to the dairy industry. This disease costs the US dairy industry about 1.7 to 2 billion USD each year (Nickerson, 1990). Most common bacteria that are known to cause mastitis include: Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Mycoplasma, Streptococcus agalactie and Streptococcus uberis (National Mastitis Council, 2009).

Medical plant extracts continue to be an important therapeutic aid to alleviate the complaint of humankind. Some plants have strong antimicrobial and disinfection activity. Both of chamomile and mint essential oils had the strongest activity against gram-positive and gram-negative bacteria (Bayoub et al, 2010). Mentha piperita has exhibit properties such antibacterial, antiseptic, anti-diarrhea, anti-astringent, antipyretic and anticonvulsant. Those antibacterial properties are resulted of materials such as menthol, menthone, and their derivatives (Imai et al., 2001). In vitro application of Mentha piperita demonstrated antibacterial activity against such as Listeria monocytogenes, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli and Campylobacter jejuni (Kenji et al., 2006). Matricaria chamomilla flowers contain 0.24- to 2.0-percent volatile oil that is blue in color. The two key constituents, alpha-bisabolol and chamazulene, account for 50-65 percent of total volatile oil content (Aggag and Yousef, 1972). Other components of the oil include alpha-bisabolol oxide A and B, alpha-bisabolone oxide A, spiroethers (cis- and trans-en-yndicyclocether), sesquiterpenes (anthecotulid), cadinene, farnesene, furfural, spathulenol, and proazulene (matricarin and matricin). Chamazulene is formed from matricin during steam distillation of the oil. Yield varies depending on the origin and age of the flowers (Luis et al., 2006). European Pharmacopoeia recommends chamomiles which contain more than 4 mL/kg of blue essential oil. Several pharmacological actions have been documented for German chamomile, based primarily on in vitro and animal studies (Thorne, 2008). Such actions include antibacterial, antifungal, anti-inflammatory, antispasmodic, anti-ulcer,
antiviral, and sedative effects (European Pharmacopoeia, 1996). Chamomile oil demonstrates antibacterial activity against such gram-positive bacteria as Bacillus subtilis, Staphylococcus aureus, Streptococcus mutans, Streptococcus salivarius, Helicobacter pylori and various strains of Escherichia coli, Mycobacterium tuberculosis and Mycobacterium avium as well as some fungicidal activity against Candida albicans (Thorne, 2008). However, implementation of medical plant extracts as teat dips in dairy farms has been remained to be evaluated. The aim of this study was to compares effects of chamomile, peppermint and menthe as antibacterial and disinfection on agent’s bovine mastitis in vitro condition.

**MATERIAL AND METHOD**

*Preparation of the Plant extracts*

The dried and powdered plants were extracted by ethanolic extraction by soaking 50 g of plant part in 500 mL of 96% ethanol for 48 hour at laboratory temperature. The supernant were then filtered. The filtrate was centrifuged at 2000 rpm on 10 minute and it was concentrated on a rotary evaporator at 42°C for ethanol elimination and the extracts were kept in sterile bottles under refrigerated conditions until use.

*Bacterial strains and culture preparation*

The bacterial strains used in this study were Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 25922, Streptococcus agalactiae ATCC 13813. All of them were obtained from the culture collection of the Iranian Research Organization for Science and Technology (Persian Type Culture Collection). Bacterial cultures were maintained on nutrient Agar and they were stored at 4°C.

*Antibacterial screening*

The screening of the ethanolic extracts of these plants for antibacterial activity was performed using agar well diffusion method. In order to determine the antibacterial spectrum, the antibacterial activity was performed by the agar-well diffusion method as described by Bayoub (2010). A volume of 10 mL of agar medium (0.7% w/v) was inoculated with 0.1 ml of fresh overnight culture of the indicator strain (approximately 107 CFU/mL) and poured into a Petri disk containing layer of the plat-count agar (PCA). Wells of 6 mm in diameter were punched in the agar and filled with 5, 10 and 50 μL of the ethanol extract. After holding the plates at room temperature for 2 h to allow diffusion of the extract into the agar, the plates were incubated at 37°C for 24 h. Then, they were examined for inhibition of the bacterial lawn and the diameters of the inhibition zones were measured.

*Minimal inhibitory concentration assay*

Plant extracts of chamomile, peppermint and menthe were identified to have potent antibacterial activity and their minimum inhibitory concentrations (MIC) were determined for Staphylococcus aurus, Escherichia coli, Streptococcus agalactiae. The micro-dilution method using serially diluted (2-fold) plant extracts recommended by the National Committee for Clinical Laboratory Standards (NCCLS) was performed and the MICs were determined by agar well diffusion method, inoculated plates were incubated at 37°C for 18 h. The MICs were determined on base of diameters of the inhibition zones as non-inhibition zone: resistance, moderate-inhibition zone: semi-sensitive and large-inhibition zone: sensitive.

**RESULT AND DISCUSSION**

Table 1 shows the effect of Mentha piperita, peppermint and Matricaria chamomilla extracts was survey on Staph. aureus, E. coli and Strep. agalactiae in vitro condition. The result shows that Mentha piperita and peppermint don’t have influence on all bacteria and they were resistance. But Matricaria chamomilla had an effect on Staph. aureus at 50 μl concentration and this bacterium has sensitive. On the other hand, Staph. aureus was semi-sensitive at 50 μl concentration. Matricaria chamomilla (treatment group) has been shown in vitro condition that can effect on the bacteria that cause mastitis in farm condition. The result shows that treatment group had favorable effect on mastitis agents such as Staph. aureus, and somewhat E. coli at 50 and 10 μl concentration. The researches show that antimicrobial properties of Matricaria chamomilla related compound include alpha-bisabolol, luteolin, quercetin, and apigenin based primarily on in vitro and animal studies. Herniarin may also have antibacterial and antifungal properties in the presence of ultraviolet light. Preliminary in vitro studies on the antimicrobial activity of chamomile have yielded promising results. Chamomile oil, at a concentration of 25 mg/mL, demonstrates antibacterial activity against such gram-positive bacteria as Bacillus subtilis, Staphylococcus aureus,
Streptococcus mutans, and Streptococcus salivarius, as well as some fungicidal activity against Candida albicans (Thorne, 2008; Amirghofran et al., 2000).

Table 1. effects of medical plant extracts on some mastitis agents with different concentration in vitro

<table>
<thead>
<tr>
<th>Medical plant extracts</th>
<th>Amount (µl)</th>
<th>Microorganisms</th>
<th>E. coli</th>
<th>Strep. agalactie</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Staph. aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mentha piperita</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>50</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Peppermint</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>50</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Matricaria chamomilla</td>
<td>10</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>±</td>
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<td>+</td>
</tr>
</tbody>
</table>

+: Resistant, ±: Semi-sensitive, -: sensitive

CONCLUSION

Overall, results of study conducted that Matricaria chamomilla had desired effects on prevention of mastitis and it could to use as organic teat disinfectant compare to chemical disinfectant in vitro condition.

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REFERENCES