Antistreptococcal and Antioxidant Activity of Essential Oil from *Matricaria chamomilla* L.

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Abstract: Streptococci have gained increasing attention as pathogens of public health importance owing to large numbers of outbreaks of streptococcal infections. Because of negative consumer perception of chemical drugs and development of drug resistance, attention is shifting towards natural alternatives. Particular interest has been focused on the potential application of plant essential oils. The objective of the present study was to determine antibacterial efficacy and antioxidant property brought about by essential oils from *Matricaria chamomilla* L. Disk diffusion and tube dilution methods were employed to evaluate inhibition, Minimal Inhibitory (MIC) and Minimal Bactericidal (MBC) concentrations and bactericidal kinetics of the oil. Streptococcus pyogenes, Streptococcus mutans, Streptococcus salivarius, Streptococcus faecalis and Streptococcus sanguis were exposed to essential oils of *Matricaria chamomilla* L. The oil composition was analyzed by GC and GC/MS. Antioxidant activity was determined by â-carotene bleaching test. The oil from the above plant was found to be strongly antimicrobial. MICs/MBCs of *Matricaria chamomilla* L. oil determined for *Streptococcus pyogenes, Streptococcus mutans, Streptococcus salivarius, Streptococcus faecalis* and *Streptococcus sanguis* in terms of ig/ml were 0.1/0.2, 0.5/1.5, 0.5/0.8, 4/7 and 0.5/1.1, respectively. The oil analysis lead to identification of 18 components of which the major ones were: guaniol (25.6%), (E)-â-faranesens (20.1%), chamazulene (12.4%), â-bisabol oxide B (7.3%), â-bisabol (7.3%) and hexadecaneol (5.6%). In the â-carotene bleaching test, the oil gave the best inhibition result of 82.5% after 120 minutes. The antistreptococcal effects of *Matricaria chamomilla* L. oil is stronger at lower concentrations against various streptococci strains tested. It is concluded that low concentrations of *M. chamomilla* essential oil could be considered as alternative antistreptococcal and antioxidant agent.

Key words: *Matricaria chamomilla* L., essential oils, antioxidant, antistreptococcus, MIC, MBC

INTRODUCTION

Finding healing power in plants is a traditional and ancient concept. However, since the advent of potent synthetic antibiotics in the 1950s, the use of plant derivatives as antimicrobials has become almost nonexistent. The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives. Within the wide range of the above-mentioned products, a common need is availability of natural extracts with a pleasant taste or smell combined with a preservative action, able to inhibit lipid deterioration, oxidation and spoilage by microorganisms. The essential oils and extracts of many plant species have become popular in recent years and attempts to characterize their bioactive principles have recently gained momentum in many pharmaceutical and food-processing applications (Cowan, 1999). The antimicrobial activities of essential oils isolated from many plants have been recognized, albeit empirically, for centuries. Only recently such properties have been confirmed. The essential oils produced by different plant species are in many cases biologically active (Couplidus et al., 2004). Chamomile is one of the most widely used and well-documented medicinal plants in the world (Salomon, 1992a). It is included in the pharmacopoeia of 26 countries (Salomon, 1992b). In Germany, where chamomile sales exceeded $8.3 million in 1996 (Cirigliano, 1999), more than 4,000 tons of chamomile are produced yearly (Berry, 1995). The use of chamomile as a medicinal plant dates back to ancient Greece and Rome. It is believed to possess anti-inflammatory, vulnerary, deodorant, bacteriostatic, antimicrobial, antitussive, carminative, sedative,.
antiseptic and spasmylytic properties (Mannad and Staba, 1986). Compounds in the essential oil of chamomile were effective against Staphylococcus and Candida (Agag and Yousef, 1972). Of chamomile’s essential oil components, "-bisabolol had the strongest activity against Gram-positive and Gram-negative bacteria. Chamazulene also had strong antimicrobial activity. Spiroethers had weak activity against Gram-positive bacteria but were inactive against Gram-negative bacteria (Kedzia, 1991). German chamomile esters and lactones showed activity against Mycobacterium tuberculosis and M. avium (Lu et al., 1998). Chamazulene, "-bisabolol, flavonoids and umbelliferone displayed antifungal properties against Trichophyton mentagrophytes, T. rubrum and Candida albicans (Kedzia, 1991; Szalontai et al., 1976; Szalontai et al., 1977). An ethanolic extract of German chamomile inhibited the growth of Herpes and Poliovirus (Suganda et al., 1983). Chamazulene affects free radical processes and inhibits lipid peroxidation in a concentration- and time-dependent manner (Kokka et al., 1996). Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year (Clark, 1996). After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. Worldwide spending on finding new anti-infective agents including vaccines is expected to increase 60% from the spending levels in 1993 (Aiper, 1998). New sources, especially plant sources, are also being investigated. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. All in all, data about specific antimicrobial properties of Matricaria chamomilla L. are scarce, although many reports give reason to believe that some utility may reside in these phytochemicals (Hamburger and hostettmann, 1991). Antibiotic resistance has become a global concern (Westh et al., 2004). Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000; Cowan, 1999). Traditional healing systems around the world that utilize herbal remedies are an important resource for the discovery of new antibiotics (Okpokon et al., 2004). Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kong et al., 2004; Sato et al., 2000). With a view to the aforementioned facts, we designed this study to explore antistreptococcal and antioxidative properties of Matricaria chamomilla L. essential oils.

MATERIALS AND METHODS

Microbial strains and growth media: Streptococcus pyogenes (PTCC 1447), Streptococcus mutans (PTCC 1601), Streptococcus salivarius (PTCC 1448), Streptococcus faecalis (ATCC 29212) and Streptococcus sanguis (PTCC 1449) were grown on blood agar. Bacterial suspensions were made in Mueller Hinton broth.

Plant and oil isolation: The plant, Matricaria chamomilla L., was identified and provided by Zardband company. The plant origin was from Yasoj region of Iran collected during May-June 2004. The shadow dried flowers were hydro distilled for 90 min in full glass apparatus. The oil was isolated using a Clevenger type apparatus. The extraction was carried out for 2 h after 4-h maceration in 500 ml of water. The oil so extracted had a specific gravity of 0.95 at 20 °C and refractive index 1.48-1.505 at 25°C was stored in dark glass bottles in a refrigerator until they were used.

Oil analysis: GC analysis was performed by GC (9-A-Shimadzu) gas chromatograph equipped with a flame ionization detector. Quantitation was carried out on Euro Chrom 2000 from KNAUR by area normalization method. The analysis was carried out using a DB-5 fused-silica column (30 m × 0.25 mm, film thickness 0.25 μm) using a temperature program of 40-250°C at a rate of 4°C/min, injector temperature 250°C, detector temperature 265°C, carrier gas: helium (99.99%). The GC/MS unit consisted of Varian-5400 gas chromatograph coupled to a Saturn II ion trap detector. The column was same as of the GC under the same conditions stated above. The constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with literature data.

Oil dilution solvent: Disk diffusion method was employed to assess anti streptococcal properties of the solvents. Bacterial strains were streaked on blood agar plates using sterile cotton swabs. 20 μl from each of Methanol, Tween-80 (20%) and Dimethyl Sulfoxide (DMSO) loaded on sterile blank disks and were then placed on the blood agar plates and were incubated at 37°C for 24 h. There was no antistreptococcal activity on the plates. Methanol was not a good solvent for our oil, Tween-80 brought about the
hemolysis on blood agar. DMSO being a good solvent did not hemolyze which was selected as a diluting agent for
the oil. Stock solution of the oil was prepared by
dissolving 200 µg essential oil per mL of DMSO. 20 µL
of diluted oil containing 4 µg oil was added to each sterile
blank disk. Further dilutions were made from the stock
solution as and when needed. The solvent also served
as control.

**Antistreptococcal analysis:** The fresh oil was tested for
its antistreptococcal activities. The disk diffusion
method was used for antistreptococcal screening of
both the diluent, DMSO and *Matricaria chamomilla* oil
as follows (Anhalt and Washington, 1985): Sterile
Mueller-Hinton agar medium (Merek) was prepared and
distributed into Petri dishes of 80 mm diameter. Twenty-four
hours old bacterial suspensions were adjusted to a turbidity
of 0.5 McFarland Units by the addition of isolate colonies
in sterile normal saline. Turbidity was verified through
spectrophotometry comparison with a 0.5 McFarland
Standard. The dilutions were used within 15 min of their
preparation and were vortexed prior to each use. Microbial
suspending were streaked over the surface of the
Mueller-Hinton agar using sterile cotton swabs in order to
gain a uniform microbial growth on the plates. Under
aseptic conditions, the blank disks (6 mm diameter) were
loaded with 20 µL from essential oil stock solution and
were then placed on the agar plates in order to rule out its antistreptococcal effect. This
also served as control. 20 µL from essential oil stock
solution was added to each disk in triplicates. After 24 h
incubation in 37°C, the zones of inhibition were measured using a vernier caliper. The Minimal Inhibitory
Concentration (MIC) and Minimal Bactericidal
Concentration (MBC) were assessed according to NCCLS
procedure (1991) as follows: Measured quantities of the
essential oil were added to each of Mueller-Hinton broth
tubes to achieve final oil concentrations from 1 through
10 µg/mL at 1 µg/mL increments. The exact concentrations
were then evaluated at every 0.1 µg mL⁻¹ increments after
finding MIC and MBC values so as to arrive at exact
minimal inhibitory and bactericidal values of each bacterial
strain. Tubes without oil served as control. Measured
counts from each of the 24 h old streptococcal suspensions prepared in normal saline at 0.5 McFarland
units were added to each tube containing various oil
concentrations so as to achieve final cell load of 5×10⁶
CFU mL⁻¹. The tubes were then incubated at 37°C for 24
hours on an incubator shaker as to evenly disperse the oil
troughout the broth in tubes. MIC determination was
conducted by bacterial count instead of turbidimetry to
avoid misleading false turbidity caused by oil interference.
The lowest concentration, showing no increased growth
compared to that of the control tubes, was regarded as
MIC. MBC was determined as the lowest concentration at
which 99.9% bacterial death occurred on the plates.
Bacterial counts were carried out at time zero both in
control and test. All tubes including the control were run
simultaneously. All the tests were carried out in triplicate.
Bactericidal kinetics of the oil: 5 mL Mueller Hinton broth
tubes containing essential oil at the concentrations
determined by MBC were inoculated with each
streptococcal suspension as described above and were
then incubated at 37°C. Samples were taken at time 0 h
and after every 30 min till 300 min. The samples were
immediately diluted with normal saline in order to stop any
carried over bactericidal effect of the oil. Viable counts
were achieved from serial dilutions in triplicates. The mean
total number was converted into log10 viable cells using
routine mathematical formulae. The data were used to
illustrate bactericidal kinetics of the oil.

**Antioxidant activity:** Antioxidant activity of essential oils
was determined using β-carotene bleaching test (Taga
*et al.*, 1984). Approximately 10 mg of β-carotene (type
1 synthetic, Sigma-Aldrich) was dissolved in 10 mL of
chloroform. The carotene-chloroform solution, 0.2 mL, was
pipetted into a boiling flask containing 20 mg linoleic acid
(Sigma-Aldrich) and 200 mg Tween 40 (Sigma-Aldrich).
Chloroform was removed using a rotary evaporator at
40°C for 5 min and, to the residue, 50 mL of distilled water
were added, slowly with vigorous agitation, to form an
emulsion. Five mL of the emulsion were added to a tube
containing 0.2 mL of essential oils solution prepared
according to Choi *et al.* (2000) and the absorbance was
immediately measured at 470 nm against a blank,
consisting of an emulsion without β-carotene. The tubes
were placed in a water bath at 50°C and the oxidation of
the emulsion was monitored spectrophotometrically by
measuring absorbance at 470 nm over a 60 min period.
Control samples contained 10 µL of water instead of
essential oils. All determinations were performed in
triplicate.

The antioxidant activity was expressed as inhibition
percentage with reference to the control after 30, 60, 90
and 120 min incubation using the following equation:

\[ AA = 100(\text{DR}_{C} - \text{DR}_{S})/\text{DR}_{C}, \]

where AA = Antioxidant activity;
\[ \text{DR}_{C} = \text{Degradation rate of the control} = [\ln(a/b)/\text{incubation time}]; \]
\[ \text{DR}_{S} = \text{Degradation rate in presence of the sample} = [\ln(a/b)/\text{incubation time}]; \]
\[ a = \text{Absorbance at time 0, b = absorbance at 30, 60, 90 and 120 min}. \]
RESULTS

Chemical analysis of the components of the oils led to identification of 18 components (Table 1). The major components of Matricaria chamomilla L. oil were guaiazulene (25.6%), (E)-β-faranesens (20.1%), chamazulene (12.4%), α-bisabolol oxide B (7.3%), α-bisabolol (7.3%) and hexadecanole (5.6%). Preliminary experiments were carried out in vitro using the disk diffusion and tube dilution methods to investigate antimicrobial action of the essential oil. Various concentrations of the essential oil were tested on the relevant agar plates and broth tubes showed a very strong antimicrobial property (Table 2). The oil was inhibitory/bactericidal at the concentrations of 0.1/0.2, 0.5/1.5, 0.5/0.8, 4/7 and 0.5/1.1 μg mL⁻¹ against S. pyogenes, S. mutans, S. salivarius, S. faecalis and S. sanguis with growth inhibition zones of 9±0.5mm, 10±0.1mm, 9±0.5mm, 8±0.5mm and 8±0.5 mm, respectively (Table 2). Study of bactericidal kinetics of essential oils revealed complete elimination of S. pyogenes and S. sanguis after 4 and 5 h of exposure to the essential oil respectively (Fig. 1). Other streptococci studied were also affected gradually at a slow rate (Fig. 1). Percent The antioxidant activity expressed as inhibition percentage

![Fig. 1: Kinetics of streptococci death](image)

with reference to the control after a 30, 60, 90 and 120 minutes were 69.76, 78.83, 81.62 and 82.50, respectively.

DISCUSSION

The results indicate that the streptococci strains under study were eliminated or inhibited after exposure to the essential oils in culture broth (Table 2 and Fig. 1). Such delay in or inhibition of microbial growth is particularly useful in terms of public health and safety. This indicates higher efficacy of Matricaria chamomilla L. oil. The difference in microbial susceptibility is attributable to the chemical composition of essential oil. The ineffectiveness of some oils might reflect the lack of antibacterial compounds in the plants against the microorganism under study. A possible explanation for this is that some of the plant extracts may have contained antibacterial constituents, but were not present in sufficient concentrations to be effective. In response to bactericidal effect of the oil, the Streptococcal strains employed in the study could be categorized from the most susceptible to the least susceptible as S. pyogenes> S. salivarius> S. sanguis> S. mutans> S. faecalis (Table 2). This could be attributed to the resistant nature of S. faecalis to most bactericidal agents which was expectedly evident in our experiments. On the contrary susceptibility of S. pyogenes to most bactericidal agents was also apparent in its elimination with the minimum amount of the oil in shorter period of time. The oil used in the present study had chemical components (Table 1) such as guaiazulene (25.6%), (E)-β-faranesens (20.1%), chamazulene (12.4%), α-bisabolol oxide B (7.3%), α-bisabolol (7.3%) and hexadecanole (5.6%) which have probably imparted antibacterial properties to the oil. Ethanolic tinctures and aqueous extracts of Matricaria chamomilla produced a 12mm average zone of clearance when tested against Staphylococcus aureus (Romero

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Table 1: Chemical composition of essential oil from Matricaria chamomilla L.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>R.I.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Limonene</td>
<td>1029</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>γ-terpinene</td>
<td>1062</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>(E)-β-faranesens</td>
<td>1459</td>
<td>20.1</td>
</tr>
<tr>
<td>4</td>
<td>germacrene-D</td>
<td>1481</td>
<td>3.1</td>
</tr>
<tr>
<td>5</td>
<td>α-muurolene</td>
<td>1496</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>germacrene-A</td>
<td>1504</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>2-Z-β-bisabolene</td>
<td>1515</td>
<td>2.6</td>
</tr>
<tr>
<td>8</td>
<td>caryophyllene oxide</td>
<td>1570</td>
<td>1.2</td>
</tr>
<tr>
<td>9</td>
<td>Spathulenol</td>
<td>1578</td>
<td>1.7</td>
</tr>
<tr>
<td>10</td>
<td>α-bisabolol oxide B</td>
<td>1654</td>
<td>7.3</td>
</tr>
<tr>
<td>11</td>
<td>α-bisabolol</td>
<td>1685</td>
<td>7.3</td>
</tr>
<tr>
<td>12</td>
<td>Chamazulene</td>
<td>1729</td>
<td>12.4</td>
</tr>
<tr>
<td>13</td>
<td>α-bisabolol oxide A</td>
<td>1746</td>
<td>1.9</td>
</tr>
<tr>
<td>14</td>
<td>Guaiazulene</td>
<td>1756</td>
<td>25.6</td>
</tr>
<tr>
<td>15</td>
<td>Hexadecanole</td>
<td>1882</td>
<td>5.6</td>
</tr>
<tr>
<td>16</td>
<td>n-nonadecane</td>
<td>1891</td>
<td>1.4</td>
</tr>
<tr>
<td>17</td>
<td>Sclarene</td>
<td>1968</td>
<td>0.4</td>
</tr>
<tr>
<td>18</td>
<td>n-pentacosane</td>
<td>2506</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 2: Antimicrobial effect of Matricaria chamomilla L. essential oil on the basis of growth inhibition zone (mm) with corresponding inhibitory or lethal properties

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>S. pyogenes</th>
<th>S. mutans</th>
<th>S. salivarius</th>
<th>S. faecalis</th>
<th>S. sanguis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone of inhibition (mm±SD) after exposure to 5μg oil per disk</td>
<td>9±0.5</td>
<td>10±0.1</td>
<td>9±0.5</td>
<td>8±0.5</td>
<td>8±0.5</td>
</tr>
<tr>
<td>MIC (μg mL⁻¹)</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>MBC(μg mL⁻¹)</td>
<td>0.2</td>
<td>1.5</td>
<td>0.8</td>
<td>7</td>
<td>1.1</td>
</tr>
</tbody>
</table>
et al., 2005). Dulger and Ahmet Gonuz (2004) did not find antibacterial effect of ethanolic extract of Matricaria chamomilla on any of the 12 microorganisms under their investigation. The chemical complexity of essential oils, often a mixture of dozens of compounds with different functional groups, polarity and chemical behaviour, could lead to scattered results. The oil with high terpenic percentages is more effective, probably as a consequence of a higher specificity of the assay for hypophylic compounds (Sacchetti et al., 2005). The reason that antioxidants are important to human physical well being comes from the fact that oxygen is a potentially toxic element since it can be transformed by metabolic activity into more reactive forms such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radicals, collectively known as active oxygen. The essential oil from Matricaria chamomilla L. exhibited a good antioxidantive potential in the present study implying feasibility of its application as an antioxidant agent. Traditional healing systems around the world that utilize herbal remedies are an important resource for the discovery of new antibiotics (Okpekfe et al., 2004), some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kon’ e et al., 2004).

The findings suggest that Matricaria chamomilla L. oil has good potential as an antistreptococcal agent in combating such pathogens as it may be more acceptable to consumers and the regulatory agencies in comparison to chemical compounds. Although high concentrations of essential oils may have toxic effects, lower concentrations may be sufficient for public health in actual situations where bacterial load is high, in addition to pleasant flavor of chamomile. The results on the mechanism of action of Matricaria chamomilla L. oil on the inactivation of various streptococci will help in the development or modification of health care substances or the implementation of a new factor to complement the prevailing factors employed in human safety.

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