

## 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  $\beta$ -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  $\mu\text{g/ml}$  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: guaiazulene (25.6%), (E)-  $\beta$ -farnesens (20.1%), chamazulene (12.4%),  $\alpha$ -bisabolol oxide B (7.3%),  $\alpha$ -bisabolol (7.3%) and hexadecanole (5.6%). In the  $\beta$ -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 (Couladius *et al.*, 2004). Chamomile is one of the most widely used and well-documented medicinal plants in the world (Salamon, 1992a). It is included in the pharmacopoeia of 26 countries (Salamon, 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, anticatarrhal, carminative, sedative,

antiseptic and spasmolytic properties (Mannand Staba, 1986). Compounds in the essential oil of chamomile were effective against *Staphylococcus* and *Candida* (Aggag and Yousef, 1972). Of chamomile's essential oil components,  $\alpha$ -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,  $\alpha$ -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 (Rekka *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 (Alper, 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 (Okpekon *et al.*, 2004). Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kon'e *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 Yasooj 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 KNAUER 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 $\mu$ m) 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-3400 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 $\mu$ l 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 (Merck) was prepared and distributed into Petri dishes of 80 mm diameter. twenty four h 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 suspensions were streaked over the surface of the Mueller-Hinton agar using sterile cotton swabs in order to get a uniform microbial growth on the plates. Under aseptic conditions, the blank disks (6 mm diameter) were loaded with 20°L DMSO 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 inhibitions were measured using a vernier clipper. 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 10ig/ml at 1ig/ml increments. The exact concentrations were then evaluated at every 0.1 µg mL<sup>-1</sup> 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 quantities 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 × 10<sup>5</sup> CFU mL<sup>-1</sup>. The tubes were then incubated at 37°C for 24 hours on an incubator shaker as to evenly disperse the oil throughout the broth in tubes. MIC determination was carried out by bacterial count instead of turbidometry 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: 5ml 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 log<sub>10</sub> 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 beta-carotene (type I synthetic, Sigma-Aldrich) was dissolved in 10 mL of chloroform. The carotene-chloroform solution, 0.2mL, 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:

$$AA = 100(DR_c - DR_s)/DR_c,$$

where AA = Antioxidant activity;

DR<sub>c</sub> = Degradation rate of the control = [ln(a/b)/incubation time];

DR<sub>s</sub> = Degradation rate in presence of the sample= [ln(a/b)/ incubation time];

a = 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)- $\beta$ -faranesens (20.1%), chamazulene (12.4%),  $\alpha$ -bisabolol oxide B (7.3%),  $\alpha$ -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 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 $\mu$ g mL<sup>-1</sup> against *S. pyogenes*, *S. mutans*, *S. salivarius*, *S. faecalis* and *S. sanguis* with growth inhibition zones of 9 $\pm$ 0.5mm, 10 $\pm$ 0.1mm, 9 $\pm$ 0mm, 8 $\pm$ 0mm and 8 $\pm$ 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

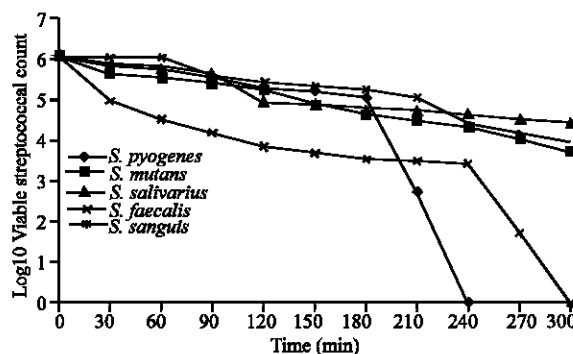


Fig. 1: Kinetics of streptococci death

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)- $\beta$ -faranesens (20.1%), chamazulene (12.4%),  $\alpha$ -bisabolol oxide B (7.3%),  $\alpha$ -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

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

No.	Compound	R.I.	%
1	Limonene	1029	0.5
2	$\gamma$ -terpinene	1062	0.5
3	(E)- $\beta$ -faranesens	1459	20.1
4	germacrene-D	1481	3.1
5	$\alpha$ -muurolene	1496	0.8
6	germacrene-A	1504	0.5
7	Z- $\gamma$ -bisabolene	1515	2.6
8	caryophyllene oxide	1570	1.2
9	Spathulenol	1578	1.7
10	$\alpha$ -bisabolol oxide B	1654	7.3
11	$\alpha$ -bisabolol	1685	7.3
12	Chamazulene	1729	12.4
13	$\alpha$ -bisabolol oxide A	1746	1.9
14	Guaiazulene	1756	25.6
15	Hexadecanole	1882	5.6
16	n-nonadecane	1891	1.4
17	Sclarene	1968	0.4
18	n-pentacosane	2500	0.5

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

Microorganisms	<i>S. pyogenes</i>	<i>S. mutans</i>	<i>S. salivarius</i>	<i>S. faecalis</i>	<i>S. sanguis</i>
Zone of inhibition (mm $\pm$ SD) after exposure to 4 $\mu$ g oil per disk	9 $\pm$ 0.5	10 $\pm$ 0.1	9 $\pm$ 0	8 $\pm$ 0	8 $\pm$ 0.5
MIC ( $\mu$ g mL <sup>-1</sup> )	0.1	0.5	0.5	4	0.5
MBC ( $\mu$ g mL <sup>-1</sup> )	0.2	1.5	0.8	7	1.1

*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 lipophilic 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 antioxidative 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 (Okpekon *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.

#### ACKNOWLEDGEMENTS

The authors wish to thank Dr. Lila Savabi (Faculty of Medicine, Shahed University) for technical helps. We thank Zardband Company (Tehran- Iran) for extraction and GC/MS analysis of the essential oil.

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