



## Antibacterial activity of extract *Chamomilla nobile* against some human pathogenic bacteria

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### Abstract

The present study was carried out to determine the potential antibacterial activity of extract *Chamomilla nobile* against some human pathogenic bacteria. The antimicrobial effect of ethanol extracts of *Chamomilla nobile* on pathogenic bacteria namely *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619, *Staphylococcus saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacter baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274, *Staphylococcus aureus* ATCC® 2592 were determined using broth microdilution method. The levels of MIC was observed ranges from 2.5 to 10 mg/ml. The highest MIC value was observed against *Enterococcus faecalis* and *Serratia marcescens*.

**Keyword:** *Chamomilla nobile*, Antibacterial activity, Standard bacteria

### Introduction

Plants are of great medicinal importance to the health of man. The curative potentials of these plants are locked-up and embedded in some chemical components that effect physiological responses in man (1). Many of these medicinal plants are used as spices and food plants; they are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (2,3). The genus *Chamaemelum nobile* of the plant family Asteraceae is a low-growing evergreen perennial that forms a spreading mat of aromatic foliage typically growing 3-6" tall and spreading by decumbent stems to 12" wide. Daisylike flowers with white rays and yellow centers bloom throughout the summer and into early fall. It is native to the Southwest Europe (France, Spain and Portugal) but the plant is present in all over Europe, North Africa and Southwest Asia. The plant is used to flavor foods, in tisanes, perfumes, and cosmetics. It is used to make a rinse for blonde hair, and is popular in aromatherapy; its practitioners believe it to be a calming agent to reduce stress and aid in sleep. Chamomile is considered to be an antiseptic, antibiotic, disinfectant, bactericidal and vermifuge. Components of the oil include (-)-alpha-bisabolol oxide A and B, (-)-alpha-bisabolone oxide A, spiroethers (cis- and trans- enyndicycloether), sesquiterpenes (anthecotulid), cadinene, farnesene, furfural, spathulenol, and proazulene (matricarin and matricin). The present study was carried out to determine the potential antibacterial activity of extract *Chamomilla nobile* against some human pathogenic bacteria.

### Bacterial Strains and Culture Conditions

Bacterial strains were obtained from standard laboratory. Evaluate the antibacterial activity of the plant extracts were investigated using strain of bacteria *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacter baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274, *Staphylococcus aureus* ATCC® 25923. The typed cultures of bacteria was sub-cultured on Nutrient agar (Oxoid) and stored at 4°C until required for study.

### Agar disk diffusion assay:

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended by CLSI (11). The procedure followed is briefly described here. *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacter baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274, *Staphylococcus aureus* ATCC® 25923 plates were grown overnight on blood agar, Nutrient agar and colony suspension was prepared using the sterile saline water equivalent to a 0.5 McFarland standard. Suspension (100 µl) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media plate. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis viz. Ceftazidim (30 µg), erythromycin (15 µg), ceftazidime (30 µg) and tetracyclin (30 µg).

**Plant materials:**

The flower *Chamomilla nobile* was collection in the region of Iran (Kerman- south-eastern, Iran) and dried at room temperature .Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

**Preparation of extracts:**

Plants were properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 ml ethanol 95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4°C in air tight screw-cap tube.

**Minimum Inhibitory Concentration (MIC) of plant extracts:**

The broth microdilution method was used to determine MIC. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3mg/ml to 10mg/ml . To each well, 10 µl of indicator solution and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension ( $10^6$  CFU/ml) was added to each well to achieve a concentration of  $10^4$  CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity.

**Result and Discussion**

Plants extracts showed inhibitory activity against bacteria with varying magnitudes and these effects were dose dependent manner. The levels of MIC was observed ranges from 2.5 to 10 mg/ml. The highest MIC value was observed against *Enterococcus faecalis* and *Serratia marcescens* (Table1). Two such studies demonstrated that gram-positive bacteria were more susceptible than gram-negative bacteria to chamomile oil (4). It was most effective against *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus salivarius*; with also *Bacillus megatherium*, *Leptospira icterohaemorrhagiae*, and *Trichomonocidal* bactericidal activity (5). Two additional *in vitro* studies showed that chamomile blocked the aggregation of *Helicobacter pylori* and numerous strains of *Escherichia coli* (6,7).

**Table1: Antimicrobial susceptibility, MIC extract plant for Standard bacteria**

Bacterial	MIC extract plant	Antibiotic resistance
<i>Staphylococcus aureus</i>	2.5	E,CE,TE
<i>Streptococcus pyogenes</i>	2.5	-
<i>Streptococcus pneumoniae</i>	2.5	E,CE,CF
<i>Hafnia alvei</i>	5	E,TE
<i>S. saprophyticus</i>	5	E,CF,TE
<i>Acinetobacter. baumannii</i>	5	CE,TE
<i>Enterococcus faecalis</i>	10	E,CE
<i>Proteus mirabilis</i>	5	E,TE
<i>Serratia marcescens</i>	10	CE

E= Erythromycin, CE= Cefixime ,CF= Ceftazidime, TE= Tetracyclin

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