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In vitro Assessment of the Antibacterial Activity of *Matricaria chamomile* Alcoholic Extract against Pathogenic Bacterial Strains

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Authors' contributions

This work was carried out in collaboration between all authors. Author HMA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors AIAG, AKA and SA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

An alarming increasing in the occurrences of antimicrobial resistance inside the existing clinical use and so the recent appearance of multidrug resistant bacteria that attempts the treatment of infections necessities to find out novel antimicrobial agents. In the attendance of Chamomile there is quite a lot of information on various studies concerning the antibacterial effects of this herb and fractioned bioassay conducted with the consent of energetic standards. Chamomile powder (*Matricaria chamomilla* L.) was purchased from private pharmacies. Fifty grams of *chamomile* powder was extracted with 250 ml 10% ethanol by soxhelt device at 45Ċ. *In vitro* determination of antibacterial activity of alcoholic extract of *Matricaria chamomilla* was done. The chamomile alcoholic extract showed higher action against *Klebsiella pneumoniae* (35±1.57 mm) inhibition zone

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and lower effect alongside *Enterococcus faecalis* (10±1.43 mm), but all the results were significant because inhibition values were near the result of active control. MIC values of *chamomile* alcoholic extract near values of tetracycline mainly against *Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus*. While MBC values of the chamomile alcoholic extract were in corresponding with tetracycline values similar to MIC, except that chamomile produced less corresponding values with tetracycline against *Enterococcus faecalis* and *Pseudomonas aeruginosa*.

Conclusion Chamomile possesses significant and potent antibacterial activity against various bacterial strains like *Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae*.

Keywords: Chamomile; alcoholic extract; antibacterial activity.

1. INTRODUCTION

An alarming increasing in the occurrences of antimicrobial resistance inside the existing clinical use and so the recent appearance of multidrug resistant bacteria that attempts the treatment of infections necessities to find out novel antimicrobial agents [1]. Therefore, demand prerequisite to discover an innovative agent with antibacterial activity designate as second-line for competition alongside resistant microorganisms and so these natural products may be a new therapeutic agent for infectious diseases [2]. Recent studies on products primarily focus on herbs since they may be a source of antimicrobial agents [3]. The antimicrobial compounds created from herbs are effective against various pathogenic bacteria which causing different types of infection [4]. In the attendance of *chamomile,* there is quite a lot of information on various studies concerning the antibacterial effects of this herb and fractioned bioassay conducted with the consent of energetic standards [5,6].

Chamomile herb derived from the *Asteraceae* family, this name came from French and Latin earth apple [7].

Moreover, *chamomile* treats chemotherapy-induced mouth sores [8,9]. Chamomile essential oil was also a conducted for the treatment of malaria and parasitic worm infections [10]. German *chamomile* flowers contain 2% volatile oil, 8% flavone glycosides, 10% mucilage polysaccharides, 0.3% choline;, 0.1% coumarines and 1% tannin [11].

Chamomile contains more than thirteen types of amino acid like lysine, leucine, methionine and serine [12]. Therefore, the present study is intending for evaluation and estimation of efficacy and tolerability of chamomile against pathogenic bacterial strains.

2. MATERIALS AND METHODS

This study was carrying out at Department of Clinical Pharmacology, College of Medicine, Almustansiriyia University, in cooperation with Department of Microbiology, College of Sciences, and Baghdad University in 2014, Iraq- Baghdad. This study confirmed and established by Scientific Jury and approved by the scientific committee board.

2.1 Preparation of Alcoholic Extract

Chamomile powder (*Matricaria chamomilla L.*) purchased from private pharmacies. Fifty grams of *chamomile* powder was extracted with 250 ml 10% ethanol by soxhelt device at 45Ċ, then the solvent reduced and concentrated via rotator evaporator at 45Ċ, this extract was reserved in deep freeze until the time of the experiment [13].

2.2 *In vitro* Determination of Antibacterial Activity of Alcoholic Extract of *Matricaria chamomilla* L.

Bacterial strains: five bacterial strains used in this study, were *Pseudomonas Aeruginosa (NCTC8313), Enterococcus faecalis (ATCC3223), Staphylococcus aureus (NCIM 2243), Escherichia coli (ATCC25922) and Klebsiella pneumoniae (NGIM2719)*.These microbial strains obtained from the National Chemical Laboratory (NCL), Iraq. These bacterial strains were inoculating in an appropriate infusion agar, incubated at 37Ċ, and poured into a sterile petri.

2.3 Assessment of Antibacterial Activity

- Disc diffusion test: according to the modified Kirby-Bauer method, one ml of plant extract balanced with 3 ml of 0.9% NaCl solution, then this dissolved in 10% dimethylsulfoxide to reach stock final concentration 30 mg/ml, this finally filtered via 0.45 µm milipore filters. Then each 100 µl contained 108 cfu/ml spilling over with 5 µg of chamomile alcoholic extract, which placed onto agar. Dimethylsulfoxide (DMSO) regarded as negative control which aided as 1% (v/v) that not affect the growth of microorganism, while tetracycline regarded as positive control, test plats cultures were incubated at 37Ċ for 24 hr, then a zone of inhibition was recorded by a ruler in mm, therefore, clear zone regarded as positive effect and reflect alcoholic extract antibacterial activity, this experimental method repeated three times [14].
- Agar well method: the inoculums of bacterial strains were prepared from 12 hr broth culture and accustomed to 0.5 Farland standard.

Microdilution method used for assessment and determination of MIC (minimal inhibitory concentration) and MBC (minimal bactericidal concentration) of chamomile alcoholic extract for bacterial strains that was sensitive and susceptible to this extract.

Eighteen wells plates used through dispensing 90 µl of broth plus 10 µl of inoculum into each well, bacterial growth measured via plating 5 µl from each clear zone of agar media. Thus, MIC regarded as the lower concentration of alcoholic extract that inhibit bacterial growth, while MBC equal to the minimal concentration when no possibly bacterial appeared in the culture [15].

2.4 Statistical Analysis

Results expressed and presented as numbers, mean±SD, the data was analysed through using unpaired t- test when p value < 0.05 as lowest limit of significance.

3. RESULTS

The *chamomile* alcoholic extract showed substantial antibacterial effects against both G+ and G- bacteria Table 1.

The *chamomile* alcoholic extract showed a higher action against *Klebsiella pneumonia*e by giving (35 mm±1.57) inhibition zone and lower effect alongside *Enterococcus faecalis* (10±1.43), but all the results were significant because these values near the result of active control.

Regarding MBC and MIC of *chamomile* alcoholic L. extract all results indicated significant antibacterial effects were most of MIC ranged between 8-32 mg/ml while MBC results ranged between 16-64 mg/ml and MIC and MBC values equivalent in the majority tested bacterial strains except *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* Table 2.

MIC values of *chamomile* alcoholic extract near values of tetracycline mainly against *Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus* Fig. 1.

While, MBC values of the *chamomile* alcoholic extract are in corresponding with tetracycline values similar to MIC, except that *chamomile* produced less corresponding values with tetracycline against *Enterococcus faecalis* and *Pseudomonas aeruginosa* Fig. 2.

4. DISCUSSION

Recently convey for novel plants and herbs with antibacterial activity have been the topic of many investigations because their essential oils with antibacterial activity may possibly be the competent agents for treatment of resistant bacteria [16-19].

An abundant biological event has assigned for *chamomile*; such assessment encompasses antibacterial, antifungal and anti-inflammatory effects [20]. The present study showed significant antibacterial effects of *chamomile* extract this supported by Annuk et al. [21] Study 1999 which revealed that alcoholic extract of *chamomile* at a concentration of 2-2.5 mg/ml exterminate aerobic organism and inhibit growth of *Escherichia coli*.

Chamomile extracts inhibit the production of urea via *H. pylori* and block adhesions of this microorganism via phospholipid lecithin [22].

Moreover, Tory et al. [23] study revealed that antiviral effects of *chamomile* against poliovirus and herpes virus also, locations and esters of *chamomile* exhibited significant anti-mycobacterial effects mainly against *Mycobacterium avium*.

Table 1. Zone of inhibition (mm) of *chamomile* alcoholic extract in comparison with positive control

Bacterial types	*Matricaria chamomilla*	Tetracycline	P value
S.A	23±0.83	22±0.82	>0.05*
E.C	13±0.54	15±1.03	>0.05*
P.A	16±0.07	22±1.14	>0.05*
K.P	35±1.57	26±0.72	>0.05*
E.F	10±1.43	11±1.02	>0.05*

**P value nonsignificant S.A = Staphylococcus aureus, E.C= Escherichia coli, P.A =Pseudomonas aeruginosa, K.P= Klebsiella pneumoniae, E.F= Enterococcus faecalis*

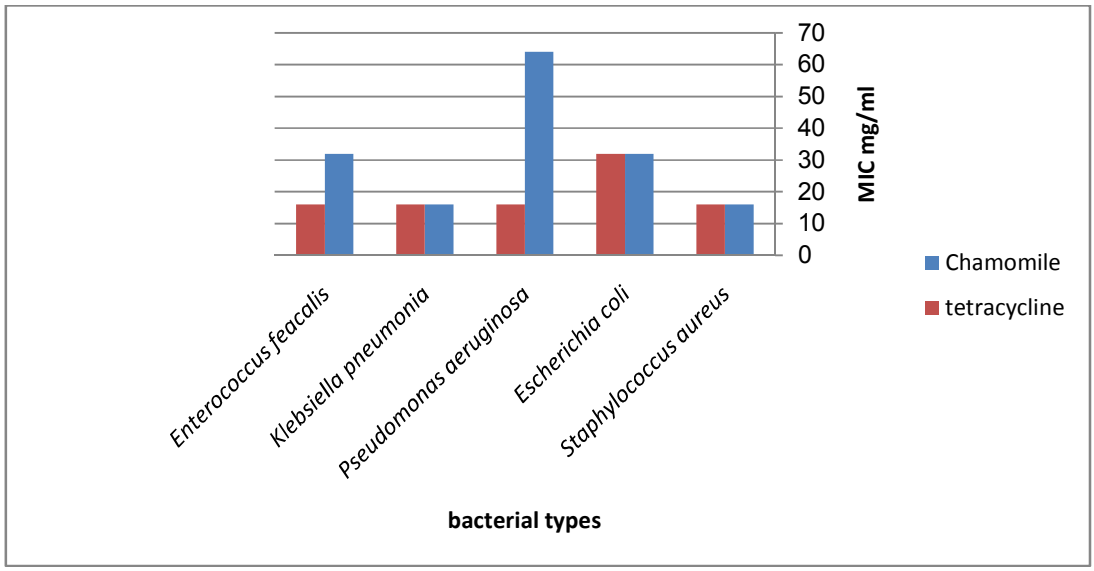

Fig. 1. MIC of alcoholic *chamomile* extracts in as compared with positive control (tetracycline) against different bacterial strains

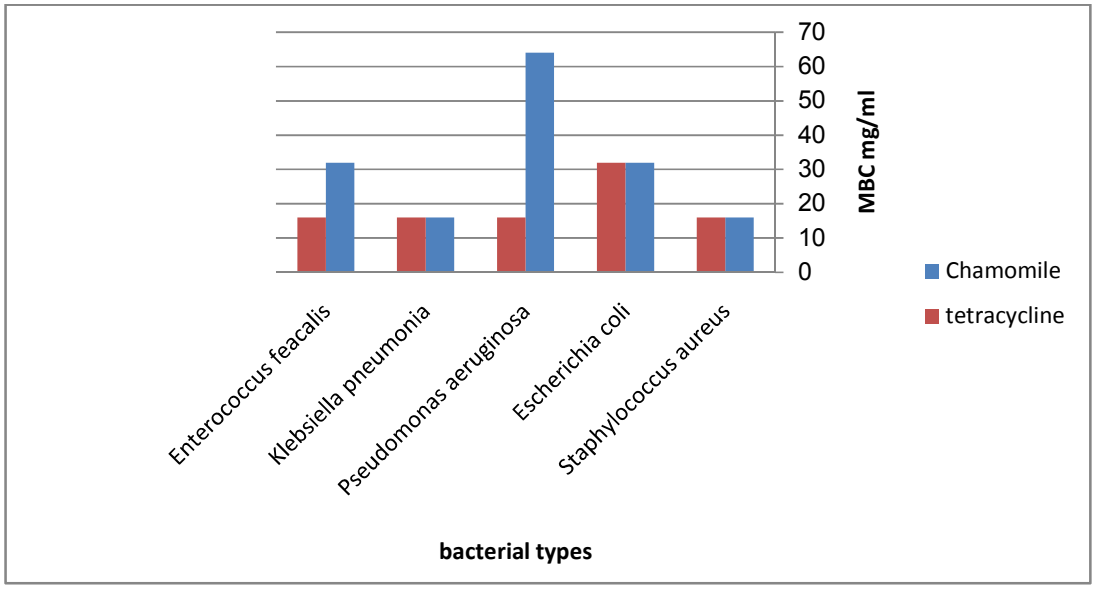

Fig. 2. Minimal bactericidal concentration (MBC) of alcoholic *chamomile* extracts in comparison with the positive control (tetracycline) against different bacterial strains

Additionally, Berry, a 1995 study indicated that alcoholic extracts of *Matricaria chamomile L.* in concentration of 25 mg/ml established noteworthy bactericidal effects against, *Streptococcus mutans, Bacillus subtillis, and Staphylococcus aureus,* in addition to a few fungicidal activities against *Candida albicans* [24].

Table 2. MIC and MBC of *chamomile* alcoholic extract (mg/ml)

Bacterial types	MIC	MBC
Staphylococcus aureus	16	16
Escherichia coli	32	32
Pseudomonas aeruginosa	32	64
Klebsiella pneumoniae	8	16
Enterococcus faecalis	32	32

Consequently, most of preceding studies corresponded with present study results.

Thus, the mechanism of antibacterial effect of *chamomile* is not well defined but may be linked to its active constituents and may be related for α- bisabolol which was effective at a low concentration alongside different pathogenic bacteria like *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis,* and *Pseudomonas aeruginosa* that are resistant against typical anti-infective agents [25] while dicycloethers, showed bacteriostatic activity at 100 mg/ml concentration, additionally chamazulene produced significant antifungal effect [26]. So, chamomile gave dual antimicrobial action bactericidal via chamazulene and bacteriostatic via bisabolol.

As well as, most of protein synthesis inhibitors are bacteriostatics like tetracycline and this is equivalent with values of *chamomile* and tetracycline so the antibacterial effects may be due to bisabolol that act as bacteriostatic. The results suggest that *chamomile* oil and alcoholic extracts have significant bacteriostatic antimicrobial activity [27]. So, the *chamomile* may be bacteriostatic via protein synthesis inhibition [28].

The broad-spectrum antibacterial effects of *chamomile* may be through the capability of it to interrupts cell wall permeability barrier and inhibition of cell membrane enzymes, the sesquiterpenoid compounds of *chamomile* like bisabolol have ability for interruption of G+ bacterial cell membrane this can be explained via the presence of the outer membrane in gram-negative bacteria and it has been shown that when permeabilising agents used in combination with *chamomile* the MBC standards of *chamomile* alongside usually liberal *Pseudomonas aeruginosa* strains are comparable for sensitive strains. Moreover, it has been defined that normally active constituent in the *chamomile* extracts revealing significant activity against bacterial cell membrane, leading to increasing in the outer cell membrane diffusion which allowing accumulation of toxic levels of monoterepenes within cytoplasm which induced bacterial cell membrane expansion ,augmentations of membrane fluidity and inhibition of bacterial cellmembrane-embeded enzymes [29-33]. this may explained the antibacterial activity of *Matricaria chamomile* in the present experimental work.

Furthermore, bisabolol inhibits 5-lipoxygenas and cyclooxygenase so *chamomile* have antipyretic effects which prevent bacterial and fungal induced pyrexia in animal model study , and other studies demonstrated that bisabolol inhibit stress and indomethacin-induced ulcer and because azulenes of chamomile active constituents, has anti-inflammatory effect via suppression of histamine and 5-hydroxytryptamine releasing, reduction in capillary permeability and anti-hyaluronidase activity [34].

Therefore, chamomile active constituents (bisabolol) inhibit cyclooxygenase and this may simulate the antibacterial activity of aspirin and diclofenac via inhibition of DNA synthesis in bacterial cells [35,36].

Moreover, *chamomile* active constituents inhibit creation and production of *Aspergillus* aflatoxin and *Trichothecene* mycotoxin [37].

So, the antibacterial effects of chamomile may directed by way of its active constituents or indirectly through action at the receptor level and this need in vivo study to verify these speculations.

5. CONCLUSION

Chamomile possesses significant and potent antibacterial activity against various bacterial strains like *Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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