

## Synergistic Interaction between Chamomile Flower (*Matricaria chamomilla* L.) Extracts and Tetracycline Against Wound Infection Bacteria

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### Abstract

*In vitro* antibacterial activity of Aqueous, ethanol and methanol flower extracts of chamomile alone and in combination with tetracycline by the use of agar well diffusion method against *Staphylococcus aureus* as gram positive bacterium and *Escherichia. coli*, *Proteus sp.* and *Klebsiella sp.* as gram negative bacteria was studied. It was found that chamomile aqueous extract has no inhibitory effects against gram positive and gram negative bacteria. Ethanolic and methanolic extracts exhibited antibacterial activity against *S. aureus* at the concentration 1000mg/ml. The diameter of the inhibition zones of *S. aureus* was 15 mm for Ethanol extract and 12mm at for methanol extract. No antibacterial effect on *E. coli*, *Proteus* and *Klebsiella* for the two extracts. The synergistic effect between the combination of tetracycline with chamomile extracts (ethanol and methanol) (vol./vol.) on *S. aureus* was studied. It was found that all the combinations proved strong inhibitory effect against *S. aureus* with different inhibition zone diameters ranged from 23 mm to 35 mm.

### 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]. Chamomile (*Matricaria chamomilla* L.) is one of the most widely used and well-documented medicinal plants in the world [4]. The use of chamomile as a medicinal plant dates back to ancient Greece and Rome. The name "chamomile" comes from two Greek words meaning "ground apple" for its apple-like smell [5]. *In vitro* data: The antiparasitic, and antiviral effects of chamomile have been well documented [6,7]. Compounds in the essential oil of chamomile were effective against Gram-positive and Gram-negative bacteria [8,9]. Synergism has been defined as phenomena on in which two different compounds are combined to enhance their individual activity. If the combination results in worsening effect, it is called antagonism; effect which is less than synergistic but not antagonistic is termed as additive or indifference [10]. This study investigated the inhibitory activity of aqueous, ethanolic and methanolic extracts of chamomile flower against bacterial pathogens that are implicated in wound infections. The

effect of combinations of the extracts and tetracycline on their resistance modifying potencies was also studied to explore for new bioactive molecules of pharmaceutical importance in the management of wound infections.

### Materials and Methods

#### Tested bacteria

The pure cultures of four bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Proteus sp.* and *Klebsiella sp.*) were isolated from wound infection of Al-Yarmouk teaching hospital, Baghdad city during the period from November/2010 to March/2011. They were maintained on Brain Heart Agar slants, and stored until use.

#### Plant collection and Extracts preparation

The plant was collected from herbarium (grassy) in Iraq. It was air dried and packed in plastic containers until used. About 50 gm of plant powder was mixed with 250 ml of sterilized distilled water, 80% methanol and 75% ethanol. After 24 hrs, the extracts were filtered through Whatman No.1 filter paper and concentrated to dryness and kept in labeled dark bottle at 4°C until used.

#### Determination of interaction between chamomile extracts and tetracycline

Tetracycline capsule (250 mg) (SDI) was used in this study. Solutions of known concentrations (250 mg/ml, 1000 mg/ml)

of tetracycline and chamomile extracts, respectively, were prepared.

The antimicrobial activity was determined by the well diffusion method [11]. Wells (6mm) were made in Brain Heart Agar for bacteria. Plates were seeded with 0.1 ml of  $10^8$  CFU/ml of bacteria, and then 0.1 ml of chamomile extracts and tetracycline were added alone to the wells, and then in combination (vol. /vol.) were added. Duplicates of each concentration for each microorganism species were prepared. The inoculated plates were incubated at 37 °C for 24 hrs. The diameter of the inhibition zones were measured for each plate.

### Results and Discussion

Numerous researches showed that the essential oils and plant extracts have potential in medical procedures and applications in the pharmaceutical, cosmetic and food industry [12]. In the present investigation the inhibitory effect of tetracycline, a broad spectrum antibiotic, and chamomile extracts were investigated against four bacteria (*S. aureus*, *E. coli*, *Proteus sp.* and *Klebsiella sp.*) by using agar well diffusion method. After 24 hrs of incubation at temperature of 37°C it was found that chamomile aqueous extract has no inhibitory effects against Gram positive and Gram negative bacteria. Methanolic and Ethanolic extracts exhibited antibacterial activity against *S. aureus* at the concentration 1000mg/ml. The diameter of the inhibition zones of *S. aureus* was 15 mm for Ethanolic extract and 12mm for methanolic extract. No antibacterial effect on *E. coli*, *Proteus* and *Klebsiella* for the two extracts were observed as shown in Table (1). These results were agreed with other two studies demonstrated that Gram-positive bacteria were more susceptible than Gram-negative bacteria to chamomile oil (7). It was found that most effective against *S. aureus* bactericidal activity [13]. As is the chamomile constituent bisabolol at concentrations of at least 1mg/ml. Alpha-Bisabolol, luteolin, quercitin, and apeginin have been theorized to possess antibacterial properties [14]. From Table (1) the inhibition zone of tetracycline for *S. aureus*, *Proteus sp.* *E. coli* were (15 mm) and *Klebsiella sp.* was (17mm), which considered intermediate sensitive, *E. coli* was

sensitive (20mm) to this antibiotic according to NCCL (11). Based on the previous results the synergistic effect between the combination of tetracycline with chamomile extracts (methanol and ethanol) (vol./vol.) on *S. aureus* was studied, as shown in Table (2). It was found that all the combination proved strong inhibitory effect against *S. aureus* at different inhibition zone diameters ranged from 23 mm to 35 mm. The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms either by inhibiting the cell wall synthesis or by causing its lyses or death [15].

Combinational antibiotic therapy to control infections is a viable approach, but will not be effective for a long period of time because of the possible alteration in the susceptibility of bacteria [16].

Therefore, the development of new classes of antimicrobial compounds is of significant importance. One possible approach is to determine whether bioactive compounds from natural products and traditional medicinal plants, which have strong bactericidal activity against pathogenic microorganisms, either show synergistic interaction with antibiotics or enhance the susceptibility level of resistant strains to antibiotics [17]. The synergistic effect from the combination of antibiotics with plant extracts against resistant bacteria leads to new ways for the treatment of infectious diseases. The increasing and indiscriminate use of antibiotics has led to the development of bacterial resistance to antibiotics. The use of synergistic combinations in antimicrobial chemotherapy is often used commercially for the treatment of various infections [18]. Previous *in vitro* studies have reported synergistic effect of combinations of different plant-derived pure compounds, such as baicalin, tellima-grandin I and rugosin B, and known antibiotic  $\beta$ -lactams against MRSA strains [19-21]. Synergistic effect between plant-derived compounds and antibiotics enables the use of the respective antibiotics when their effectiveness as single agents are reduced [22]. Data from the literature as well as our results reveal the potential of plants for therapeutic treatment. Therefore, more studies need to be conducted to search for such

compounds and plant extracts before being used in new therapeutic treatments, should have their toxicity tested *in vivo*.

**Table (1)**  
**Effect of Chamomile extracts, Tetracycline on Wound infection bacteria.**

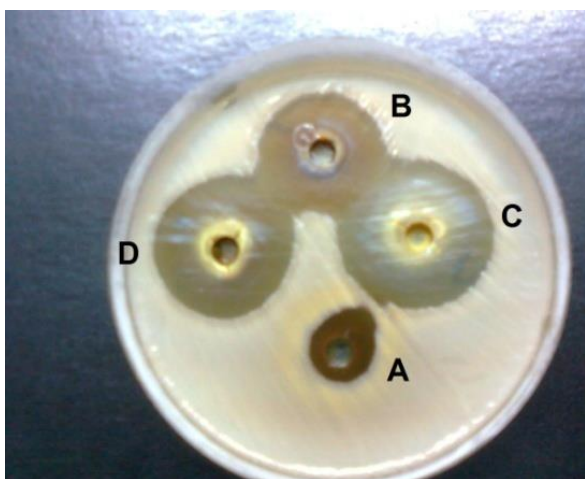
Plant extract	Concentration mg/ml	Diameter of inhibition zones (mm)			
		<i>S. aureus</i>	<i>E. coli</i>	<i>Proteus sp.</i>	<i>Klebsiella sp.</i>
Aqueous extract	250	-	-	-	-
	500	-	-	-	-
	1000	-	-	-	-
Ethanol extract	250	-	-	-	-
	500	-	-	-	-
	1000	15	-	-	-
Methanol extract	250	-	-	-	-
	500	-	-	-	-
	1000	12	-	-	-
Tetracycline	250	15	20	15	17
*Control (-)	-	-	-	-	-

(-): no inhibition zone, \*: sterilized distilled water

**Table (2)**  
**Synergistic interaction of Chamomile extracts with Tetracycline.**

Bacteria	vol./vol. ( $\mu\text{l}/\mu\text{l}$ )	Diameter of inhibition zones for	
		Ethanol extract/ tetracycline	Methanol extract/ tetracycline
<i>Staphylococcus aureus</i>	75/25	31.5	35
	50/50	31.25	34.5
	25/75	33	23
*Control (-)	-	-	-

\*: sterilized distilled water



**Fig. (1) Synergistic interaction of Chamomile methanolic extract and Tetracycline.**  
A: methanol extract only. B: 25/75 methanol extract/tetracycline. C: 75/25 methanol extract/tetracycline. D: 50/50 methanol extract/tetracycline.

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## الخلاصة

درست الفعالية التثبيطية للبكتريا خارج جسم الكائن الحي للمستخلصات المائية، الميثانولي والايثانولي لزهرة البابونج على حده ومع مضاد التتراسايكلين باستخدام طريقة الأنتشار بالحفر ضد البكتريا *Staphylococcus aureus* كبكتريا موجبة لصبغة غرام وضد البكتريا *Klebsiella sp.* و *Proteus sp.* و *Escherichia coli* كبكتريا سالبة لصبغة غرام. حيث وجد أن ليس هنالك أي تأثير للمستخلص المائي ضد البكتريا الموجبة والسالبة، في حين اظهر المستخلصان الكحولي والميثانولي فعالية تثبيطية ضد البكتريا *S. aureus* عند التركيز ١٠٠٠ ملغم/مل حيث كان قطر التثبيط ١٥ ملم للايثانولي و ١٢ ملم للمستخلص الميثانولي. ولم يوجد أي فعالية تثبيطية للمستخلصين (الايثانولي والميثانولي) على البكتريا *E. coli*، *Proteus sp.* و *Klebsiella sp.* كما درس التأثير التآزري بين المستخلصين (الميثانولي والايثانولي) (vol./vol.) على البكتريا *S. aureus* حيث وجد أن تأثير تثبيطي قوي ضد بكتريا *S. aureus* وبأفطار تثبيط مختلفة تراوحت من ٢٣ ملم إلى ٣٥ ملم ولجميع التراكيز.