

**Antibacterial activity of *Borage officinalis* leaves extracts against
Gemifloxacin Resistance *Klebsiella pneumonia***

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Abstract

This study aimed to detect the activity of hot aqueous and hot alcoholic leaves extracts of *Borage officinalis* against Gemifloxacin -resistance *Klebsiella pneumonia* isolated from burns infections. Ten Gemifloxacin resistance *Klebsiella pneumonia* isolates collected from Al-Karama teaching hospital in Baghdad during the period of 10th of August 2023 to 10th of April 2024. All isolates were resistance to Gemifloxacin with diameter of inhibition ranged from (0-13) mm. Three concentrations (0.001, 0.05 and 0.1) mg/ml of hot aqueous and Hot alcoholic extract of *Borage officinalis* were studied. The hot aqueous extract gives an inhibition zone ranged from (3-28) mm. The hot alcoholic extract gives an inhibition zone ranging from (5-30) mm. In conclusion, as the first study to investigate the effect of both aqueous and alcoholic extract of borage plant against Gemifloxacin *K. pneumonia*. The result of both, the hot aqueous and alcoholic extract of *B. officinalis* were effective against Gemifloxacin resistant *K. pneumonia*. As the fact that it contains many active substances, including Rosmaric acid, alkaloids and tannins which effect on transmembrane enzymes in the cell membrane.

Keywords: Gemifloxacin, *Klebsiella pneumonia*, *Borage officinalis*, Multi-drug Resistance.

الفعالية التثبيطية لمستخلص اوراق نبات لسان الثور *Borage officinalis*

ضد *Klebsiella pneumonia* المقاومة للمضاد الحيوي Gemifloxacin

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مستخلص:

هدفت الدراسة الحالية الى تقييم كفاءة مستخلص اوراق نبات لسان الثور في تثبيط بكتيريا *Klebsiella pneumonia* المقاومة للمضاد الحيوي Gemifloxacin المعزولة من التهابات الحروق . جمعت عشر عزلات من بكتيريا *Klebsiella pneumonia* المقاومة للمضاد الحيوي Gemifloxacin من مستشفى الكرامة التعليمي في محافظة بغداد، وذلك خلال الفترة 10/ آب/ 2023 الى 10/ نيسان/ 2024. وكانت جميع العزلات مقاومة Gemifloxacin وتراوح اقطار التثبيط بين (0-13) ملم. درست فعالية ثلاث تراكيز من المستخلص المائي والكحولي الساخن لنبات لسان الثور وكانت التراكيز (0.1، 0.05، 0.001) ملغم/ مل . بينت نتائج الدراسة الحالية ان المستخلص المائي الساخن اعطى منطقة تثبيط تراوحت بين (3-28) ملم، في حين كانت منطقة تثبيط المستخلص الكحولي الحار بين (5-30) ملم. توصلت الدراسة ان كل من المستخلص المائي والكحولي لنبات لسان الثور كان لهما تأثير تثبيطي اعلى من التأثير التثبيطي ل Gemifloxacin ضد البكتيريا من نوع *Klebsiella pneumonia* لكونها حاوية على عدة مواد فعالة ومنها Rosmaric acid والقلويدات alkaloids و التانينات tannins والتي تكون ذات فعالية على الانزيمات المتنقلة عبر غشاء الخلية.

الكلمات المفتاحية: بكتيريا *Klebsiella pneumonia* ، نبات لسان الثور ، المقاومة المتعددة للمضادات الحيوية، المضاد الحيوي Gemi-floxacin .

Introduction

In medical science, the resistance of antibiotics by bacteria and fungi is one of the most important concerns in the medical world. The biologist Alexander Fleming was the first to examine the resistance in microorganisms [1–3]. In modern times and in some cases, drug resistance formed to fight the pathogenic microorganisms such as bacteria and fungi. Medical herbs used for many years to treat some cases caused by bacteria and fungi; they were also used in the pharmaceutical industry [4]. The emergence of chemistry in the twentieth century and the development of complex organic building systems led to an evolutionary step in the pharmaceutical industry and the use of medicinal plants instead [5]. But with the progress in production, new chemicals and antibiotics began to appear, and the side effects of these drugs gradually began to appear, and since the fifties of the last century, many bacteria show a high resistance to antibiotics [6]. All over the world, medical plants are used by human beings to treat harmful diseases that infect humans and animals [7]. One of the main problems in using antibiotics as therapeutic agents is

the resistance and the side effects of these drugs. Accordingly, after much research to detect the effect of plants, humans use them in many industries [8]. After penicillin discovery and the use of it as treatment, new generations of antibiotics introduced and used to heal many types of infections [9]. The overuse of drugs led to drug resistance by various types of bacteria [10–12]. This was the most important reason for the increasing use of medicinal products, as they are less dangerous, have lower prices, and are made of natural ingredients that are not harmful to the body compared to antibiotics [13–15]. Studies have shown that only about 10% of the 250,000 species of medicinal plants have been studied all over the world [16].

This study aimed to detect the activity aqueous and alcoholic extract of *B. officinalis* leaves against Gemifloxacin .resistant *K. pneumonia*

Methodology

Ten isolates of Gemifloxacin resistance *Klebsiella pneumonia*, isolated from burns infection, collected from Karama teaching hospital in Baghdad during the period from 10th of August 2023 to 10th of April 2024. All isolates

cultured on MacConkey agar to determine its morphological characteristics then identified with Biochemical tests and Vitek-2 system. Leaves of the *Borage officinalis* plant collected from the local markets in the city of Baghdad. Washed with distilled water to remove dirt and impurities, then left to dry at room temperature, crushed, and kept in the refrigerator until use. To prepare hot water extract, 60 g of leaf powder weighed and placed in Soxhlet using distilled water with a volume of 450 ml as a solvent at a temperature of 80 °C. The same method mentioned before used to prepare the hot alcoholic extract, but using an alcoholic solvent (ethanol) with a volume of 450 ml at a temperature of 60 °C [17]. To prepare the stock solution, as mentioned in [18] with little modification to concentrate the extract, 5 g of the powder were dissolved in 50 ml of distilled water to reach the concentration of 0.1 g/ml filtered with 0.2 Millipore filter paper, and several concentrations were prepared. Muller-Hinton agar used to detect the activity of both extracts. After inoculation of nutrient agar plates with previously prepared bacterial stock by using swabs, 5 mm wells were made in the plates inoculated and filled with

each extract, incubated for overnight, and the diameter of inhibition was calculated [19]. The antibiotic sensitivity test performed as mentioned in [20], and the results compared with the Clinical and Laboratory Standards Institute M100 32th edition 2022 from the Iraqi Ministry of Health.

Results and Discussion

The results of the current study showed that all isolates were resistant to the antibiotic Gemifloxacin after detecting their sensitivity to the antibiotic on Muller-Hinton agar. The diameter of the antibiotic for each of the isolates (2, 4, 5, 7) ranges between (3-9) mm, while the diameter of inhibition for each of the isolates (1, 3, 8, 9, 10) ranged between (10-13) mm. isolate (6) showed resistance to antibiotic as shown in Figure (1). The results of Alkaabi (2013) [21] indicate that *K. pneumonia* are the second most frequent pathogen in burn infections. The findings of Wu, *et al.* (2011) [22] indicated that *K. pneumonia* is one of the pathogens that could be acquired from nosocomial infections.

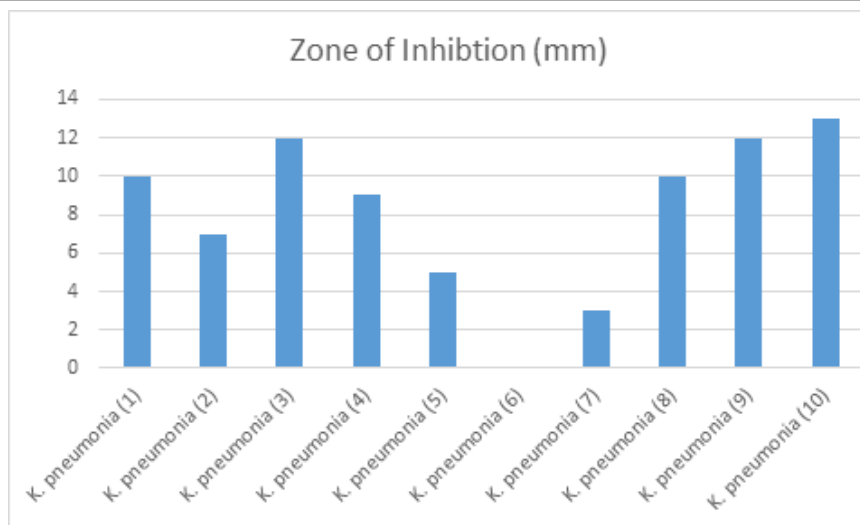


Figure (1): Gemifloxacin susceptibility by *K. pneumonia* isolates

The result concluded that the both hot aqueous and alcoholic extracts of *B. officinalis* leaves were effective against Gemifloxacin -resistant *K. pneumonia*. As shown in figure (2), the aqueous extract with concentration 0.001 g/ml was not effective in inhibiting bacterial isolate No. (6), as the diameter of its

inhibition was (0) mm, while the diameter of the inhibition zone was at the same concentration for the isolates (2, 3, 5, 7, 9, 10) are ranged between (3-7) mm. As for the isolates (1, 4, 8), the diameter of their inhibition was (10, 10, 11) mm, respectively.

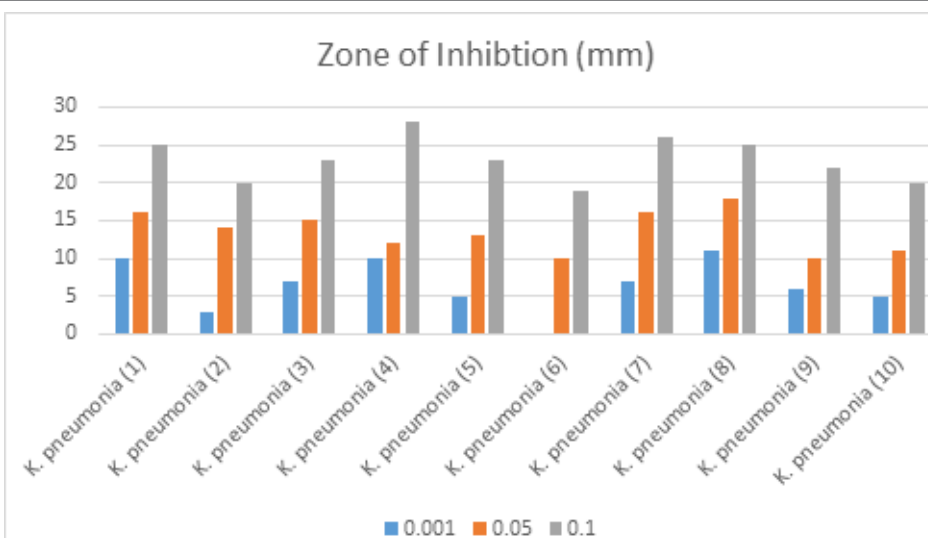


Figure (2): Antibacterial activity of hot aqueous extract of *B. officinalis*.

At the concentration of 0.05 g/ml, the inhibition diameter ranged between (10-18) mm. The concentration of 0.1 g/ml, the effectiveness of *B. officinalis* extracts increased in inhibiting all isolates. The lowest diameter of the inhibition area was (19) mm for isolate (6), while the highest diameter at the same concentration reached (28) mm for isolate (4). As for the hot alcoholic extract *B. officinalis*, the results showed that the concentration 0.001 g/ml showed an inhibition zone with a diameter ranging from (4-10) mm for each of the isolates (1, 3, 4, 5, 9, 10), and for isolates (2, 6, 7, 8) the diameter of its inhibi-

tion reached at the same concentration (12, 11, 17, 14) mm, respectively. The concentration of 0.05 g/ml of the alcoholic extract showed a diameter of inhibition ranging between (10-21) mm. As for the concentration of 0.1 g/ml, it was the most effective concentration of the alcoholic extract compared to the aqueous extract and the antibiotic Gemifloxacin. The lowest diameter of the inhibition zone at 0.1 g/ml reached (18) mm for isolate (9), while the highest diameter of inhibition at the same concentration reached 30 mm for the isolate (3) as shown in figure (3).

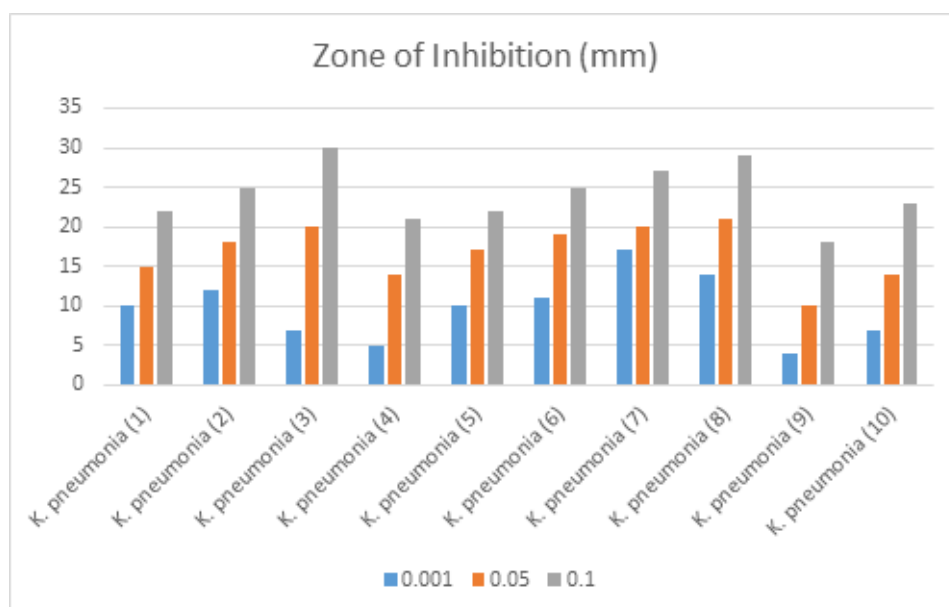


Figure (3) Antibacterial activity of Alcoholic extract of *B. officinalis*.

This study considers the first to investigate the effect of both aqueous and alcoholic extract of borage plant against Gemifloxacin *K. pneumonia*. Results of Abdullah *et al.* (2017) showed that *B. officinalis* aqueous extract was effective in inhibiting *Staphylococcus aureus* and the diameter of the inhibition ranged between (7-11) mm, and for *Streptococcus pyogenes* was between (5-10) mm, while the diameter of the inhibition of the aqueous extract of *Staphylococcus epidermis* was (5-7) mm [23]. They also indicates that alcoholic extract of *B. officinalis* gives an inhibition zone ranged from (6-10) mm. In addition, the results from [24], conducted to find out the effect of the *B. officinalis* plant on *Bacillus subtilis*, and their results show the effectiveness of the plant in inhibiting this bacteria. Another study [25] indicated the effectiveness of *B. officinalis* leaves extract against *Escherichia coli* and *Pseudomonas aeruginosa*. The effectiveness of the extracts in this plant is due to the fact that it contains many active substances, including rosmarinic acid, which was mentioned by [26], and it contains alkaloids and tannins which effect on transmembrane enzymes in the cell membrane, as well as soapy materials

that remove cell membranes [15, 23].

Conclusions

In conclusion as the first study to investigate the effect of both aqueous and alcoholic extract of borage plant against Gemifloxacin *K. pneumonia*, the result of both, the hot aqueous and alcoholic extract of *B. officinalis* were effective against Gemifloxacin resistant *K. pneumonia*. As the fact that it contains many active substances, including Rosmaric acid, alkaloids and tannins which effect on transmembrane enzymes in the cell membrane.

الاستنتاجات

وفي الختام، وكأول دراسة بحثية لدراسة تأثير كل من المستخلص المائي والكحولي لنبات لسان الثور ضد بكتيريا *K. pneumonia* المقاومة للمضاد الحيوي Gemifloxacin، فقد أظهرت نتائج كلتا الدراستين أن المستخلص المائي والكحولي الساخن لنبات لسان الثور كان فعالاً ضد بكتيريا *K. pneumonia* المقاومة للمضاد الحيوي Gemifloxacin، وذلك لاحتوائه على العديد من المواد الفعالة، بما في ذلك حمض الروزماريك والقلويدات والعفص التي تؤثر على الإنزيمات عبر الغشائية في غشاء الخلية.

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