



The efficacy of *Dianthus caryophyllus* leave extracts against some Bacterial species

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فعالية مستخلصات أوراق القرنفل ضد بعض الأنواع البكتيرية

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Abstract:

the average diameters and areas of inhibition zones indicative of growth suppression by *Dianthus caryophyllus* extracts were systematically analyzed against *staphylococcus aureus*. Streptomycin exhibited the most significant antibacterial effect, surpassing plant extracts in efficacy. Notably, the first extract of *D. caryophyllus* leaves demonstrated the highest antibacterial activity with a diameter area of 17.88 ± 3.72 , while extract 3 showed a diameter area of 7.88 ± 2.57 , with a statistically significant variation at ($P < 0.05$). The average diameters and areas of growth inhibition zones produced by *D. caryophyllus* extracts against *pseudomonas sp* bacteria were accurately recorded for analysis. Among the tested agents, Gentamycin exhibited the most pronounced effect, achieving a diameter measure of 20.88 ± 6.03 . The first extract of *D. caryophyllus* leaves yielded a diameter area of 13.44 ± 1.23 , while the third extract showed a lower diameter area of 6.88 ± 1.36 , with a notable variation at ($P < 0.05$). This evaluation involved systematic determination of average diameters and areas of growth inhibition zones elicited by the extracts against *Klebsella sp* bacteria. Streptomycin displayed the most significant antibacterial effect, with an impressive diameter of 21.77 ± 2.77 . The first extract of *D. caryophyllus* leaves generated a significant diameter area of 12.55 ± 1.94 , while extract 3 exhibited a lower diameter area of 6.44 ± 2.24 , both showing a statistically significant variation at ($P < 0.05$).

Keywords: *D. caryophyllus*, extracts, Bacteria, antibiotic.

الملخص

تم قياس متوسط أقطار ومساحات مناطق التثبيط التي تُشير إلى تثبيط النمو بواسطة مستخلصات القرنفل (*Dianthus caryophyllus*) بشكل منهجي ضد بكتيريا المكورات العنقودية الذهبية. أظهر الستربتوميسين التأثير المضاد للبكتيريا الأكثر أهمية، متفوقاً على المستخلصات النباتية من حيث الفعالية. والجدير بالذكر أن المستخلص الأول لأوراق القرنفل أظهر أعلى نشاط مضاد للبكتيريا بمساحة قطر 17.88 ± 3.72 ، بينما أظهر المستخلص الثالث مساحة قطر 7.88 ± 2.57 ، مع وجود فرق ذي دلالة إحصائية عند ($P > 0.05$). تم تسجيل متوسط أقطار ومساحات مناطق تثبيط النمو التي تنتجها مستخلصات القرنفل ضد بكتيريا الزائفة الزنجارية بدقة للتحليل. من بين العوامل المختبرة، أظهر الجنتاميسين التأثير الأكثر وضوحاً، محققاً مقياس قطر 20.88 ± 6.03 .

أظهر المستخلص الأول لأوراق نبات القرنفل مساحة قطرها 13.44 ± 1.23 ، بينما أظهر المستخلص الثالث مساحة قطرها أقل بلغت 6.88 ± 1.36 ، مع تباين ملحوظ عند ($P > 0.05$). تضمن هذا التقييم تحديداً منهجياً لمتوسط أقطار ومناطق تثبيط النمو التي أثارها المستخلصات ضد بكتيريا كليبسيلا. أظهر الستربتوميسين التأثير المضاد للبكتيريا الأكثر أهمية، بقطر بلغ 21.77 ± 2.77 . أما المستخلص الأول لأوراق نبات القرنفل، فقد أظهر مساحة قطرها أقل بلغت 12.55 ± 1.94 ، بينما أظهر المستخلص الثالث مساحة قطرها أقل بلغت 6.44 ± 2.24 ، وكلاهما يُظهر تبايناً إحصائياً ذا دلالة إحصائية عند ($P > 0.05$).

الكلمات المفتاحية: مستخلصات، القرنفل، بكتيريا، تضاد حيوي.

Introduction

Carnations and pinks are generally names for several species of the genus *Dianthus*, which belongs to the Caryophyllaceae. The family contains 80 genera and 2,000 species which are either annual or perennial and majority of these herbs occur in the northern hemisphere. Over 300 *Dianthus* species have been known (Galbally and Galbally 1997, Jurgens *et al.* 2003). The carnation flower fragrance is predominantly due to eugenol, β -caryophyllene and benzoic acid derivatives. The cultivar 'Eliat', indicated that the level of these compounds rises during flower development and coincides with a rise in flower fragrance (Zuker *et al.* 2002).

Clove extracts have demonstrated broad-spectrum antimicrobial activity, making them suitable for use in food preservation. They are effective against common foodborne pathogens and spoilage microorganisms, which are critical targets in the food industry (Pinto *et al.*, 2023). While the antimicrobial properties of carnation extracts are promising, it is important to consider the broader implications of using plant-based antimicrobials. The development of resistance to plant-derived compounds, although less common than with synthetic antibiotics, remains a potential concern. Additionally, the variability in the efficacy of plant extracts due to environmental factors and extraction methods necessitates further research to standardize and optimize their use. Nonetheless, the integration of carnation extracts into agricultural and medical applications represents a sustainable and effective strategy for managing microbial threats (Cheng *et al.*, 2022). The antimicrobial activity is likely due to the production of secondary metabolites by these rhizobacteria, which can disrupt bacterial cell walls or interfere with their metabolic processes, leading to inhibited growth or death of the pathogens (Sharma & Kaur, 2010).

Carnation essential oil, rich in eugenol, exhibits significant antimicrobial properties, effectively inhibiting pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus*. The study demonstrated that eugenol concentrations (0.2 to 0.8 $\mu\text{l/ml}$) significantly reduced bacterial growth, while it did not affect *Saccharomyces cerevisiae*. The addition of eugenol enhanced the survival of lactic acid bacteria in yoghurt, with the most effective concentration being 0.6 $\mu\text{l/ml}$, which prolonged yoghurt shelf life without microbial spoilage (Assem *et al.*, 2019).

Specific concentrations of aluminum sulfate, 8-hydroxyquinoline sulfate, and 12% essential oils from *Artemisia* and *Anethum* can significantly extend the vase life of cut carnations by reducing ethylene production and bacterial contamination, and by promoting better water uptake. Hydroxyquinoline sulfate and aluminum sulfate on cut carnation flowers. These treatments reduce bacterial colonies at the stem end, minimizing ethylene production and enhancing water uptake, which ultimately prolongs vase life. The study highlights the importance of these compounds in managing bacterial contamination rather than detailing the antimicrobial properties of carnation extracts themselves (Hashemabadi *et al.*, 2015).

The data on antimicrobial susceptibility highlights considerable heterogeneity in antibiotic efficacy among various bacterial species, with Augmentin (AMC) demonstrating robust effectiveness, particularly against *E. coli*, *Klebsiella* spp., and *Staphylococcus aureus*. Conversely, Oxacillin (OX) and Ofloxacin (OFX) often revealed substantial resistance, emphasizing the necessity for vigilant monitoring of local resistance trends and the prioritization of targeted therapeutic strategies informed by culture and sensitivity assessments (Elkouly *et al.*, 2025a, , Elkhoully, 2025b)

Material and methods

The current investigation elucidates the *in vitro* antimicrobial prowess of Carnation leaf extracts against three distinct bacterial species.

Samples were gathered from various origins (Ahtwesh ornamental center- Surman). The samples were air-dried to perfection. Using an electrical crusher, the samples were finely ground. The pulverized material was rinsed and stored in specialized bags. The extraction process involved both water and alcohol, adhering to the following protocol: Water extract: 20 grams of the plant leaves were dissolved in 200ml of water. Alcohol extract: 20 grams of the plant leaves were dissolved in 200ml of ethyl alcohol for a duration of 24 hours. The solutions underwent filtration, and the extraction was centrifuged to segregate its constituents. The extraction process was facilitated by a rotary evaporator under pressure at 55°C. The water and alcoholic solvents were evaporated, leaving the extracted compounds concentrated as a stock. The balance was established by weighing an empty container, to which 3 grams of the extraction were added and left for 24 hours before being refrigerated until needed. The Carnation extracts were prepared in a descending series of concentrations in the following order: 100, 50, and 25 mg/ml. The MIC represents the minimal concentration of the antimicrobial agent that entirely halts growth. The antimicrobial efficacy was assessed by measuring the diameter of the inhibition zone. Three antibiotics were scrutinized in relation to their effectiveness against bacterial growth activity. Streptomycin, Ampicillin and Gentamycin were evaluated in comparison with the three leaves extracts concentration sdiscrped.

Starting solution (Stock)

The concentration of the stock solution is quantified as 1 g in 100 ml (extract1), which equates to 10 mg/mL (1% w/v).

Dilution 1 — to 50 mL (1:50) (extract 2)

Designate a 50-mL volumetric tube or flask with the label "Dilution 1 (1:50)". Using a pipette, transfer 1.00 mL of the stock solution into the designated tube. Subsequently, introduce a diluent, such as distilled water, until the

total volume reaches 50.0 mL, and proceed to mix the contents gently. The concentration can be calculated as follows ($C_1 \rightarrow C_2$): $C_2 = C_1 \times \left(\frac{V_1}{V_2}\right) = 10 \text{ mg/mL} \times \left(\frac{1.00}{50.0}\right) = 0.200 \text{ mg/mL}$, which corresponds to 0.02% w/v. The dilution factor is thus determined to be 50×

Dilution 2 — to 25 mL (additional 1:25; overall 1:1250)

Assign a label to a 25-ML (Extract 3) volumetric tube or flask stating “Dilution 2 (1:25 from Dilution 1)”. Employing a pipette, extract 1.00 mL of Dilution 1 and introduce it into the tube. Augment the volume with a diluent to achieve a final volume of 25.0 mL, followed by gentle mixing. The concentration is ascertained through the following calculation: $C_3 = 0.200 \text{ mg/mL} \times \left(\frac{1.00}{25.0}\right) = 0.00800 \text{ mg/mL}$, equating to 0.0008% w/v, or 8 mg/L. The overall dilution factor is computed as $50 \times 25 = 1250\times$ from the stock.

Statistical analysis:

the analysis of variance, specifically termed as ANOVA single factor, was meticulously employed for the purpose of examining and interpreting the data derived from the study, utilizing a significance level set at ($P < 0.05$). In conjunction with this statistical method, both the means and the standard deviation were systematically utilized to facilitate a comprehensive analysis of the collected data pertaining to the study.

Results and discussion

The data that is meticulously presented in Table (1) illustrates that the evaluation of the antibacterial activity against *staphylococcus aureus* associated with the extracts derived from the leaves was comprehensively conducted. Furthermore, the average measurements pertaining to both the diameters and the areas of inhibition zones, which serve as indicators of growth suppression effects produced by the extracts of *D. caryophyllus* against a series of standard bacterial organisms, were systematically determined and analyzed. It is noteworthy to mention that the most pronounced antibacterial effect was achieved by the antibiotic known as Streptomycin, which exhibited superior efficacy ($23.00 \pm 2.23 \text{ mm}$) compared to the plant extracts. Conversely, it is of particular interest that the highest level of antibacterial activity attributed to the leaves dilution 1 of *D. caryophyllus* was documented, revealing a diameter area of effect quantified at $17.88 \pm 3.72 \text{ mm}$, while the dilution 3 showcased a diameter area of effect measured at 7.88 ± 2.57 , with a statistically significant variation established at ($P < 0.05$). These results are supported by those of *D. caryophyllus* extracts have demonstrated significant antimicrobial activity against *Staphylococcus aureus*, with minimum inhibitory concentrations (MICs) ranging from 62.5 to 2000 $\mu\text{g/mL}$, depending on the extract type and concentration used (Abdelkader and Halawani, 2014). on the other hand, Dababneh (2008) concluded that, *D.caryophyllus* extracts exhibited significant antimicrobial properties against *Staphylococcus aureus*, with a minimum inhibition concentration (MIC) of 2000 ppm, resulting in a diameter of inhibition zone (DIZ) of 20 mm. This indicates that the extract effectively inhibited the growth of this Gram-positive bacterium, showcasing its potential as a natural antimicrobial agent. The study highlights the efficacy of *D. caryophyllus* in combating pathogenic microorganisms, contributing to its potential use in pharmaceutical applications.

Table (1) diameter and area of the culture treated by antibiotics and extracts on *staphylococcus aureus*

Treatment	diameter of the culture (mm) \pm sd	N	F
Streptomycin	23.00 ± 2.23	9	25.00
Ampicillin	14.44 ± 2.69	9	
Gentamycin	12.77 ± 1.48	9	
Dilution 1	17.88 ± 3.72	9	
Dilution 2	12.66 ± 5.29	9	
Dilution 3	7.88 ± 2.57	9	

The data that has been meticulously compiled and presented in table (2) provides a comprehensive analysis indicating that the antibacterial activity against *pseudomonas sp* of the dilution extracts derived from the leaves has been systematically evaluated and examined in detail. Specifically, the average measurements concerning both the diameters and the areas of the growth inhibition zones that were produced by the extracts of *Dianthus caryophyllus* when tested against standard bacterial organisms were accurately determined and recorded for further analysis. Among the various antibacterial agents tested, the antibiotic known as Gentamycin exhibited the most pronounced effect, with a range of effect measured in terms of diameter yielding a remarkable value of (20.88 ± 6.03). Conversely, when considering the highest antibacterial effect observed from the first Dilution of *D. caryophyllus* leaves, it was found to correspond to a diameter area of effect measured at (13.44 ± 1.23), while the third Dilution demonstrated a significantly lower diameter area of effect measured at (6.44 ± 2.24), which was determined to have a notable variation with a statistical significance set at ($P < 0.05$). Several studies have demonstrated that plant extracts can significantly inhibit the biofilm formation of *Pseudomonas aeruginosa*. For instance, extracts from Indonesian medicinal plants showed inhibitory effects on both planktonic growth and

biofilm formation of *Pseudomonas aeruginosa* at low concentrations, indicating their potential as antibiofilm agents (Pratiwi *et al.*, 2015).

Table (2) diameter and area of the culture treated by antibiotics and extracts on *pseudomonas sp*

Treatment	diameter of the culture \pm sd	N	F
Streptomycin	16.00 ± 1.118	9	37.08
Ampicillin	5.66 ± 2.39	9	
Gentamycin	20.88 ± 6.03	9	
Dilution 1	13.44 ± 1.23	9	
Dilution 2	11.22 ± 1.20185	9	
Dilution 3	6.44 ± 2.24	9	

The data meticulously delineated within table (3) provides a comprehensive overview of the evaluative assessment conducted on the antibacterial efficacy of the extracts derived from the leaves of the plant species, specifically *D. caryophyllus* against *Klebsiella sp*. This assessment involved the systematic determination of the average diameters as well as the areas corresponding to the zones of growth inhibition that were elicited by the aforementioned dilutions when tested against standard bacterial organisms. Notably, the most pronounced antibacterial effect was observed with the application of the antibiotic Streptomycin, which demonstrated an impressive range for the diameter of its inhibitory effect, specifically measured at (21.77 ± 2.77). Conversely, when considering the antibacterial activity of the leaf dilution extraction categorized as dilution 1 from *D. caryophyllus*, it was found to have generated a significant diameter area of effect measured at (12.55 ± 1.94), while a further analysis of dilution 3 revealed it to exhibit a comparatively lower diameter area of effect quantified at (6.88 ± 1.36), with both extracts exhibiting a statistically significant variation at a probability level of ($P < 0.05$). Shaik *et al.*, (n.d.) reviewed various plants with antimicrobial properties against *Klebsiella pneumoniae*, including essential oil yielding plants. However, it does not specifically mention *D. caryophyllus* or its extracts in relation to their inhibitory effects on *Klebsiella sp*. Therefore, while the potential exists for bioactive compounds in *Dianthus caryophyllus* to exhibit such effects, they did not provide direct evidence or findings regarding this specific plant. Further research would be needed to confirm any inhibitory effects. Winnett *et al.*, (2017) focused on 106 extracts from 40 native Australian plant species, with significant inhibitory activity against *Klebsiella pneumoniae* observed in extracts from plants such as *Tasmannia lanceolata*, *Eucalyptus spp.*, and others. The study highlights the potential of these extracts in preventing and managing ankylosing spondylitis, but does not provide information on *D. caryophyllus* as a mean factor against *Klebsiella sp*

Table (3) diameter and area of the culture treated by antibiotics and extracts on *Klebsella sp*

Treatment	diameter of the culture \pm sd	N	F
Streptomycin	21.77 ± 2.77	9	72.30
Ampicillin	8.22 ± 2.43	9	
Gentamycin	7.66 ± 1.80	9	
Dilution 1	12.55 ± 1.94	9	
Dilution 2	9.55 ± 1.58	9	
Dilution 3	6.88 ± 1.36	9	

Conclusion:

the diameters and areas of inhibition zones indicative of growth suppression by *Dianthus caryophyllus* extracts were rigorously examined against *Staphylococcus aureus*. Streptomycin demonstrated superior antibacterial efficacy, with the first extract of *D. caryophyllus* leaves exhibiting the highest activity at 17.88 ± 3.72 mm. Similarly, evaluations against *Pseudomonas sp* and *Klebsiella sp* confirmed Gentamycin's prominent effect, with *D. caryophyllus* extracts displaying significant variations in inhibition zones at ($P < 0.05$).

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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