

Antibacterial properties of alcoholic and aqueous extracts of ginger and anise plants against various types of bacteria

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الخصائص المضادة للبكتيريا للمستخلصات الكحولية والمائية من نبات الزنجبيل واليانسون
ضد أنواع مختلفة من البكتيريا

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تاريخ النشر: 2025-10-20

تاريخ القبول: 2025-10-10

تاريخ الاستلام: 2025-09-07

المخلص:

تمت دراسة الخصائص المضادة للبكتيريا لكل من المستخلصات المائية والكحولية للزنجبيل (Zingiber officinale) واليانسون (Pimpinella anisum) ضد أربعة أنواع من البكتيريا المعزولة من مرضى التهابات المسالك البولية، وهي: المكورات الذهبية (Staphylococcus aureus)، والكليبيسيلا (Klebsiella)، والباسودوموناس (Pseudomonas)، والإشريكية القولونية (E. coli)، تم تحديد أنواع البكتيريا باستخدام الاختبارات الكيموحيوية. تقييم النشاط المضاد للبكتيريا لكل مستخلص تم إجراؤه باستخدام طريقة انتشار القرص، أظهر المستخلص الخلوي للزنجبيل أعلى فعالية ضد المكورات الذهبية (قطر منطقة تثبيت النمو 0.36 مم)، تليها الإشريكية القولونية (0.11 مم)، بينما لم يظهر أي تأثير على الباسودوموناس والكليبيسيلا. أما المستخلص المائي للزنجبيل، فقد أظهر أعلى فعالية ضد الإشريكية القولونية (0.11 مم)، دون أي تأثير على الأنواع الأخرى. أظهر المستخلص الخلوي لليانسون أعلى فعالية ضد المكورات الذهبية (0.46 مم)، تليها الإشريكية القولونية (0.44 مم) والباسودوموناس (0.2 مم)، دون أي تأثير على الكليبيسيلا. أما المستخلص المائي للكمون، فقد أظهر أعلى فعالية ضد الإشريكية القولونية (0.25 مم) والكليبيسيلا (0.23 مم)، دون أي تأثير على المكورات الذهبية والباسودوموناس.

الكلمات الدالة: النشاط المضاد للبكتيريا - اليانسون والزنجبيل - طريقة انتشار القرص البكتيري - بكتيريا القولون (E. coli).

Abstract

Aqueous and alcoholic extracts of ginger (*Zingiber officinale*) and cumin (*Pimpinella Anisum*) were investigated For their antibacterial activity against four bacterial isolates. The bacteria isolated from UTI patiens , they were *Staphylococcus aureus* , *klebsiella*, *pseudomonas* and *E.coli* the bacterial isolates were identified using biochemical tests , the antibacterial actions or tasks performed by each extracts was evaluated by the disc diffusion method the alcoholic extract of ginger (*Zingiber officinale*

) showed a highest effect on *Staphylococcus aureus* with inhibition zone diameter 0.36mm folloed by *E.coli* with inhibition zone diameter 0.11mm and there is no any inhibition zone on *pseudomonas* and *klebsilla* , the aqueous extract of ginger (*Zingiber officinale*) showed a highest effect on *E.coli* with inhibition zone diameter 0.11mm while there is no inhibition zone on *Staphylococcus aureus*, *pseudomonas*, and *klebsilla* . The alcoholic extract of cumin (*Pimpinella Anisum*) showed a highest effect on *Staphylococcus aureus* with inhibition zone diameter 0.46 mm folloed by *E.coli* with inhibition zone diameter 0.44mm , , *pseudomonas* with inhibition zone diameter 0.2mm and there is no any inhibition zone on *klebsiella*.

the aqueous extract of cumin (*Pimpinella Anisum*) showed a highest effect on *E.coli* with inhibition zone diameter 0.25mm ,and *klebsiella* with inhibition zone diameter 0.23mm while there is no inhibition zone on *Staphylococcus aureus* and *pseudomonas*.

Keywords: Antibacterial activity - Disc diffusion method- *E. coli* - *Pimpinella anisum* (Cumin) and *Zingiber officinale* (Ginger)

Introduction:

Medicinal plants serve as valuable sources of potent compounds and have been utilized for therapeutic purposes across various regions. According to the World Health Organization, these plants hold significant potential as a key resource for developing a wide range of drugs. There is a need for ongoing research to gain deeper insights into their properties, efficacy, and safety. Numerous medicinal plants are recognized for their antimicrobial activities and have been widely applied in this context (okme *et al.*, 2017).

Historically, aromatic and higher plants have been utilized in traditional medicine and as natural preservatives to prolong food shelf life, demonstrating their ability to inhibit bacteria, fungi, and yeasts. (Bupesh *et al*, 2007).

The growing reliance on natural sources for medicines has resulted in the process of extracting and developing a range of drugs and chemotherapeutic agents from traditional herbal sources.. Several foods also possess antibiotic properties, often unrecognized by consumers, which help inhibit the growth of bacteria within the body. Additionally, numerous plants have long been utilized for their antimicrobial effects. (Lucky *et al*, 2017).

Medicinal plants have been integral to humanity's well-being for centuries. According to the World Health Organization (WHO), approximately 80% of the global population primarily depends on traditional remedies, which often involve the use of plant extracts or their active compounds(Emhmd *et al*, 2017). These plants play a vital role in traditional healthcare systems, offering effective solutions for curing numerous ailments. Their medicinal value lies in specific chemical constituents that induce targeted physiological effects on the human body(Lamma *et al*, 2019). Among these plants, ginger stands out as an essential species with remarkable medicinal, ethnomedicinal, and nutritional properties(Alhadad,2022). It is derived from the underground rhizome of the ginger plant, characterized by its firm and striated texture. Scientifically known as *Zingiber officinale* Roscoe, ginger belongs to the family *Zingiberaceae*. Initially developed in China, it later spread to India, Southeast Asia, West Africa, and the Caribbean. (Gupta & sharma,2014).

Aromatic plants like anise seeds have a long-standing history of use in both traditional and conventional medicine, as well as in the pharmaceutical industry. Anise (*Pimpinella anisum*) is an herbaceous annual plant native to the Mediterranean region, cultivated mainly for its fruits and seeds. It is reported to also be indigenous to Iran and Turkey (Sun *et al.*, 2019).

The use of aromatic anise seeds dates back nearly 5,000 years, though pinpointing the first recorded instance of its utilization remains challenging. Some researchers suggest its origins lie in ancient Egypt, while others argue for Greece or Persia, which corresponds to modern-day Iran (Dumitrescu *et al.*, 2023). Anise belongs to the Apiaceae family and is among the oldest medicinal plants known in Iraq (Lamma& Moftah,2016). This family is recognized for its distinctive flavors, derived from essential oils and aromatic compounds. Research indicates these compounds can effectively combat microorganisms without encouraging the development of resistance (Awheda *et al* 2025) They exhibit low toxicity to

mammalian cells and are relatively easy to obtain. Additionally, they degrade quickly in water and soil, making them environmentally friendly options (Al-Wendawi et al., 2021).

2-Materials and Method

2.1. Isolation of bacteria

11 urine samples were collected aseptically from the middle of the urethra in a sterile scientific container and transported to a laboratory within 30 minutes. Each sample was cultured on a CLED agar plate and incubated at 37°C aerobically overnight. Identification of bacterial isolates. After incubation, the bacterial species were identified based on their morphological characteristics and biochemical tests. (Alsideeq et al., 2023)

2.1.1 Preparing Plant Extract

2.1.2 Aqueous Extracts:

80 grams of ground *Zingiber officinale* powder and *Pimpinella Anisum*. were added to 250 ml of distilled water each one separately. The mixture was thoroughly shaken and left at room temperature for 24 hours then filtered using filter paper to obtain the clear aqueous extract. (Alsideeq et al., 2023)

2.1.3. ALCOHOLIC EXTRACTS:

80 grams of ground *Zingiber officinale* powder and *Pimpinella Anisum*. were added to 250 mL of 70% methanol each one separately. The mixture was thoroughly shaken and left at room temperature for 24 hours then filtered using filter paper to obtain the clear alcoholic extract.



Figure: 1 *Zingiber officinale*



Figure: 2 *Pimpinella anisum*

2.1.4. ANTIBACTERIAL ACTIVITY TESTING:

The inhibition zone measurements were analyzed using appropriate statistical methods. The mean of the inhibition zones for each extract were calculated. The results were compared across different plant extracts and bacterial strains to determine the most effective extracts for inhibiting bacterial growth.

Testing the Efficiency of Aqueous Extract of Ginger and Cumin in Inhibiting the Growth of Bacteria. Four colonies of bacteria pure culture were taken using a sterile loop and incubated in a tube containing 10 ml of distilled water. The tube was shaken by rocking before 0.100 ml of bacterial culture was placed on the surface of the plate containing nitrate agar and distributed by swab on the surface of the media. The plates were left for 15 minutes to absorb the culture medium.

Three replicates were made for each isolate. Then, discs of filter paper with a diameter of 6 mm were taken. Each group of discs was placed in a kind of extract and left for 10 minutes to be imbibed with the immersed extract. The discs were then lifted using forceps and distributed on the dishes grown with bacterial isolates. The dishes were incubated at 37°C for 24 hours. (Alsideeq et al., 2023)

The results were recorded by measuring the diameters of the inhibition zones in millimeters. The method was repeated for all the isolates under study.

3.RESULTS:

Table 1: Bacterial isolates Four types of bacteria were isolated: *Staphylococcus aureus*, *Klebsiella*, *Pseudomonas*, and *E.coli*

Bacterial isolates	Colony morphology
<i>Staphylococcus aureus</i>	Colonies are small to medium circular in shape yellow in color with a smooth surface moist texture and elevated in height
<i>Klebsiella</i>	Colonies are large in size circular in shape with defined edges creamy white in color smooth in surface slightly raised and mucoid
<i>Pseudomonas</i>	Colonies are medium to large irregular in shape greenish blue in color with a smooth surface and are flat or slightly raised in elevation
<i>E.coli</i>	Colonies are medium to large in size circular in shape yellow in color smooth and elevated on the plate

Table 2: Biochemical tests using to identify *E.coli*

Biochemical test	Result
Indole Test	+
Gram Staining	-



Figure: 3 Indole Test of *E.coli*

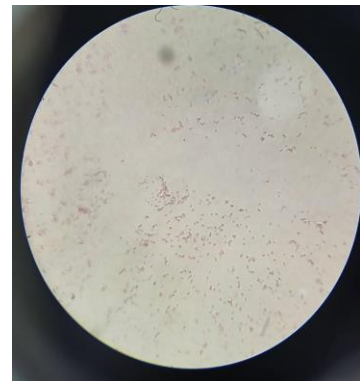


Figure: 4 Gram staining of *E.coli*

Table 3: Biochemical tests using to identify *Staphylococcus aureus*

Biochemical test	Result
Gram Staining	+
Coagulase Test	+

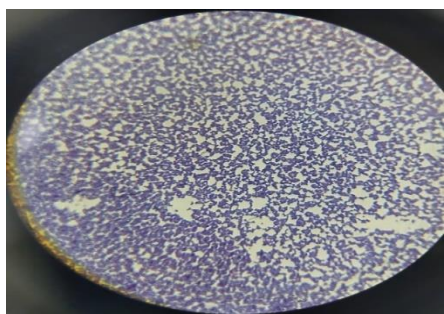


Figure 5: Gram Staining of *Staphylococcus aureus*

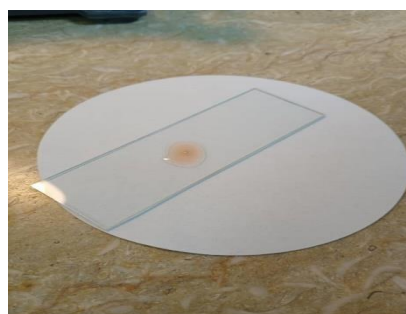


Figure 6: Coagulase Test of *Staphylococcus aureus*

Table 4: Biochemical tests using to identify *Pseudomonas*

Biochemical test	Result
Oxidase Test	+
Gram Staining	-



Figure 7: Oxidase Test of *Pseudomonas*

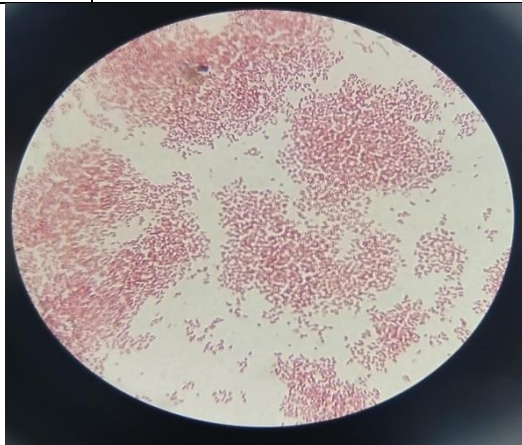


Figure 8 :Gram Staining of *Pseudomonas*

Table 5: Biochemical tests using to identify *Klebsiella*

Biochemical test	Result
Gram Staining	+

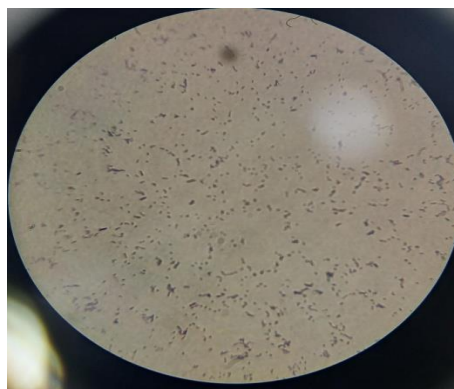


Figure 9: Gram Staining of *Klebsiella*

The table shows the effect of *Zingiber officinale* (ginger) extracts, both aqueous and alcoholic on the growth of selected **bacterial isolates**:

Aqueous extract:

Showed slight inhibitory activity against E.coli with a value of 0.1. No effect was observed on the other bacterial isolates.

Alcoholic extract:

Showed significant inhibitory activity against *Staphylococcus aureus* with a value of 0.36. Displayed slight activity against E.coli with a value of 0.11. No effect was observed on *Klebsiella* and *Pseudomonas*, the results indicate that the alcoholic extract is more effective than the aqueous extract against E.coli.

As Shown in the table.

Table 6: The mean of inhibition zone of aqueous and alcoholic extract of *Zingiber officinale* against *Staphylococcus*, *Klebsiella*, *Pseudomonas* and *E.coli*.

Bacterial isolates	Aqueous extract	Zingiber officinale Acoholic Zingiber officinale extract
<i>Staphylococcus aureus</i>	0	0.36
<i>Klebsiella</i>	0	0
<i>Pseudomonas</i>	0	0
<i>E.coli</i>	0.1	0.11

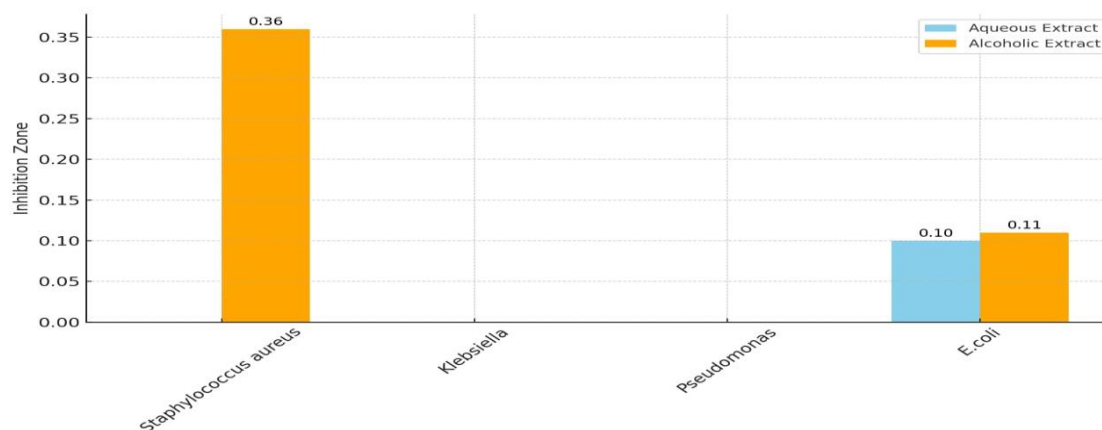


Figure 10: Inhibition zone of Aqueous and Alcoholic *Zingiber officinale* Extracts on Bacterial Isolates



Figure11: Inhibition Zone of aqueous extract Zingiber officinale against Staphylococcus aureus



Figure 12: Inhibition Zone of alcoholic extract Zingiber officinale against Staphylococcus aureus



Figure 13: Inhibition Zone of alcoholic extract Zingiber officinale against Klebsiella. (the Upper part). inhibition Zone of aqueous extract Zingiber officinale against Klebsiella. (the Lower part).



Figure 14: Inhibition Zone of alcoholic extract Zingiber officinale against Pseudomonas. (the Upper part). inhibition Zone of aqueous extract Zingiber officinale against Pseudomonas. (the Lower part).



Figure 15: Inhibition Zone of alcoholic extract Zingiber officinale against E.coli. (the Upper part). inhibition Zone of aqueous extract Zingiber officinale against E.coli. (the Lower part).

The table shows the mean inhibition zones of Cuminum cyminum(cumin) extracts, both aqueous and alcoholic against selected bacterial isolates:

Aqueous extract:

Showed slight inhibitory activity against Klebsiella (0.23) and E.coli (0.25). No inhibitory effect was observed against Staphylococcus aureus and pseudomonas(values=0).

Alcoholic extract:

Exhibited significant inhibitory activity against *Staphylococcus aureus* (0.46) and *E.coli* (0.44). Showed slight activity against *Pseudomonas* (0.2). No effect was observed on *Klebsiella* (value=0)

The results indicate that the alcoholic extract of *Cuminum cyminum* is more effective than the aqueous extract, particularly against *Staphylococcus aureus* and *E.coli*

As shown in the table.

Table 7: The mean of inhibition zone of aqueous and alcoholic extract of *Pimpinella Anisum* against *Staphylococcus aureus*, *Klebsiella*, *Pseudomonas* and, *E.coli*.

Bacterial isolates	Aqueous <i>Pimpinella Anisum</i> extract	Acoholic <i>Pimpinella Anisum</i> extract
<i>Staphylococcus aureus</i>	0	0.46
<i>Klebsiella</i>	0.23	0
<i>Pseudomonas</i>	0	0.2
<i>E.coli</i>	0.25	0.44

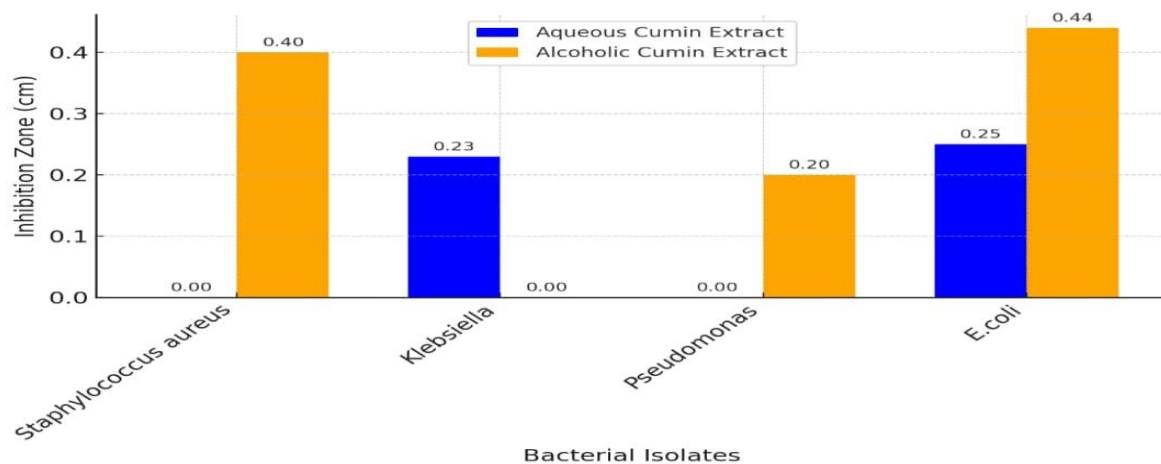


Figure 16: Inhibition zone of Aqueous and Alcoholic *Pimpinella Anisum* Extracts on Bacterial Isolates



Figure 17: Inhibition Zone of aqueous extract Pimpinella Anisum against Staphylococcus aureus.



Figure 18: Inhibition Zone of alcoholic extract Pimpinella Anisum against Staphylococcus aureus.



Figure 19: Inhibition Zone of alcoholic extract Pimpinella Anisum against Klebsiella. (the Upper part). inhibition Zone of aqueous extract Pimpinella Anisum against Klebsiella. (the Lower part).



Figure 20: Inhibition Zone of alcoholic extract Pimpinella Anisum against Pseudomonas. (the Upper part). inhibition Zone of aqueous extract Pimpinella Anisum against Pseudomonas. (the Lower part).



Figure 21: Inhibition Zone of alcoholic extract Pimpinella Anisum against E.col. (the Upper part). inhibition Zone of aqueous extract Pimpinella Anisum against E.col. (the Lower part).

Table 8: the effect of antibiotics on the following bacterial species *staphylococcus klebsiella pseudomonas* and *E.col*

Bacterial isolates	CN10	DAP30	CFM5	APX30	CAZ30
<i>Staphylococcus aureus</i>	0.4	1.16	0	0	0
<i>Klebsiella</i>	1	0	0	0	0
<i>Pseudomonas</i>	1.16	0.2	0.1	0.2	0
<i>E- Coli</i>	0.4	0.1	0.1	0.1	0

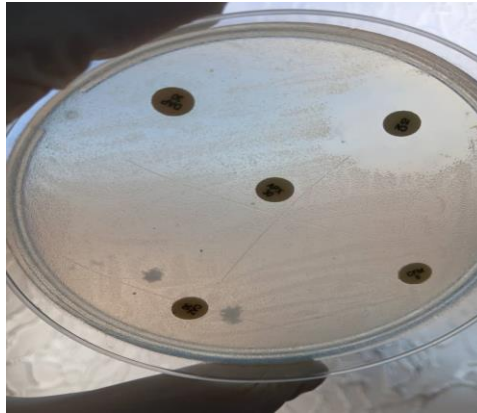
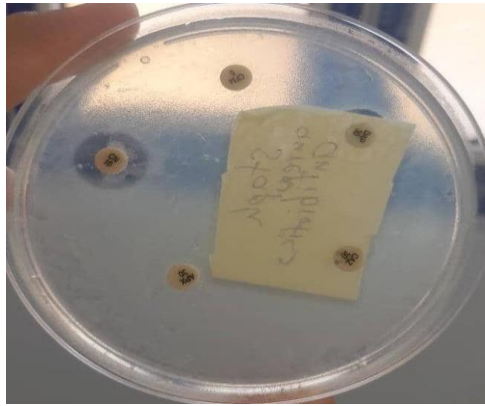


Figure 23 Antibiotics sensitivity test of *Staphylococcus*.

Figure 22 Antibiotics sensit *Klebsiella*. "aureu:

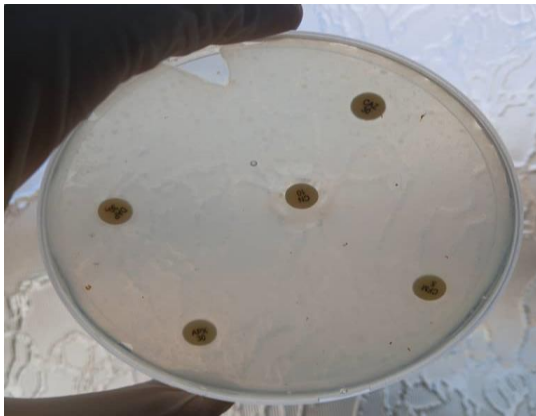


Figure 24 Antibiotics sensitivity test of *Pseudomonas*."

Figure 25 Antibiotics sensitivity test of *Escherichia coli*".

Conclusion :

Pseudomonas is highly resistant and showed very weak responsiveness to the tested extracts.

1. Effect of Plant Extracts on *Klebsiella*:

This bacterium exhibited moderate resistance.

The alcoholic ginger extract showed a minimal effect (0.1 cm inhibition zone).

All other extracts, including aqueous and alcoholic cumin, were ineffective (X).

Conclusion: The tested plant extracts showed very limited efficacy against *Klebsiella*.

2. Effect of Plant Extracts on *E. coli*:

The alcoholic cumin extract demonstrated the highest efficacy against this bacterium, with an inhibition zone of (0.2 cm).

The alcoholic ginger extract produced a comparable result (0.175 cm), indicating good activity.

The aqueous cumin extract exhibited moderate activity (0.125 cm), while the aqueous ginger extract was less effective (0.1 cm).

Conclusion: *E. coli* displayed moderate sensitivity to the extracts, with alcoholic extracts performing better.

Alcoholic extracts were generally more effective than aqueous extracts, as they were able to extract bioactive compounds with stronger antibacterial activity.

The alcoholic ginger extract was the most effective overall.

Staphylococcus aureus showed the highest sensitivity to the extracts, making it the least resistant species.

Pseudomonas was the most resistant, displaying very limited response to the extracts.

E. coli showed moderate sensitivity, while *Klebsiella* exhibited intermediate resistance.

Discussion

Many plants, owing to their phytochemical components, exhibit diverse properties and have been utilized globally for a range of purposes. Some of these plants not only serve as a source of nutrition for humans but also contribute to improving health. Over the past decades, numerous studies have highlighted the medicinal, antimicrobial, phytochemical, anti-inflammatory, and antioxidant properties of various plants. Researchers have discovered that these plants not only enhance the efficacy of antibiotics but may also serve as alternatives when microorganisms develop total resistance to antibiotics. This study was conducted to evaluate the phytochemical and antimicrobial properties of *Zingiber officinale* (ginger).

The plant contains bioactive compounds such as alkaloids, flavonoids, phenols, terpenoids, and saponins in both ethanol and aqueous extracts. These findings align with previous research that successfully isolated these compounds from ginger rhizomes. The antimicrobial activity of *Zingiber officinale* observed in this study targeted specific microorganisms, including *Staphylococcus aureus*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The results presented here corroborate earlier studies which reported similar inhibitory effects of ginger extracts on these bacteria. Additionally, *Pimpinella anisum* (anise) extracts have been found to contain numerous active chemical constituents with notable antibacterial effects. Research has shown that *Pimpinella anisum* influences its antibacterial activity through hydrophobic lipids that degrade bacterial cell structure, including damaging cell walls. Various investigations have demonstrated the antibacterial effects of both aqueous and alcoholic extracts of cumin against human pathogenic bacteria, especially those resistant to antibiotics. One prominent compound from cumin extract, pimpinella aldehyde, is a potent aromatic compound (C₁₀H₁₂O) with significant antibacterial properties. This compound disrupts the outer membrane of bacterial cells, impairing ion transport in and out of the cells, thereby inhibiting their function.

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