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# Contribution of Biological Activity of Some Secondary Metabolites of Petroselinum Crispum (Mill.) Fuss, Against Bacteria Isolated From Patients' Urinary Tract Infections

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**ABSTRACT**

Due to their potential to generate a diverse array of physiologically active metabolites, medicinal plants play a significant role in the creation of novel pharmaceutical and medical products. They are being researched as possible targets for discovering new antibiotic compounds. Results from the agar well diffusion method demonstrated Petroselinum crispum's antibacterial efficacy against a few human pathogenic bacteria.

Four different plant extract concentrations were used: 25, 50, 75, and 100 mg/ml. The maximum inhibition zone for the ethanolic extract of P. crispum against P. mirabilis was 36.0 mm at a 25 mg/ml dose. In comparison, the minimum inhibition zone for P. aeruginosa measured 6.66 mm. At 100 mg/ml, the inhibition zone of P. crispum hexane extract was the largest (34.3 and 31.0 mm.) against P. mirabilis and E. coli, respectively. In contrast, Staphylococcus aureus had the lowest inhibition zone (5.00 mm). While harmful bacteria were suppressed to varying degrees at concentrations less than 100 mg/ml, several bacterial strains shown resistance to plant extracts at low doses. According to the results of the gas chromatography-mass spectrometry (GC-MS) examination, the chemical composition analysis of the Petroselinum crispum extract had a high concentration of organic components.

## 1- INTRODUCTION

Using plant extracts for treating many diseases has increased, particularly after increasing bacteria resistance that causes a major problem for an individual's health, whether in developed or developing countries for the treatment of these diseases, many of the antibacterial agents. However, the rising use of these medications, which is frequently random and long-term, has resulted in the rise of side effects that are harmful to individuals (Askary and Malih,2021). Suppose the plant contains a group of active compounds that have been known since ancient times in the treatment of many diseases, as some studies have shown that some plants have wide physiological and pharmacological efficacy and as protection and preservation factors for foods. In that case, they are natural alternatives to chemicals used in the pharmaceutical field or in the field of food preservation or prolonging its life when stored, using natural additives with antibiological properties (Nychas *et al* ,.2003). ***Petroselinum crispum*** is a green herbaceous belonging to Umbelliferae (Apiaceae), a plant with scented plants and compound floral umbels. *P. crispums* comes from the Latin name for celery, selinum, and the Greek word pétros, meaning rock (Najajreh,2021). *P. crispum* is a green, upright plant with angular stems and branches—oval, plain, tripinnate parsley leaves. The 75-cm blooming stem has few leaves, flat-tipped yellow umbels, and many yellow to yellowish-green flowers. Round, 2-3 mm seeds with a

distinct style at the tip (Boutsika *et al*,.2021). It is commonly available throughout Europe and the Mediterranean area and is high in vitamin C and minerals. Fresh P. crispum (100 g) contains 36 kcal, 133 mg vitamin C, 554 mg potassium, and 138 mg calcium, as well as trace amounts of vitamin E and other vitamins, minerals, protein, and fibre. P. crispum is a very scented plant that is used as a herb (Sarwar et al., 2019 ). P. crispum has antibacterial properties against Bacillus subtilis and Escherichia coli in its leaves, stems, and seeds (Ahmed et al., 2021). Both hot and cold water extracts of P. crispum leaves inhibited Pseudomonas aeruginosa, Staphylococcus aureus, and S. pyogenes from a burn infection patient. Petroselinum crispum leaf ethanol extract inhibited Lactobacillus Plantarum and Leuconostoc mesenteroides growth (Chauhan *et al*.,2018 ). This study used ethanolic and hexane solvents to examine the biological effects of isolated chemical components from Petroselinum crispums.

## 2. Material and Methods

### 2.1 Collection and Classification of study stations

In Thi Qar Governorate, between October and December 2022, seeds of Petroselinum crispum were gathered from local markets. The samples were then transferred to the laboratory and cleansed of dust and grime before drying for 7-14 days in the shade within the laboratory at room temperature. Dr. Haider Radhi also classified seeds in the Plant Classification Laboratory - Department of Biology / College of Science / Thi - Qar University.

### 2.2 Preparation of Extracts

#### 2.2.1 Collection and classification of study stations

Petroselinum crispum plant seed samples were obtained from local marketplaces in Thi Qar Governorate between October and December 2022. The samples were transferred to the laboratory and cleansed of dust and grime before drying for 7-14 days in the shade within the laboratory at room temperature. Dr. Haider Radhi also classified the samples in the Plant Classification Laboratory of the Department of Biology, College of Science, Thi - Qar University.

#### 2.2.1.1 Preparation Hexane Extract

Soxhlet continuous extraction was used to mix 20 g of dried seed powder with 200 ml of hexane. The solution was filtered using Whatman No. 13 filter paper, concentrated using a rotary evaporator at 50 degrees Celsius, and dried at 25 degrees Celsius under low pressure. The extract was kept until needed in sterilized glass tubes (Bobby *et al* .,2012).

#### 2.2.1.2 Preparation of Ethanol Extract

The extraction procedure was performed employing ethanol at a concentration of 96% and at a temperature of 78°C, except for utilizing a Rotary evaporator at 50°C to concentrate the sample before placing it in a glass dish to dry (Harborne, 1984).

#### 2.2.1.3 GC-MS Analysis of *P. crispum* Extracts

Nahran Omar / Southern Oil Company has utilized the device in Basrah. The Shimadzu GCQP 2010 ultra-gas chromatograph was utilized for the GC-MS analysis. The G.C. oven's temperature was set to increase by 10 C/min from 40°C to 280°C. The use of helium as a carrier gas was made. 7.0699 psi of pressure was present. The purge flow was 3 ml/min, while the column flow rate was 1 ml/min. In split injection mode, the injector reached a temperature of 290 degrees Celsius. The settings for the MS scan are as follows: the source temperature is set to 200 degrees Celsius, the interface temperature is set to 290 degrees Celsius (MSD transfer line), the solvent cut time is set to 4 minutes, the scan speed is set to 1562 (N2), and the range is 35 m/z to 650 m/z. P.crispum chemical compounds were discovered by matching the spectra to known

chemicals (NIST library,2005).

### 2.4 Culturing of Samples

Swabs were only cultivated on MacConkey agar, and both the blood and the swab samples were incubated at 37 degrees Celsius for a full 24 hours in an aerobic and anaerobic atmosphere, respectively. Proteus mirabilis, for example, develops in waves on a blood agar plate, generating a thin, filmy coating of swarming concentric rings. However, P. mirabilis does not swarm on a plate of MacConkey agar, so its colonies are smooth, pale, or colorless. The colonies of P. aeruginosa are colorless because they do not ferment lactose. This is a crucial method for distinguishing P. aeruginosa from other bacteria in the sample, especially gram-positive bacteria such as S. aurous, which outgrows blood agar in 18 to 24 hours. 1-2 mm in diameter, round, elevated, opaque yellow to golden yellow colonies are visible with or without beta hemolysis. Hashim et al. (2012) isolated a single colony from the culture, placed it on a sterile microscope slide, desiccated and heat-fixed it, stained it with gramme stains, and examined it under a microscope (oil immersion). The suspected biopsy sample is used to create subcultures of E. coli on blood agar and MacConkey agar. Because they are fermented lactose sugar, which produces lactic acid, all specimens exhibit lactose fermenter colonies on MacConkey. This is shown by the appearance of a neutral red pigment as an indication and the pink color (Frol and Iurp, 2012).

### 2.5 Analytical Profile Index

S. aureus and Enterobacteriaceae isolates were identified using the API-20 system, which consists of a container bar with 20 biochemical reactions dispersed throughout individual microtubes.

## 3. Results

### 3.1 Extract of Organic Compounds from P. crispum Using Ethanol

Table 1 displays the results of a chemical investigation that identified 10 different chemicals based on their retention times. Apiol had the highest percentage (58.9308), while Naphthalene's decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-,[4aR (4a.alpha.,7-alpha.,8.beta)]- had the lowest (0.5914).

**Table 1: GC-MS analysis of chemical compounds in the ethanol Extract of P. crispum**

| P | R.Time | commen name | Area % | Formala |
|---|---|---|---|---|
| 1 | 9.953 | .alpha.-Pinene | 1.0316 | C10H16 |
| 2 | 10.838 | Cyclohexene, 4-methylene-1- (1-methylethyl)- | 1.5857 | C10H16 |
| 3 | 11.819 | D-Limonene | 0.9687 | C10H16 |
| 4 | 18.781 | Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]- | 0.5914 | C15H24 |
| 5 | 19.29 | 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- | 19.1957 | C11H12O3 |
| 6 | 19.497 | Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- | 1.6256 | C12H16O3 |
| 7 | 21.351 | Apiol | 58.9308 | C12H14O4 |
| 8 | 21.768 | Sedanolide | 2.9948 | C12H18O2 |
| 9 | 23.929 | n-Hexadecanoic acid | 1.4973 | C16H32O2 |
| 10 | 25.721 | Linoelaidic acid | 11.5784 | C18H32O2 |
| | | | **%** 100 | |

### 3.2 Organic Compounds Extract from *P. crispum* by Hexane Extract

GC-Mass technology was used to get the organic compounds out of P. crispum. Table 2 shows

that the hexane extract contains 16 organic compounds, each taking a certain distance within the retention time. The chemical compounds that were the most cut off from other organic compounds were the ones that proved to be the most efficient against the isolating bacteria. The highest percentage recorded for the compound Apiol was 41.71292%, while the lowest percentage recorded was 0.46386%. gamma.-Terpinene.

**Table 2: GC-MS analysis of chemical compounds in hexane Extract of P. crispum**

| P | R.Time | Commen Name | Area % | Formula |
|---|---|---|---|---|
| 1 | 9.808 | 3-Carene | 5.271783 | C10H16 |
| 2 | 10.751 | Cyclohexene,4-methylene-1-(1-methylethyl)- | 4.895593 | C10H16 |
| 3 | 11.776 | D-Limonene | 2.968096 | C10H16 |
| 4 | 12.312 | .gamma.-Terpinene | 0.46386 | C10H16 |
| 5 | 14.673 | Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl- | 0.754626 | C10H14O |
| 6 | 18.77 | Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]- | 0.794881 | C15H24 |
| 7 | 19.309 | 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- | 18.08062 | C11H12O3 |
| 8 | 19.513 | Benzene,1,2,3-trimethoxy-5-(2-propenyl)- | 2.809393 | C12H16O3 |
| 9 | 21.371 | 1H-Benzimidazole,2-(5-ethyl-2-pyridinyl)- | 3.196604 | C14H13N3 |
| 10 | 21.418 | Apiol | 41.71292 | C12H14O4 |
| 11 | 21.824 | 3-Oxabicyclo[3.3.0]oct-6-en-2-one, 4-methoxy-7-methyl-, trans- | 2.776368 | C9H12O3 |
| 12 | 23.941 | n-Hexadecanoic acid | 1.646519 | C16H32O2 |
| 13 | 24.114 | Hexadecanoic acid, ethyl ester | 0.515131 | C18H36O2 |
| 14 | 25.642 | 1-(2-Methoxyphenyl)-5-methyl-4-hexene-1-ol | 0.540554 | C14H20O2 |
| 15 | 25.714 | cis-3-Methyl-endo-tricyclo[5.2.1.0(2.6)]decane | 3.343962 | C11H18 |
| 16 | 25.776 | Ethyl Oleate | 10.22909 | C20H38O2 |
| | | | 100% | |

### 3.3 Activity of *P. crispum* extracts:

Tables (3) (4) illustrate the antibacterial effectiveness of P. crispum hexane and ethanol extracts against selected human pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, and Escherichia coli. The hexane and ethanolic extracts of P. crispum (25, 50, 75, and 100 µg/µl) were tested against four types of bacteria: Gram-negative (Pseudomonas et al. mirabilis, and Escherichia coli) and Gram-positive (Staphylococcus aureus). The zone of bacterial growth inhibition is used to assess the antibacterial activity of the tested extract. The findings of the P. crispum hexane and ethanol extracts revealed that the inhibition diameters of the several bacterial species under test varied.

The inhibitory zone was clearly visible in both extracts (hexane and ethanolic). On the other hand, these four bacteria were killed by both P. crispum extracts. The Pcrispum extract possesses antibacterial characteristics, as shown by the results of the antibacterial tests. Both Gram-positive bacteria like Staphylococcus aureus and Gram-negative bacteria like Pseudomonas aeruginosa

and Proteus mirabilis are susceptible to this activity. Alternatively, according to the zone inhibition in table (3), the activity was low, intermediate, and high. The complete ethanolic extract of P. crispum demonstrated considerable activity against S. aureus at all four concentrations (25%, 50%, 75%, and 100%). While concentration 50 exhibited a low activity and the Concentration 25 showed no activity, the hexane extract had an inhibitory activity against S. aureus that was more than 20 mm in diameter (Table(4)). Clearly, the 100% concentration of the P. crispum hexane and ethanol extract inhibited S. aureus more efficiently than the other concentrations tested, as shown in Table (3)(4).

Table 3 shows that the Gram-negative bacteria P. mirabilis was not very resistant to P. crispum ethanol extracts. While intriguing activity was shown in P. crispum Table(4) for both the whole extract and the extract made in hexane. According to the findings in Table 3, adding hexane and ethanolic extracts resulted in a low zone of inhibition (less than 10 mm) for the P. crispum extract against P. aeruginosa and E. coli. This finding suggests that these bacteria are only moderately sensitive to the extract.

Table(3) shows that at all doses tested, the ethanolic extract of P. crispum inhibited growth of S. aureus, P. aeruginosa, P. mirabilis, and E. coli with an inhibition zone larger than 20 mm, as seen in Figure(1). In contrast, at 75% and 100% concentrations, the hexane extract of P. crispum displays a substantial antibacterial activity against the P. mirabilis and E. coli pathogens. (see Figure (2) and Table (4))

**Table 3: The diameter of inhibition achieved for various P. crispum extracts against different bacteria (Mean ± SD).**

| Bacteria / Mg/ml concentration | *P. mirabilis* | *E. coli* | *P. aeruginosa* | *S. aureus* |
|---|---|---|---|---|
| | **Inhibition Zone of *P. crispum* seeds Ethanolic extract Mean ± SD** | | | |
| **100** | 36.0 ± 2.00[a] | 33.3 ± 1.52[a] | 28.3 ± 1.52[a] | 27.3 ± 1.15[a] |
| **75** | 23.6 ± 1.52[b] | 27.0 ± 1.73[b] | 20.6 ± 1.52[b] | 24.0 ± 1.00[a] |
| **50** | 20.0 ± 1.00[c] | 20.3 ± 0.57[c] | 15.3 ± 1.52[c] | 15.0 ± 2.00[b] |
| **25** | 13.3 ± 0.57[d] | 10.0 ± 0.00[d] | 6.33 ± 1.15[d] | 8.33 ± 2.05[c] |
| **control** | 0.00 ±0.00[e] | 0.00 ±0.00[e] | 0.00 ±0.00[e] | 0.00 ±0.00[d] |
| **p. value** | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** |
| **LSD** | **2.60** | **2.24** | **2.71** | **3.72** |

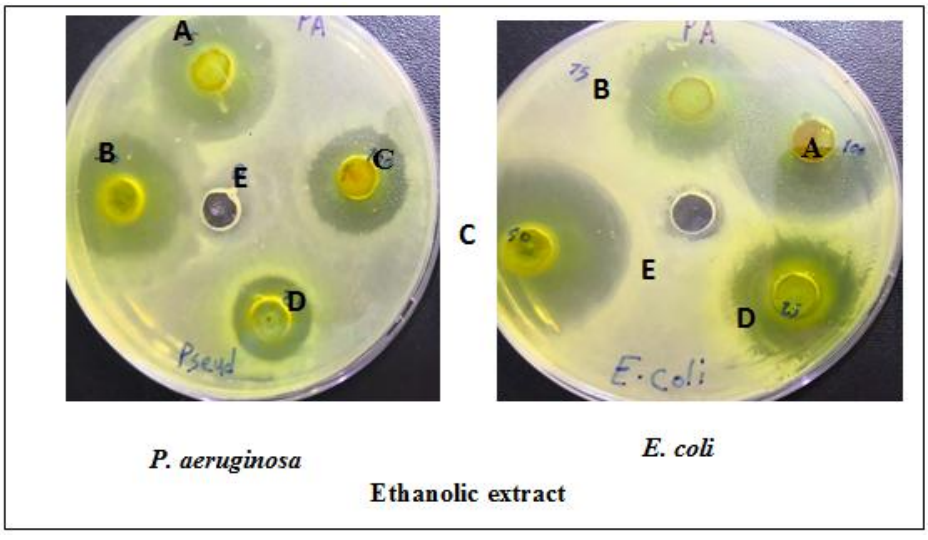


**Figure (1) c Activity of *P. crispum* L. extract ethanol against isolated bacteria**

Well A concentration 100% Well C concentration 50%

Well B concentration 75% Well D concentration 25%

Well E concentration control

**Table 4: Diameter of inhibition obtained for the different extracts hexane of *P. crispum* against different used strains (Mean ± SD).**

| Bacteria / Mg/ml concentration | *P. mirabilis* | *E. coli* | *P. aeruginosa* | *S. aureus* |
|---|---|---|---|---|
| | **Inhibition Zone of *P . crispum* seeds hexane extract Mean ± SD** | | | |
| **100** | 34.3 ± 0.57[a] | 31.0 ± 1.00[a] | 26.6 ± 0.57[a] | 24.0 ± 0.00[a] |
| **75** | 32.3 ± 2.08[a] | 24.6 ± 1.15[b] | 17.3 ± 0.57[b] | 23.3 ± 1.15[a] |
| **50** | 27.0 ± 1.00[b] | 16.3 ± 1.15[c] | 13.0 ± 2.00[c] | 5.00 ± 1.73[b] |
| **25** | 20.6 ± 1.52[c] | 5.33 ± 1.15[d] | 0.00 ± 0.00[d] | 0.00 ± 0.00[c] |
| **control** | 0.00 ±0.00[d] | 0.00 ±0.00[e] | 0.00 ±0.00[e] | 0.00 ±0.00[d] |
| **p. value** | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** |
| **LSD** | **2.66** | **2.10** | **2.03** | **1.95** |

- The DMSO was used as a control group in the above and below tables and had not biological action.
- In the table above, every pair of averages has the same small letter, thus they are not significantly different. Each letter pair differs significantly.
- Adding letters such as (a, b, c, etc.) increases the extract's effectiveness.

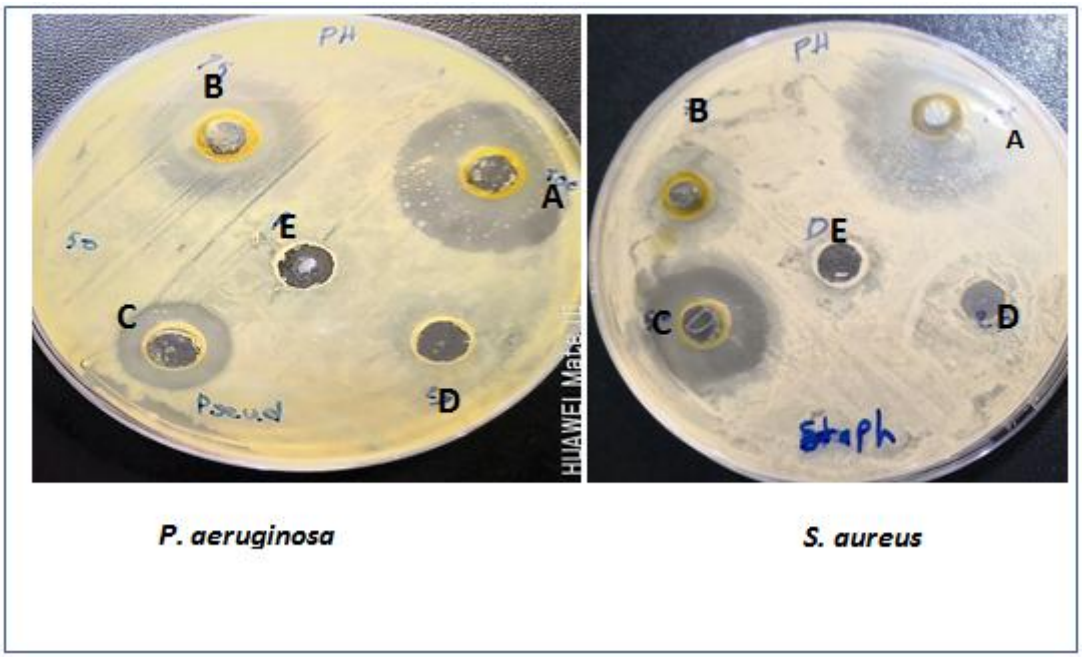


**Figure (2) c Activity of *P. crispum* L. extract hexane against isolated bacteria**

Well A concentration 100% Well C concentration 50%

Well B concentration 75% Well D concentration 25%

Well E concentration control

## 4. Discussion

Antibiotic-resistant bacteria may be treated with compounds found in medicinal plants that have not previously been utilised to treat bacterial infections. The findings of this research show that different amounts of active compounds in the same seed plant have different effects on different pathogenic organisms. This meant that gram-positive bacteria were more vulnerable to the active ingredient in the extract than gram-negative bacteria. Possibly because gram-negative organisms have an outer membrane that functions as an effective barrier (Girish, 2008). Also, the findings revealed that gram-positive bacteria were the most vulnerable. This may be because gram-positive bacteria have a single membrane, which makes it easier for the active principles of the extract of active chemicals to get inside (Al Marzoqi *et al.*, 2008). In the present investigation, the seeds of the plant P. crispum, which had strong antibacterial properties, had different amounts of bioactive substances, which could play an important role in making them kill bacteria. The useful pharmaceutical qualities of plant materials are often due to the mixture of their secondary products, such as phenol compounds, flavonoids, alkaloids, steroids, and tannins (Davidson, 2001). Bioactive phytochemicals from plants may be better at fighting resistant germs than chemical or manufactured antimicrobials because they may have stronger mechanisms (Al-Doweriej *et al*., 2016). The antibacterial activity of plant seed extracts is influenced mostly by the kind of bacterial strain and extraction solvent. Certain Gramme-negative and Gramme-positive bacteria strains were suppressed by ethanolic and hexane extracts. Methanol and ethanol have higher antibacterial action in medicinal plants, according to Majhenic et al. (2007). A better antibacterial compound was also created by El-Safey and Salah (2011) using methanol and ethanol, an organic extraction solvent. Consequently, the findings of earlier investigations support those presented in this study. in agreement with Yuhannes et al. Seed extracts were tested for antibacterial activity using hexane and ethanol in the agar well diffusion technique. This study tested the antibacterial efficacy of solvent extracts against bacterial strains using disc diffusion. The ethanolic fraction of P. crispum seeds was the most effective of the studied solvents, with maximal activity (zone of inhibition) against P. mirabilas (36mm in concentration 100%). Amel et al. (2012) discovered similar results. They also utilized several solvent extracts against a variety of bacteria. Another group reported the same bacterial strains and the antibacterial activity was evaluated by Djarmouni et al. (2018) used the agar diffusion method to test 11 different microorganisms. Crude plant extracts generally contain active and inactive chemicals against bacterial strains. Scientists have been very interested in testing products from plant seeds as they look for new drugs that will help treat diseases better. Thus, plant seed extracts and phytochemicals with recognised antibacterial activity may be important in treatment. Al-Kareemi (2012) found that plant seeds influence both gram-positive and gram-negative bacteria from urinary tract infections. Gram-positive bacteria were more sensitive than Gram-negative bacteria. Gram-positive bacteria may lack the natural sieve effect against big molecules owing to their cell envelope's narrow pores and simpler cell walls (Daly *et al*., 2010). The results indicated that these plants are rich in all secondary metabolic components, allowing them to be employed medically in various disciplines. So this study accords with previous studies such as (Frank et al,.2020). The antibacterial activity of the extracts of the seeds was varied, some of which had a weak effect, such as hexane extract at a concentration of 25%, which was less effective than the rest of the other solvents, and this may be attributed to the difference in the polarity of the organic solvents, or perhaps the inhibitory activity of plant seeds is because they contain the active compounds. Especially tanning materials, including tannic acid, may affect microorganisms (Tagrida *et al.*,2021). The percentage of impact is proportional to the concentration of the extract. Therefore the insensitivity of the tested bacteria to some concentrations may be attributable to a decrease in the active chemicals at such concentrations.

Accordingly, the percentage of inhibition increases as the extract concentration rises, correlating

with findings reported by (Elshanawany, 1996). Some plant extracts are more effective against the tested bacteria than these antibiotics. It makes us turn to alternative medicine and use such plants to resist microbes instead of using commercial antibiotics, especially after recent publications and reports issued by the World Health Organization, which indicated the mutations that occurred and are still occurring in microbial cells and made them more resistant to most known antibiotics, which previously had great effectiveness against microbes (WHO, 2017).

## Conclusion

Due to their phytochemical composition, medicinal plants may be used as a source of antibacterial agents. The gram-positive and gram-negative bacteria strains examined were both vulnerable to the ethanolic extract of P. crispum seeds. Hexane extracts, on the other hand, were less efficient against all pathogenic bacteria tested. Extracts with secondary metabolites may have a suppressive chemical composition. However, these extracts may provide a safe and effective therapeutic alternative to antibiotics for the treatment of bacterial infections.

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