**Antibacterial activities of *Portulaca oleracea* on *Helicobacter pylori* isolated from patients with gastritis and duodenal ulcers in Basrah, Iraq**

**Running title: *P. oleracea* effects on *H. pylori***

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**Abstract**

**Background:** One of the new approaches in the treatment and eradication of *Helicobacter pylori* is the use of new herbal compounds. *Portulaca oleracea* has many antimicrobial properties. This study aimed to evaluate the antibacterial activity of *P. oleracea* extracts on *H. pylori* isolated from patients with gastritis and duodenal ulcers in Basrah, Iraq.

**Methods:** In this cross-sectional study (August to December 2023), gastric and duodenal biopsies specimens were collected and cultured on a Modified Columbia Urea agar plates. Identification of *H. pylori* was done through Gram staining and biochemical tests. Antibacterial activities of different concentrations (200, 150, 100, 50, 25 mg/ml) of methanolic, ethanolic, and aqueous extracts of *P. oleracea* stems and leaves were assessed on *H. pylori* using disc diffusion method.

Results: The results showed that crude methanolic, ethanolic, and aqueous extracts of *P. oleracea* stems and leaves have antibacterial activity on *H. pylori*. Different concentrations of ethanolic extract of *P. oleracea* stems showed inhibition zones ranged from 10 to 26 mm against *H. pylori*. These inhibition zones were larger than leaves ethanolic extract with inhibition zones of 9 to 24 mm. Different concentrations of stems methanolic extract showed larger inhibition zones (9-23 mm) when compared to leaves methanolic extract (11-22 mm). Moreover, stem aqueous extracts showed larger inhibition zones (11-21 mm) in diameter than leaves aqueous extracts (9-18 mm).

**Conclusion:** According to the results, *P. oleracea* may provide a good potential source of antibacterial compounds against *H. pylori* that need further analysis to reveal its effective ingredients.

**Keywords:** Antibacterial activity, *Helicobacter pylori*, *Portulaca oleracea*

## Introduction

*Helicobacter pylori* is one of main causes of gastric infections worldwide as it affects half of the world's population.[1] *H. pylori* is a spiral-shaped, flagellated, microaerophilic, Gram-negative bacteria that infects the mucous layer of gastrointestinal tract.[2] It colonizes the gastric epithelial surface causes gastritis and peptic ulcer disease in addition to gastric cancer.[3,4] *H. pylori* infection has been linked to a number of risk factors as low socioeconomic status of the subjects.[5] The most common mode of transmission includes intra-familial transmission through direct person to person contact.[6]

*H. pylori* had to adapt by possessing multiple virulence genes in order to survive in the stomach.[7,8] The effectiveness of *H. pylori* eradication therapy is primarily determined by the bacteria's susceptibility to antimicrobial agents.[9] It is commonly acquired in a youngster and can persist a lifetime if not treated.[10] Antibiotic regimens used in *H. pylori* treatment give high cure rate but have some negative effects were some of the treated patients develop antibiotic resistance.[11,12]

The herbal medicines are becoming more popular since they are less cost-effective natural remedies that are widely available that have antibacterial activities against different diseases.[13,14] The World Health Organization (WHO) lists *Portulaca oleracea* (*P. oleracea*) as one of the most extensively used medicinal herbs due to contains different phytochemical compounds that play important role human health.[15,16] *P. oleracea* is also known as purslane is an annual plant that belong to the *Portulacaceae* family.[17] This plant owns thick stem as well as fleshy leaves and yellow flowers. Different parts of *P. oleracea* such as stems, leaves are used around the world as both vegetables and a medicinal herb.[18] This plant is found all over the world and is popular in

many parts of Europe, Asia, and the Mediterranean.[19,20] *P. oleracea* consider a rich source of α-linolenic acid and omega-3 fatty acids in addition to have various phytochemical compounds that including ascorbic acid, α-tocopherols, β-carotene, phenolics, flavonoids, alkaloids, terpenoids, glutathione, polysaccharides, sterols and proteins.[21,22] Also, it has high concentration of vitamins and minerals like iron, magnesium, potassium and calcium.[23] Because of the presence of these various active compounds, *Portulaca oleracea* aerial part have antioxidant and antimicrobial activities.[24,25] Many studies confirmed that *P. oleracea* has antibacterial effects against different microorganisms such as *Escherichia coli, Staphylococcus aureus*, *Klebsiella* species*, Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Bacillus subtilis*, and *Proteus mirabilis*.[26,27] Many studies have focused on the importance of *Portulaca oleracea* in the healthcare. This plant is used in treatment of different gastrointestinal and respiratory disorders.[28,29,30] This study aimed to evaluate the inhibitory effect of methanolic, ethanolic, and aqueous extracts of *P. oleracea* leaves and stems on *H. pylori* isolated from patients with gastritis and gastric ulcers in Basrah, Iraq.

## Materials and Methods

### Ethics approval

The Institutional Review Board (IRB) of AL-Sader Teaching Hospital, Basrah, Iraq and University of Basrah, Basrah, Iraq approved this study (no registered code). Written informed consent was taken from all patients.

### Isolation of *H. pylori*

This study included 40 patients with gastritis and duodenal ulcer diseases with age ranged from 20 to 80 years. These patients underwent endoscopy unit of Al-sadder teaching hospital and

private clinics during the period between Augst to December 2023. Biopsies that obtained from these patients, were examined under supervision of specialist doctors. Biopsy samples were transported in brain heart infusion broth (BHI) (Merck, Germany) to the laboratory. Specimens were homogenized and cultured on selective Modified Columbia Urea agar (Merck, Germany) for 1-2 days at 37 °C under microaerophilic conditions. Bacterial colonies were tested for Gram staining and biochemical tests.[31]

**Preparation of *P. oleracea* methanolic, ethanolic, and aqueous extracts**

*P. oleracea* was obtained from local markets in Basrah, Iraq. The fresh *P. oleracea* leaves and stems were washed to get rid of soil and debris. Then, leaves and stems were air dried at room temperature. A mechanical grinder was used to powder the *P. oleracea* leaves and stems separately. The powder was kept at room temperature in an air tight container until it was used. The three different extracts of *P. oleracea* from each part of leaves and stems were made by adding 20 gm of dry leaves as well as stems powder to 200 ml of different solvents that include methanol, ethanol, and distilled water. Then these solutions were shaken in a rotary shaker for 24 hours. These solutions were filtered through a Whatman No.1 filter paper and left dried then stored at 4$^{o}$C until used. The crude methanolic, ethanolic, and aqueous extracts were made by mixing their powders with dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml. Then diluted with DMSO to achieve different concentrations (200, 150, 100, 50, and 25 g/ml).[32,33]

**Disk diffusion test method**

Disk diffusion test was used to determine *H. pylori* susceptibility to methanolic, ethanolic, and aqueous extracts for leaves and stems of *P. oleracea*. *H. pylori* inoculum was prepared by inoculating colonies of this bacteria into tubes containing normal saline to reach turbidity of the

colony *H. pylori* suspension equivalent to a 0.5 McFarland standard. Then the bacterial suspensions were spread onto Modified Columbia Urea agar plates by using a sterilized cotton swab. Filter paper disks of 6 mm diameter were placed on the surface of the inoculated agar after impregnated (saturated) with different extracts including crude methanolic, ethanolic, and aqueous extracts of *P. oleracea* leaves and stems in addition with their different concentrations (200, 150, 100, 50, 25mg/ml). All these plates were incubated for 1-2 days at 37 °C under microaerophilic conditions. The antimicrobial activity was estimated by measuring the diameters of the inhibition zones that surrounding each impregnated disk.[34]

**Results**

All crude methanolic, ethanolic, and aqueous extracts of leaves as well as steams were found to be effective in inhibiting the growth of *H. pylori* isolates as compared to the control solution DMSO which has not revealed any inhibitory effect on *H. pylori* isolates. According to different concentrations (200, 150, 100, 50, 25mg/ml) of methanolic, ethanolic, and aqueous extracts, these extracts revealed different inhibitory effects on *H. pylori* isolates as they showed varying sizes of inhibition zones.

**Inhibitory effects of methanolic extract of *P. oleracea* stems**

The different concentrations of methanolic extract of *P. oleracea* stems showed various antibacterial activities against *H. pylori* ranged from 9 to 23 mm in diameter as shown in Table 1. The highest inhibition zone was reached at 200 mg/ml concentration with 23 mm in diameter. At 150 mg/ml concentration, the inhibition zone was 22 mm in diameter. Meanwhile, other

concentrations including 100 mg/ml, 50 mg/ml, and 25 mg/ml showed smaller inhibition zones diameters as follows: 18 mm, 12 mm, and 9 mm, respectively.

**Inhibitory effects of methanolic extract of *P. oleracea* leaves**

Different concentrations of methanolic extract of *P. oleracea* leaves exhibited various inhibitory effect against *H. pylori* ranged from 11 to 22 mm in diameters (Table 1). The maximum inhibition zone was 22 mm in diameter at 200 mg/ml concentration. At 150 mg/ml concentration, the inhibition zone was also high (20 mm) in diameter. However, 100 mg/ml and 50 mg/ml concentrations of leaves methanolic extract showed smaller inhibition zones sizes as foolows: 15 mm and 11 mm, respectively. While no inhibitory effect was found at 25 mg/ml concentration. Also, stems methanolic extract of *P. oleracea* had higher inhibitory effect against *H. pylori* than leaves methanolic extract.

**Inhibitory effects of ethanolic extract of *P. oleracea* stems**

Ethanolic extract of *P. oleracea* stems also revealed various antibacterial activities against *H. pylori* that ranged from 10 to 26 mm in diameter according to its different concentrations as shown in Table 2. At 200 mg/ml concentration, the size of inhibition zone was largest against *H. pylori* which reach 26 mm in diameter. The size of inhibition zone at 150 mg/ml concentration was 21 mm in diameter. Other concentrations of ethanolic extracts that included 100 mg/ml, 50 mg/ml, and 25 mg/ml showed different inhibition zones diameters as follows: 18mm, 12 mm, and 10 mm, respectively.

**Inhibitory effects of ethanolic extract of *P. oleracea* leaves**

The ethanolic extract of *P. oleracea* leaves also showed different inhibitory effect against *H. pylori* according to its concentrations that ranged from 9 to 24 mm in diameters. The inhibition zone at 200 mg/ml and 150 mg/ml concentrations were 24 mm and 20 mm in diameters, respectively. While other concentrations of ethanolic extracts of *P. oleracea* leaves included 100 mg/ml, 50 mg/ml and 25 mg/ml showed inhibition zones sizes as follows: 15 mm, 12 mm, and 9 mm, respectively. All concentrations of ethanolic extracts for both leaves and stems showed inhibitory effect against *H. pylori*. Moreover, stems ethanolic extract of *P. oleracea* had higher inhibitory effect than leaves ethanolic extract.

**Inhibitory effects of aqueous extract of *P. oleracea* stems**

Aqueous extract of *P. oleracea* stems at different concentrations also showed various antibacterial activities against *H. pylori* that ranged from 11 to 21 mm in diameter as shown in Table 3. At 200 mg/ml concentration, the size of inhibition zone against *H. pylori* was 21 mm in diameter. While the size of inhibition zones at 150 mg/ml and 100 mg/ml concentrations were 15 mm and 11 mm, respectively. Aqueous extract of *P. oleracea* stems did not show any inhibitory effect against *H. pylori* isolates at 50 mg/ml and 25 mg/ml concentrations.

**Inhibitory effects of aqueous extract of *P. oleracea* leaves**

Moreover, the different concentrations of aqueous extracts of *P. oleracea* leaves showed various inhibitory zones ranged from 9 to 18 mm in diameters. The extract at 200 mg/ml concentration showed the maximum inhibition zone diameter reached to 18 mm in diameter. The aqueous extract at concentrations 150 mg/ml and 100 mg/ml showed inhibition zones of 13 mm and 9 mm, respectively. While at 50 mg/ml and 25 mg/ml concentrations did not show any inhibitory

effects against *H. pylori* isolates. Aqueous extract of *P. oleracea* stems also had largest inhibitory effect against *H. pylori* than leaves aqueous extract.

## Discussion

Antibiotic therapy for patients with *H. pylori* infection has limitations due to antibiotic resistance. *P. oleracea* considered as an effective therapeutic agent for various gastric diseases due to its protective activity.[35] Because of the presence of various active phytochemical compounds, *P. oleracea* extracts have antioxidant and antimicrobial activities.[36,37] The current study investigated the antibacterial activity of three different extracts including methanolic, ethanolic, and aqueous extracts of *P. oleracea* stems and leaves on *H. pylori* that isolated from patients with gastritis and duodenal ulcer. Results showed that *H. pylori* isolates were sensitive for crude methanolic, ethanolic, and aqueous extracts of *P. oleracea* stems and leaves. These extracts have antibacterial activity on *H. pylori* isolates but at varying sizes in diameters of inhibition zones. The inhibitory effects for these extracts according to their different concentrations (200, 150, 100, 50 and 25 mg/ml) showed variation against *H. pylori* isolates. The different concentrations of stems ethanolic and methanolic extracts had the largest inhibitory effect against *H. pylori* isolates than leaves ethanolic and methanolic extracts. In the state of stems and leaves aqueous extracts inhibition affects were noticed when using higher concentrations only and it was also noticed that stems aqueous extracts are larger than leaves aqueous extracts. Our results indicated that *P. oleracea* ethanolic extract has the highest antibacterial activity against *H. pylori* isolates than methanolic and aqueous extracts. These results agreed with other study were the different extracts of *P. oleracea* have inhibitory effect against *H. pylori* isolates.[38] Also similar to another study which found that ethanolic extract have

higher antibacterial activity than methanolic and aqueous against *H. pylori*.[39] Many studies suggested that *P. oleracea* extracts have inhibitory effect against different species of gram positive and gram negative bacteria.[40,41] It was revealed that *P. oleracea* ethanolic extract had good antibacterial activity against Gram positive and Gram negative bacteria.[42] These extracts of *P. oleracea* have various biologically active compounds as alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, fatty acids and steroids.[43,44] Many studies confirmed that *P. oleracea* contains high levels of phenols and flavonoids. This suggested that this plant may have extremely high antibacterial and antioxidant activity.[45] It was also suggested that ethanolic extract out performed methanolic and aqueous extracts in antibacterial activity against gram negative and gram positive strains due to variation in their constituents and concentrations of flavonoids and phenolic compounds.[46] One study showed that aqueous extract of *P. oleracea* contains a high concentration of phenolic compounds, whereas ethanol extract of *P. oleracea* contains a high concentration of flavonoids.[33] Presence of flavonoids in the extract can act by some mechanisms for preventing *H. pylori* infection through interacting with virulence factors and enzymes of *H. pylori*.[47] Also flavonoids can effect on urease inhibition, DNA damage and protein synthesis inhibition of *H. pylori*.[48] In addition to that mentioned, our results revealed that parts of *P. oleracea* differ in antibacterial activity against *H. pylori* were these findings support previous researches that amount of flavonoids and phenols varies depending on the part of the plant. There are differences found among *P. oleracea* parts were stems containing significantly higher levels of total phenolic compounds than leaves and flowers.[49] Based on previous studies, the emergence of resistant bacteria from different sources and countries makes the necessity of approaching new treatment methods based on herbal medicines even more clear.[50,51,52]

**Conclusion**

The findings indicate that *P. oleracea* extracts have antibacterial activity against *H. pylori*, especially ethanolic extract that have a higher inhibitory effect than methanolic and aqueous extracts. Also *P. oleracea* stems extracts have higher inhibitory effect when compared to *P. oleracea* leaves extracts. *P. oleracea* may provide a good potential source of antibacterial compounds against *H. pylori* that need further analysis to reveal its effective ingredients.

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Table 1. The inhibitory effect of methanolic extract of *Portulaca oleracea* stems and leaves on *Helicobacter pylori* isolates according to various concentrations.

| **Studied parts of *Portulaca oleracea*** | **Concentrations of methanolic extract** | | | | |
|---|---|---|---|---|---|
| | **200 mg/ml** | **150 mg/ml** | **100 mg/ml** | **50 mg/ml** | **25 mg/mg** |
| | **Diameter of inhibition zone (mm) against *Helicobacter pylori*** | | | | |
| **Stems** | 23 | 22 | 18 | 12 | 9 |
| **Leaves** | 22 | 20 | 15 | 11 | 0 |

Table 2. The inhibitory effect of ethanolic extract of *Portulaca oleracea* stems and leaves on *Helicobacter pylori* isolates according to various concentrations.

| **Studied parts of *Portulaca oleracea*** | **Concentrations of ethanolic extract** | | | | |
|---|---|---|---|---|---|
| | **200 mg/ml** | **150 mg/ml** | **100 mg/ml** | **50 mg/ml** | **25 mg/mg** |
| | **Diameter of inhibition zone (mm) against *Helicobacter pylori*** | | | | |
| **Stems** | 26 | 21 | 18 | 12 | 10 |
| **Leaves** | 24 | 20 | 15 | 12 | 9 |

Table 3. The inhibitory effect of aqueous extract of *Portulaca oleracea* stems and leaves on *Helicobacter pylori* isolates according to various concentrations.

| **Studied parts of Part of *Portulaca oleracea*** | **Concentrations of aqueous extract** | | | | |
|---|---|---|---|---|---|
| | **200 mg/ml** | **150 mg/ml** | **100 mg/ml** | **50 mg/ml** | **25 mg/mg** |
| | **Diameter of inhibition zone (mm) against *Helicobacter pylori*** | | | | |
| **Stems** | 21 | 15 | 11 | 0 | 0 |
| **Leaves** | 18 | 13 | 9 | 0 | 0 |