

## Brief Original Article

**Antibacterial activity of ethanolic extracts of *Plantago major* leaves against *Pseudomonas aeruginosa* from burn infections**Alaa' Turki Monawer<sup>1</sup>, Ismaeil Mohammed Abdulkahar Mammani<sup>2</sup><sup>1</sup> Department of Biology, College of Sciences, University of Duhok, Kurdistan Region, Iraq<sup>2</sup> College of Health sciences, University of Duhok, Kurdistan Region, Iraq**Abstract**

**Introduction:** The use of herbal extracts is increasing because of the increase in bacterial resistance to conventional antibiotics. *Plantago major* is frequently used in traditional medicine because of its medicinal properties. The aim of the current study was to assess the antibacterial efficacy of an ethanolic extract of *P. major* leaves against *Pseudomonas aeruginosa* isolated from burn infections.

**Methodology:** One hundred and twenty burn samples were collected from hospitalized patients at the Burn Hospital in Duhok city. The bacterium was identified using Gram stain, colony morphology, biochemical tests and selective differential media. Antibacterial activity of *P. major* leaves was assessed by using an ethanolic extract in serial dilutions of 100, 75, 50, 25, and 10 % and disc diffusion assay. Antibiotic susceptibility testing was also performed by disk diffusion using Muller-Hinton agar medium.

**Results:** Different concentrations of the ethanolic extract of *P. major* leaves exhibited different zones of inhibition against *P. aeruginosa* from 9.93 mm to 22.18 mm in diameter. The inhibition zone increased as the concentration of the extract increased. The 100% ethanolic extract had the greatest inhibitory effect, inhibiting bacteria in the zone of 22.18 mm diameter. This bacterium showed a high level of resistance to the antibiotics used.

**Conclusions:** This study demonstrated that herbal extracts could be used as a combination therapy with antibiotics and chemical drugs in the elimination of bacterial growth. Further investigations and future experiments, need to be carried out before recommending use of herbal extracts.

**Key words:** antimicrobial activity; burn; ethanol extract; *Plantago major*; *Pseudomonas aeruginosa*; Soxhlet.

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**Introduction**

Patients with severe burns have a greater susceptibility to infections due to the destruction of their skin barrier and altered systemic immune responses [1]. Bacteria can penetrate into the deeper layers of burned tissue and migrate into the bloodstream. This can lead to septicemia which is a common cause of death in patients with severe burns [2]. Over the past few years, drug-resistant bacteria have been on the rise because of the large-scale use of antibiotics [3].

The multidrug-resistant organism *P. aeruginosa* was first identified due to its widespread distribution and unique mechanism developing advanced antibiotic resistance [4]. Antibiotics are among the most widely used methods for eliminating harmful microorganisms and are essential for maintaining human health. One of the main issues affecting the use of antibiotics is the resistance of many dangerous bacteria to them [5,6].

In recent years, natural antimicrobial compounds that can be used to replace synthetic antibiotics have

been studied. Synthetic antibiotics can contribute to the development of antimicrobial resistance in microbes and may have negative effects on human health [7].

*P. aeruginosa* is a rod-shaped, Gram-negative, facultative anaerobic bacterium. It is a common and widespread organism that can adapt to many different environments, and it can be isolated from many sources within hospitals [8]. It is an important cause of community and hospital acquired infections due to its intrinsic resistance to many antibiotics. Bacterial infections with this organism have been linked to higher mortality and morbidity when compared to other pathogens [9]. *P. aeruginosa* infections are a common medical problem, and it is difficult to treat because of its high resistance to many antibiotics and the high risk of development of resistance during treatment [10,11].

Plant and seed therapy have been used for many years in folk medicine as an alternative or complementary treatment for conventional medicine [12,13]. The genus *Plantago* (Plantaginaceae) [14], popularly known as tansagem or “Barhang” in

traditional Persian medicine, is distributed worldwide and includes 275 species [12]. *P. major* originated in Northern Europe and Central Asia, and is well-suited to tropical regions. *P. major* species is easily propagated by its small, rough seeds [15].

*P. major* is used in the treatment of a variety of medical conditions [12]. It is used as an anesthetic, antiviral, anti-inflammatory, astringent, anthelmintic, analgesic, analeptic, antihistamine, antirheumatic, antitumor, anti-ulcer, diuretic, expectorant, and hypotensive [16,17].

Several bioactive substances are found in *P. major*, including flavonoids, alkaloids, terpenoids, phenolic compounds (derivatives of caffeine), iridoid glycosides, fatty acids, polysaccharides, and vitamins [18]. These chemical components are present in almost every component of the plant: seeds, leaves, flowers, and roots, and are responsible for the bioactive properties of *P. major* [18]. The purpose of this study was to investigate the antibacterial activity of an ethanol extract from the leaves of *P. major* against pathogenic bacteria *P. aeruginosa*.

## Methodology

### Ethics statement

The study protocol was approved by the arch Ethics Committee / Scientific Research Division / Directorate of Planning / Duhok Directorate General of Health / Ministry of Health / Kurdistan Regional Government / Iraq [Reference number: 22062021-6-8].

### Bacterial isolation

The study included 120 clinical samples that were collected from burn infections in patients at the burn hospital in Duhok city. Sampled patients included both genders and different ages. The samples were transported to the Microbiology Laboratory at the Department of Basic Sciences, College of Nursing within 1-2 hours for culture and bacteriological detection.

### Bacterial identification

All isolates were first cultured using sterile cotton swabs in sterile vials containing Nutrient broth and Amies transport medium swabs that were incubated at 37 °C/24 h for microbiological identifications. Each sample was then inoculated onto Nutrient, Blood and MacConkey agar to isolate *P. aeruginosa*. Bacterial identification was done by using Gram stain, biochemical tests (catalase test, oxidase test, IMViC test, Triple Sugar Iron [TSI test] and Cetrinide agar.

Confirmation test done was by using Automated Vitek 2 system (bioMérieux, Lyon, France).

### Protocol for obtaining *P. major* extract

Five kilograms of fresh *P. major* plants were collected from the villages of Duhok city, Kurdistan region and were identified by Prof. Dr. Saleem Esmael Shahbaz of the “Medical Plants of Kurdistan, Iraq”.

The plants were taken to Department of Basic Sciences, College of Nursing, University of Duhok and then transported to the Microbiology Laboratory of the same university where leaves that were in good condition were selected. The leaves were washed with tap water and left to dry in shade for 5 days. The dried leaves were ground to fine powder with a mortar and pestle and by a mechanical grinder and stored in containers in dark until extraction.

### Preparation of plant leaves extract

Two extraction methods were used in this study and the results were compared.

### Maceration method

Thirty grams of *P. major* leaves powder was macerated in 300 mL of ethanol solvent (1:10 ratio) in a flask and kept for 72 h until complete extraction of the bioactive material was achieved [19,20].

After 72 h, the extract was filtered through Whatman No. 1 filter paper. The extract was stored in screw cap bottles were kept in a refrigerator at 4 °C until use. The extraction solvent was evaporated and the extract was concentrated at room temperature or in an oven at 40 °C and 4 g of the plant extracted was obtained.

A stock solution of the extract was prepared by dissolving 0.1 g of extract with 100 mL of ethanol to produce a final concentration of 100 mg/mL. The stock solution was diluted to concentrations of 75, 50, 25, and 10 mg/mL with appropriate volumes of sterile distilled water.

### Soxhlet apparatus method

Thirty grams of *P. major* leaf powder was macerated in 300 mL of ethanol solvent (1:10 ratio) by using a Soxhlet apparatus for 10 h at a temperature not exceeding the boiling point of the solvents [21]. Then, the same procedure was carried out for this extract as was used in maceration extraction method.

### Antibacterial activity

The antibacterial effect of the plant extract was evaluated using the disk inhibition zone method. The

**Table 1.** Number and percentage of *P. aeruginosa* isolates.

Source	Number of samples	Positive	Negative
Burn	120 [100%]	48 [40%]	72 [60%]

Kirby and Bauer method [22] was used where the Muller-Hinton agar medium was inoculated with freshly prepared cells of bacteria to yield a growth. After solidification of the agar, a number of sterile disks were dipped into the extract solution and placed on plates. Following incubation for 24 h at 37 °C, the antimicrobial activity was measured based on the diameter of the inhibition zone formed around the disk. At the same time, a comparison antibiotic control test was performed using commercial disks (piperacillin, ceftazidime, cefepime, meropenem, ofloxacin and tobramycin), and the diameter of the inhibition zones were measured in mm standardized by the CLSI (Clinical and Laboratory Standard Institute) and reported as sensitive (S), intermediate (I), and resistant (R).

*Statistical analyses*

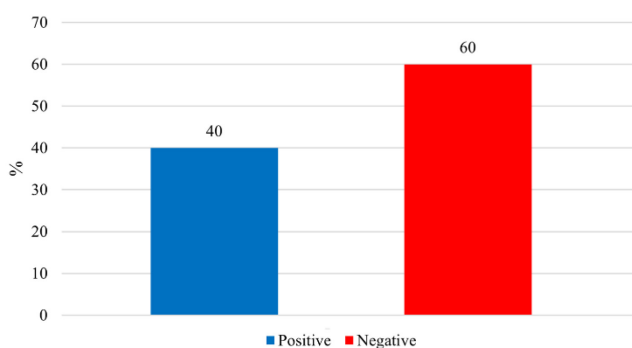
The antibacterial activity of the *P. major* extract against the multidrug-resistant bacterium *P. aeruginosa* was determined in number and percentage and the statistical calculations were performed by the predictive analytics software for scientists and engineers, JMP pro 14.3.0.

**Results**

Out of 120 clinical samples only 48 (40%) samples showed positive isolates for *P. aeruginosa* whereas 72 (60 %) samples were negative (Table 1 and Figure 1).

In this study, the antibacterial activity of various concentrations of ethanolic extracts of the *P. major* plant against the multi-drug resistant bacterium *P. aeruginosa* were first determined using the diffusion method for antimicrobial susceptibility test (Table 2). The results of the current study showed that ethanolic

**Figure 1.** Number and percentage of *P.aeruginosa* isolates.



**Table 2.** Inhibitory activity of *P. major* leaves ethanolic extract in different concentrations against *P. aeruginosa* isolates.

Concentration of extracts %	Average diameter of inhibition zone (mm) by ethanol extract
100	22.18
75	19.25
50	15.97
25	12.81
10	9.93

*P. major* leaves extract had antimicrobial effects on the growth of *P. aeruginosa* isolates ranging from 9.93-22.18 mm diameter zone of inhibition (Figure 2).

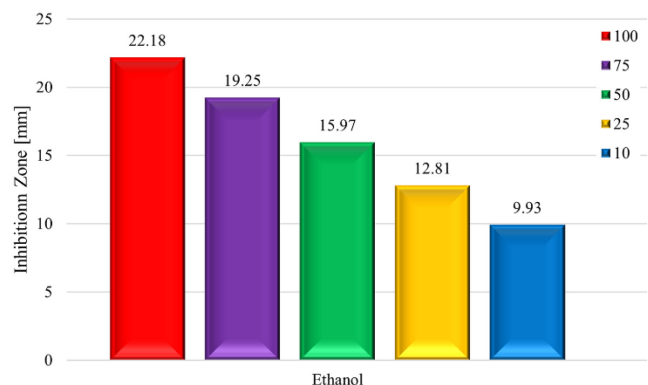
*P. aeruginosa* isolates were also tested for their susceptibility against commonly used (commercial) antibiotics by modified Kirby-Bauer method (Table 3). Figure 3 shows 100% level of resistance was recorded by all the isolates against piperacillin, ceftazidime and cefepime while a 93.8 %, 79.2% and 64.6% resistance were recorded against ofloxacin, meropenem and tobramycin respectively.

**Discussion**

One of the biggest and fastest-growing health problems in the world is the emergence of bacterial resistance to antibiotics [23] as a result of which infections caused by these microorganisms are unlikely to be properly cured. Therefore, prudent antibiotic usage is advocated and treatment strategies must be based on knowledge of the antibiotic that is used, without neglecting advancements in bacterial resistance profiles [23].

*P. major* plays an important role in the management of ailments and diseases such as ulcers, bacterial and

**Figure 2.** Inhibitory activity of *P. major* leaves ethanolic extract in different concentrations against *P. aeruginosa* isolates.



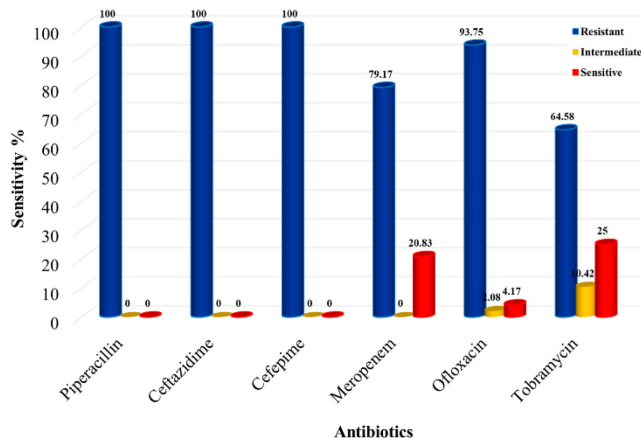
viral infections, diarrhea, pain, inflammation and cancer. This plant contains several classes of essential biologically active compounds such as flavonoids, alkaloids, iridoid glycoside, fatty acids, vitamins, phenolic compounds (caffeic acid) and terpenoids [18]. The biological activities and medicinal properties of *P. major* depend on the properties of the active chemical constituents. However, the exact mechanisms and the main bioactive compound responsible for treating certain diseases needs to be investigated [18].

*P. major* is utilized for diverse purposes in conventional medicine around the world and researchers have tested it for different types of biological activities. Most tests were performed with crude extracts without examining the nature of the active compounds [24].

At this time, it is unclear which phytochemicals in *P. major* play the most significant roles in its biological activities. The bioactivity of polysaccharides and polyphenols has been suggested, and *P. major*'s antiviral activity is described to be mostly determined from its phenolic components [21]. *P. major* leaves contain a mixture of different polyphenolic antioxidants that may contribute to its antiviral activities [22]. The present study was designed to obtain preliminary data on the antibacterial action of *P. major* leaf extract on *P. aeruginosa* bacterium. The agar well diffusion method was utilized in this study. The results showed remarkable antibacterial activity of the ethanol extract of this plant.

The result of the present study indicates that different concentrations of *P. major* extract exhibited different inhibition zones against *P. aeruginosa*. The potency of *P. major* leaf extract on *P. aeruginosa* ranged from 9.93 mm to 22.18 mm diameter zone of inhibition. Based on the dose response, the zone of inhibition increased with increasing the concentration of ethanol extracts. The lowest concentration (10 mg/mL) resulted in weak inhibition of the bacteria, while the higher concentrations of ethanol extract (100, 75, 50 and 25 mg/mL) recorded noticeable inhibition activity against the bacteria. The 100 mg/mL

**Figure 3.** Resistance of *P. aeruginosa* isolates to selected antibiotics [N = 48].



concentration of ethanol extract had the highest inhibitory effect for *P. aeruginosa* with 22.6 mm inhibition zone. These results are in agreement with previous reports [25, 26]. A study in Saudi Arabia demonstrated that *P. aeruginosa* was moderately sensitive to the ethanolic extract (95%) of *P. major* leaves with inhibition zone diameter of 12.5 mm [27].

**Conclusions**

Our findings showed good antibacterial activity of *P. major* leaves ethanolic extract that can be used as a treatment for infections, caused by *P. aeruginosa*.

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**Authors' contributions**

The corresponding author designed the study, collected data, analyzed the results, and drafted the manuscript; the co-author provided supervision; all authors read and approved the final version of the manuscript.

**Table 3.** Resistance of *P. aeruginosa* isolates to selected antibiotics [N = 48].

Antibiotics	Symbol	Disc potency [µg]	<i>P. aeruginosa</i> isolates		
			Resistant [R]	Intermediate [I]	Sensitive [S]
			No. (%)	No. (%)	No. (%)
Piperacillin	PRL	30	48 (100)	0 (0)	0 (0)
Ceftazidime	CDZ	30	48 (100)	0 (0)	0 (0)
Cefepime	FEP	30	48 (100)	0 (0)	0 (0)
Meropenem	MEM	1	38 (79.2)	0 (0)	10 (20.8)
Ofloxacin	OFX	10	45 (93.8)	1 (2.0)	2 (4.2)
Tobramycin	TOB	10	31 (64.6)	5 (10.4)	12 (25.0)

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## Corresponding author

Alaa' Turki Monawer, MSc Medical Microbiology  
 Department of Biology, College of Sciences,  
 University of Duhok, Kurdistan Region-Iraq  
 Tel: 009647504229321  
 E-mail: alaa.monawer@uod.ac

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