

Assessing the Antibacterial Activity of *Plantago major* Leaf Petroleum Ether Extract against *Pseudomonas aeruginosa* Isolated from Different Sources

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Abstract

The use of herbal extracts is becoming more popular as a result of the rise in bacterial resistance to conventional antibiotics. The benefits of *Plantago major* as traditional medicines have been acknowledged globally for years because of its medicinal properties. *P. major* leaf has been widely known for its efficacy in wound healing and also has antibacterial and antioxidant activities. Four hundred and eight samples (ear, urine, and sputum) were collected from patients who attended the main hospitals in Duhok city during the period from May 2022 to February 2023. Bacterial identification, and antimicrobial susceptibility were tested using the traditional methods and confirmed by VITEK 2 compact system. For the prevalence of *P. aeruginosa*, 87 isolates were isolated from 408 samples including, ear 39 (9.55%), urine 27(6.61%), and sputum 21(5.14%). Regarding antibiotic-resistant pattern, resistance was noticed to chloramphenicol, nitrofurantoin, cefixime, piperacillin, cefepime, and ceftazidime 100% for all isolates. Analysis of *P. major* plant components was done by Gas Chromatography-Mass Spectrometry detected 11 bioactive compounds in petroleum ether extract, and Liquid Chromatography-Mass Spectrometry revealed that pectin found in petroleum ether extracts. For the extraction of *P. major* leaves petroleum ether were used as solvents using soxhlet, then the extract obtained were evaporated in a rotary evaporator. The antibacterial activity of *P. major* leaves was assessed by petroleum extract in serial dilutions of 100, 75, 50, 25, and 12.5 % and disc diffusion assay. A remarkable results were obtained, *P. major* leaves petroleum ether had the antimicrobial effects on *P. aeruginosa* growth with the inhibition zone diameter ranging from (1.93-9.63) mm. In conclusion, the present study highlighted that *P. major* leaves showed good antibacterial activity for the selected extract that can be used as a treatment for *P. aeruginosa* infections. Hence, herbal extracts could be used as a combination.

Keywords: antibacterial activity, petroleum ether extract; *Plantago major*; *Pseudomonas aeruginosa*; Soxhlet.

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Introduction

Medicinal plants have always obtained a high interest in the management of diseases and conditions (Keshavarzi *et al.*, 2022). In this regard, *Plantago major* is a perennial plant, and is native to most of Europe, Northern, and Central Asia (Najafian *et al.*, 2018).

Plantago is a genus widely distributed all over the world (Kartini *et al.*, 2017); *P. major* is spread in temperate regions of Asia, South Australia, North America, and North Africa (Tariq and Mehmood, 2022) and the leaves of *P. major* have been used for centuries to treat diseases relating to skin, digestive organs and blood circulation like wounds, inflammation, and hypertension (Turgumbayeva *et al.*, 2022).

P. major contains biologically active compounds, such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides, alkaloids, and terpenoids (Sahakyan *et al.*, 2019), and also the presence of different vitamins, such as ascorbic acid and carotenoids (Adom *et al.*, 2017).

Pseudomonas aeruginosa is one of the most important and opportunistic pathogens that causes a high rate of mortality and morbidity in hospitalized patients with compromised immune systems (Aghamollaei *et al.*, 2019). It is one of the most important hospital-acquired infection in burnt patients (Al-Daraghi and Al-Badrwi, 2020). *P. aeruginosa* has a tendency to form biofilms, which are compound bacterial groups that stick to a variety of surfaces together with plastics, medical transplant materials, and tissue. They are very difficult to destroy (Al-Daraghi and Al-Badrwi, 2020).

The frequent occurrence of drug resistance and persistent colonization on surfaces makes *P. aeruginosa* particularly difficult to treat and eradicate. Multiple intrinsic and acquired resistant mechanisms are exploited by *P. aeruginosa*, including the modification of drug targets, attenuation of membrane permeability, and formation of biofilms, which, collectively, contribute to its distinctly low antibiotic susceptibility (Taylor *et al.*, 2014).

P. aeruginosa has been classified as an ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogen; one of the six highly antibiotic-resistant bacteria and known for their increasing bacterial virulence (Chen *et al.*, 2018), *P. aeruginosa* is designated by the World Health Organization as a top antibiotic-resistant “priority pathogen” for which new antibiotics are critically required (WHO, 2017). *P. aeruginosa* is the most non-fermenting organism isolated and identified from the clinical specimens, which is resistant to various antibacterial drugs (Valentine *et al.*, 2020). Infections caused by pathogens with multidrug-resistant (MDR) are challenging and difficult to treat (Mirzaei *et al.*, 2020). The study aimed to assess the antibacterial activity of *P. major* petroleum ether extract against *P. aeruginosa*.

Materials and Methods

Place and Period of the Study

The study was carried out in the Microbiology Laboratory, Department of Basic Science, College of Nursing, University of Duhok from May 2022 till February 2023. The study was approved by the Ethics Committee /Scientific Research Division / Directorate of Planning / Duhok General Health Directorate/ Ministry of Health/ Kurdistan Regional Government/ Iraq.

Bacterial Isolation

This study included 408 samples that were collected from different clinical sources (ear swab, urine, and burn) in the hospitals in Duhok city; patients were involved both sexes and different age groups, samples were transported to the Microbiology Laboratory, within 1-2 hrs for culturing, and bacteriological identification. All samples 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 hrs for microbiological identifications. To isolate *P. aeruginosa* each sample was first identified according to general cultural characteristics of colonies (color, shape, and size) grown on Nutrient agar, Blood agar, and MacConkey agar that were incubated at 37°C for 24hrs for further tests.

Identification of *Pseudomonas aeruginosa*

Bacterial identification was done by using Gram stain, biochemical tests (catalase test, oxidase test, IMViC test, and Triple Sugar Iron test), Cetrimide agar, and confirmation test was done by using automated VITEK 2 compact system.

Plant Collection

Fresh *P. major* was collected from the Berwari village, Duhok city, Kurdistan region, Iraq at the beginning of flowering and fruiting from August to September. The plant was taken to the Microbiology laboratory and the leaves in good condition were selected. Then, the leaves were washed with tap water. After that, the leaves were left to dry in the closed room for 5 days, grounded to fine powder by the electrical grinder, and the powdered leaves were stored in containers in the dark until the time of extraction.

Extraction Technique

Preparation of Leaves Petroleum Ether Extract of *Plantago major*

For the extraction of *P. major* leaves, water was used as a solvent, 100 grams of dried and powdered plant materials were extracted with 1000 ml (i.e. 1:10 ratio) of petroleum ether solvent using Soxhlet (Barnstead, USA) (Appendix II) for 10 hrs at a temperature not exceeding the boiling point of solvent (petroleum ether 55°C) (Abd Razik *et al.*, 2012), then the obtained extract was filtered by using filter paper (Whatman No.1).

Yield

The extract obtained from the extraction was evaporated in a rotary evaporator (Stuart, UK) under reduced pressure (650 mmHg), 70 rpm, temperature 40°C, for 5-10 min (Piva *et al.*, 2018).

The final extract was weighed and the yield was calculated using the formula (Astuti *et al.*, 2020):

$$\text{Yield} = \frac{\text{Extract weight (g)}}{\text{Sample weight (g)}} \cdot 100\% \quad (1)$$

A stock solution of the extract was prepared by dissolving 100 mg of extract with 1 ml of petroleum ether solvent to produce a final concentration of 100 mg/ml, which was then diluted to concentrations (75, 50, 25, and 12.5 mg/ml) of extract with appropriate volumes of sterile distilled water needed for the study.

Preparation and Impregnation of the Discs

Twenty microliters of each concentration (100, 75, 50, 25, and 12.5%) from the petroleum ether extract were added to sterile filter paper discs (6 mm in diameter) with the aid of a micropipette (Dragon, China), and allowed them to rest for 10 min until the concentration was completely absorbed, after wards, placed in Muller Hinton agar plates (Tanoglu, 2019).

Gas Chromatography-Mass Spectroscopy (GC-MS)

Gas Chromatography-Mass Spectrometry were performed to analyze the qualitative and quantitative identification of organic compounds in the given sample. The potential biological compounds of *P. major*



leaf extracts were analyzed using GC-MS (Varian Saturn, UAS) system. GC-MS analysis was performed by injecting 2 μ L of each sample into the column (Elite-5MS, 30m $\times$ 0.25mm ID $\times$ 0.25 μ m) with helium as a carrier gas at 1 ml/min rate of flow, and 280°C temperature. The oven temperature was raised from 40 to 280°C with an isothermal for 5 min. The bioactive compounds were identified based on retention time (r.t.), Mass Spectra (MS) fragment ions were generated and the percentage of these bioactive compounds was evaluated from the total peak area. Components have been identified by comparing their MS patterns to the standard MS available at the National Institute of Standards and Technology (NIST) Mass Spectra Database (Chirumamilla *et al.*, 2022).

Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid Chromatography-Mass Spectrometry is a powerful analytical technique used to separate, identify both unknown and known compounds, as well as elucidate the structure and chemical properties of different molecules. Its sensitivity, selectivity, and accuracy are very useful for analyzing multicomponent-containing substances. It involves the physical separation of target compounds followed by their mass-based detection. LC-MS (Agilent, USA) combines the physical separation capabilities of LC with the mass analysis capabilities of MS (Vignesh *et al.*, 2022). LC-MS was used for the determination of bioactive compound (pectin) in *P. major* leaf extracts: aqueous, ethanol, and petroleum ether extracts were centrifuged at 12,000 rpm for 10 min before analysis. The Pectin separation was performed by injecting 5 μ L of each sample into Atlantis T3-C18 column (3 μ m, 2.1 $\times$ 150 mm) at 35°C using a binary mobile phase at a flow rate of 0.2 ml/min. The mobile phase components were (A) ACN + 0.1% Formic Acid 60% and (B) H₂O + 0.1% Formic Acid 40%. The LC conditions were 5% at 0–3 min in B, a linear increase from 5 to 20% between 3 and 25 min, 20 to 40% during 25–40 min, and from 40 to 50% between 40 and 55 min, finally, it reached 50 to 95% at 55–63 min followed by (Vignesh *et al.*, 2022). Identification of the pectin was performed by using a mass spectrometer equipped with an electrospray source interface (ESI) in the negative mode. The mass spectrometer parameters were as follows: Cone Volt 20 V, Capillary Volt 4 kV, Extractor 2V, RF (ratio frequency) Lens 0.2 V, collision-induced dissociation gas pressure (Ar) 224 kPa, drying gas flow (N₂), 20 L/min, nebulizing gas flow (N₂) 200 L/h, source temperature 120°C and desolvation temperature 300°C. Pectin from *P. major* leaf extracts was identified by comparing their retention time (r.t.) and ESI dissociation patterns with those of standards. Pectin quantification was performed by constructing calibration curves using the peak areas of the first transitions. Results were expressed as a mass spectrum (Pereira *et al.*, 2020).

Statistical Analysis

The data of the study were analyzed and determined in number and percentage and the statistical calculations were performed by using Predictive Analytics Software for Scientists and Engineers program (JMP pro 14.3.0) (SAS Institute Inc, North Carolina, USA).

Results

A total of 408 clinical samples from different sources were collected, with an age range of (1-80) years old. According to gender, 216(52.9%) of the patients were females and 192 (47.1%) were male patients.

Morphological and Biochemical Identification

The identity of *P. aeruginosa* was confirmed on the basis of morphological and biochemical characteristics. The colonies appeared green in Nutrient broth and appeared green on Nutrient agar. All the isolates were non-lactose fermenting on MacConkey agar plate, production of β -hemolysis on Blood agar and capable of growing on ceftrimide agar as green colonies (at 37°C for 24 hrs.). The pink-color and rod-shaped appearance of the isolates was confirmed under microscope in Gram staining. Bubbles formation in catalase test; development of deep blue color on filter paper in oxidase test;



formation of yellow ring at surface of pepton broth in indole test; development of deep blue color in citrate utilization test; development of alkaline slant / alkaline butt (K/K reaction) in TSI agar test; fluorescent bacterial colonies under UV light, positive motility test; and an automated VITEK 2 compact system was used to confirm the identification of *P. aeruginosa* isolates, and the diagnostic accuracy of VITEK device reaches approximately 99%.

Incidence of *Pseudomonas aeruginosa* Infections

A total of 408 different clinical samples from (ear, urine, and sputum) were aseptically collected from patients. The samples were screened for isolation of *P. aeruginosa* by traditional and automated tests, including bacteriological tests, antibiotic sensitivity test, as well as VITEK 2 compact system. There were 87 isolates of *P. aeruginosa* (21.3%), while 321 (78.7%).

Gender-Associated Frequency

According to the gender of patients, there were 192/408 samples from male patients and 216/408 from female patients. Out of 160, the high percent of *P. aeruginosa* isolates was observed in females 54.4%, while in male it was 45.6%. There was a significant difference (P value < 0.0001) between males and females in terms of the prevalence of *P. aeruginosa* isolates, as shown in Table (1).

Table 1. *Pseudomonas aeruginosa* Isolates among Males and Females

Gender	No. of samples	No. of positive isolates of <i>P. aeruginosa</i>	%	P. value*
Male	192	36	45.6	< 0.0001
Female	216	51	54.4	
Total	408	87	100	

Note: Chi Square * significant

Age-associated Frequency

The frequency of age group (21-40 years) expressed the highest frequencies of *P. aeruginosa* infection than other types of different age groups with 44 (10.77%), while the lowest frequency of *P. aeruginosa* was 8 (1.96) in age group (61-8- years). A significant difference was noticed (P value < 0.0001) as shown in Table (2).

Table 2. Distribution of *P. aeruginosa* among Different Age Groups

Age Group (Y)	Number of samples (%)	Positive isolates of <i>P. aeruginosa</i> (No %)	Negative isolates of <i>P. aeruginosa</i> (No %)	P. value*
1-20	98 (24)	21 (5.14)	77 (18.86)	< 0.0001
21-40	100 (24.5)	44 (10.77)	56 (13.73)	
41-60	155 (38)	14 (3.43)	141 (34.57)	
61-80	55 (13.5)	8 (1.96)	47 (11.54)	
Total	408 (100)	87 (21.3)	321 (78.7)	

Note: Chi Square test * significant

Pseudomonas aeruginosa Distribution According to the Source of Clinical Sample

The highest expression of *P. aeruginosa* was among the samples of ear swabs (9.55%, respectively), followed by urine (6.61%), while the lowest rate was in sputum swabs (5.14 %), as demonstrated in Table (3).



Table 3. Isolates of *P. aeruginosa* from Different Clinical Samples

Sources	Number of samples (%)	Positive (%)	Negative (%)
Ear	131 (32.1%)	39 (9.55%)	92 (22.55%)
Urine	220 (53.9%)	27 (6.61%)	193 (47.29%)
Sputum	57 (14%)	21 (5.14%)	36 (8.86%)
Total	408 (100%)	87 (21.3%)	321 (78.7%)

Antibiotic Susceptibility Profiles for *Pseudomonas aeruginosa*

The resistance was 100% for chloramphenicol, nitrofurantoin, amoxicillin/clavulanic acid, cefixime, piperacillin, ceftazidime, and cefepime, as in Table (4).

Table 4. Rates of Antibiotic Resistance in Total *P. aeruginosa* Isolates

Antibiotics	Symbol	Disc potency (µg)	No. (%)
Chloramphenicol	C	5	408 (100%)
Nitrofurantoin	F	100	408 (100%)
Cefixime	CFM	10	408 (100%)
Piperacillin	PRL	100	408 (100%)
Ceftazidime	CDZ	30	408 (100%)
Cefepime	FEP	30	408 (100%)

Extraction of *Plantago major* Leaves

One hundred grams of powdered *P. major* leaves were extracted in a total of 1000 ml of petroleum ether in Soxhlet. The extraction mixture was dried in rotary evaporator. Dry extract was weighted and the Extract yield percentage was calculated.

Analysis of *Plantago major* Plant Components

Gas Chromatography-Mass Spectrometry analysis of *Plantago major*

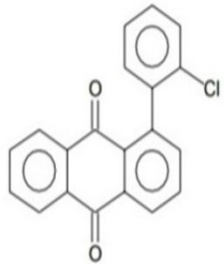
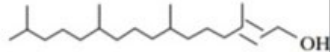
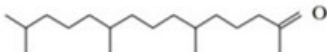
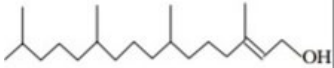
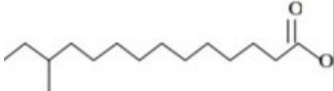
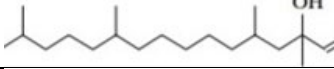
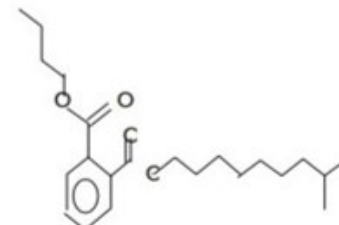
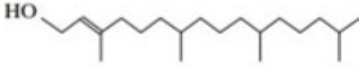
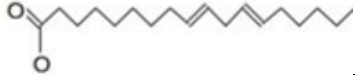
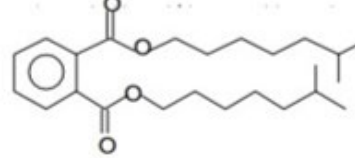
Gas Chromatography-Mass Spectrometry profiling detected potential phytochemicals in *P. major* leaf extracts by their molecular formula and retention time.

Gas Chromatography-Mass Spectrometry analysis of *Plantago major* Petroleum Ether Extract

Eleven phytochemicals that were detected from leaf petroleum ether extract as in Table (5) and Figure (1).



Table 5. GC-MS Analysis of *P. major* Petroleum Ether Extract

Retention Time [RT]	Molecular Formula	Molecular Weight [g/mol]	Compound name	Peak Area [PA%]	Chemical Structure
23.359	C ₂₀ H ₁₁ ClO ₂	318.78	Anthraquinone, 1-(p-chlorophenyl)	1.800	
24.590	C ₃₀ H ₅₀	410.73	Squalene	3.875	
15.828	C ₂₀ H ₄₀ O	296.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5.118	
15.906	C ₁₈ H ₃₆ O	268.478	6,10,14-Trimethylpentadecane-2-one	1.362	
16.080	C ₂₀ H ₄₀ O	296.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.476	
16.713	C ₁₆ H ₃₂ O ₂	256.42	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	0.966	
16.938	C ₂₀ H ₄₀ O	296.531	1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-	0.844	
17.155	C ₂₂ H ₃₄ O ₄	362.5	1,2-Benzenedicarboxylic acid, butyl 8-methynonyl ester	14.218	
18.490	C ₂₀ H ₄₀ O	296.5	Phytol	43.848	
18.737	C ₁₈ H ₃₂ O ₂	280.4	9,12-Octadecadienoic acid (Z,Z)-	23.164	
21.200	C ₂₄ H ₃₈ O ₄	390.6	1,2-Benzenedicarboxylic acid, diisooctyl ester	3.329	



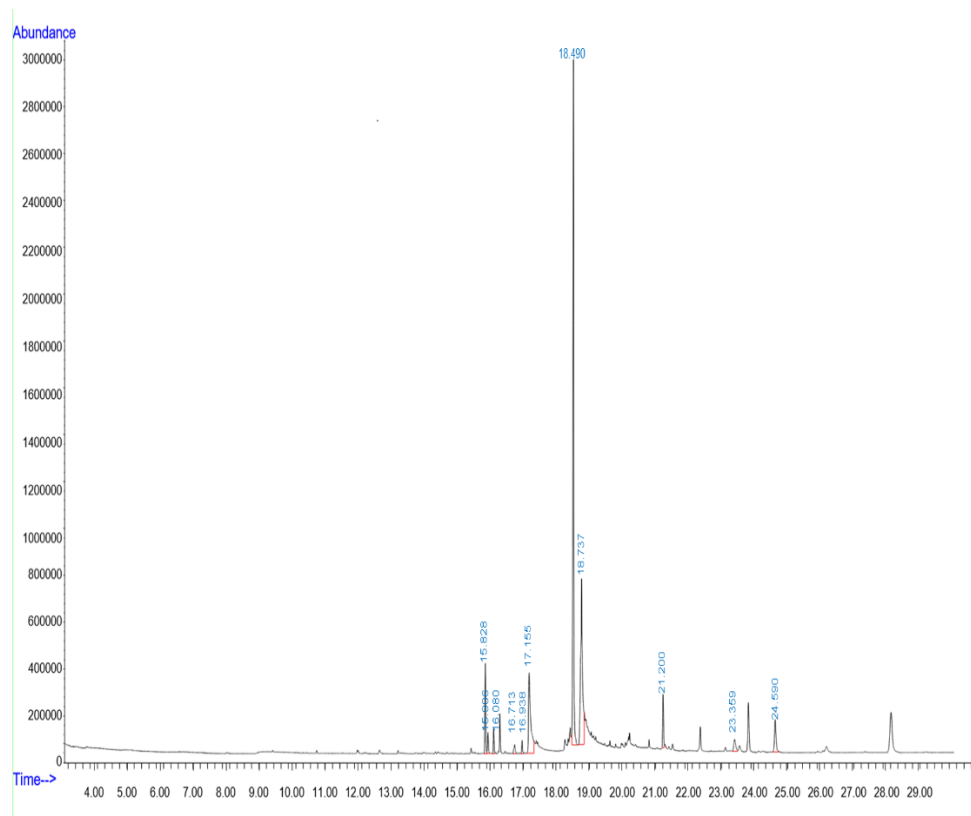


Figure 1. GC-MS Chromatogram of *P. major* Petroleum Ether Extract

Liquid Chromatography-Mass Spectrometry analysis of *Plantago major*

Pectin found in *P. major* leaf petroleum ether extracts using LC-MS analysis, as identified in Figure (2)

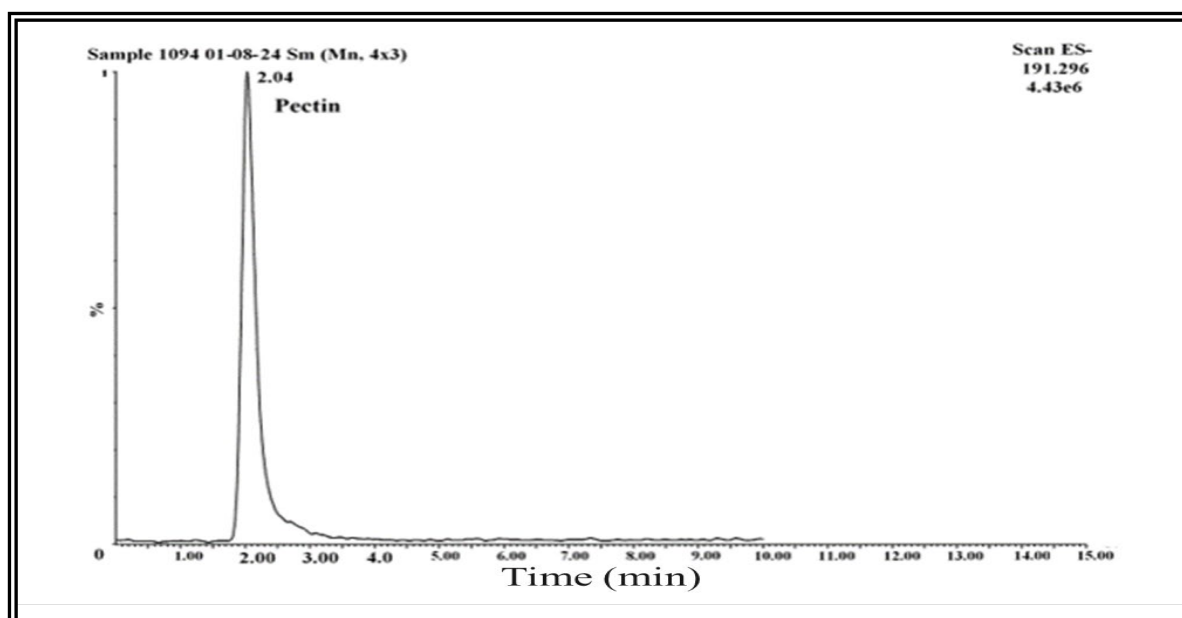


Figure 2. Chromatograms Obtained by LC-MS Showing the Pectin Compound Identified in Petroleum Ether Extract from the Obtained *P. major* Leaves



Antibacterial Activity of *Plantago major* Using Agar Well Diffusion Method

The antibacterial activity of various concentrations of aqueous, ethanol and petroleum ether extracts of the *P. major* leaves against *P. aeruginosa* bacterium were first determined using the diffusion method for antimicrobial susceptibility test (Table 6). The results of the current study showed that petroleum ether *P. major* leaves extract had antimicrobial effects on the growth of *P. aeruginosa* isolates ranging from (1.93-9.63) mm diameter zone of inhibition. (Figure 3).

Table 6. Inhibitory Activity of *P. major* Leaves Extracts in Different Concentrations against *P. aeruginosa* Isolates

Concentration of extracts %	Average diameter of inhibition zone (mm) by petroleum ether extract
100	9.63
75	7.29
50	5.24
25	2.68
12.5	1.93

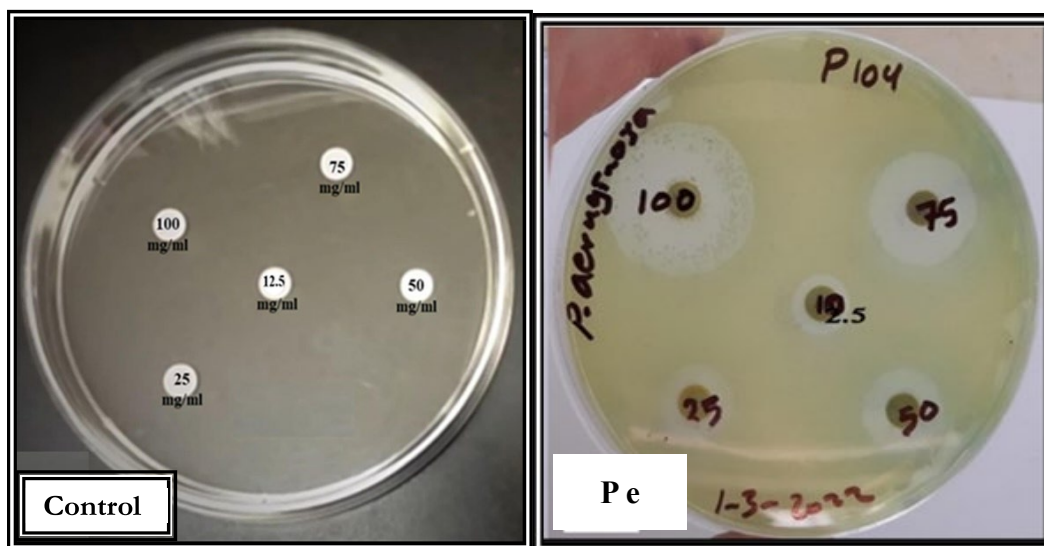


Figure 3. Antibacterial Activity of *P. major* Leaves; Petroleum Ether Extracts in Different Concentrations against *P. aeruginosa* Isolates

Discussion

Antibacterial resistance to currently utilized drugs has caused major challenges. This resistance has not only hampered the treatment of microbial diseases but has also increased the cost of treatment. Therefore, there is a need to find alternative methods of curbing infectious microorganisms (Kaigongi, 2014). It is well known that *P. aeruginosa* infections are associated with high morbidity and mortality as well as are very hard to kill due to their ability to adapt their environmental changes, to express many virulence factors, and to acquire resistance to many medicines and antibiotics (Ghanem *et al.*, 2023).

Isolation and Identification of *Pseudomonas aeruginosa*

In the current study, out of 408 clinical samples that were obtained and collected from patients, 87 (21.3%) isolates of *P. aeruginosa* were detected and identified, this result is similar to a previous study in Spain done by Causapé and colleagues, who indicated that 79/341 (23.2%) of *P. aeruginosa* were identified



(Causapé *et al.*, 2017). In addition, Al-Saffar and Jarallah, found that 20 (25%) isolates from 80 samples collected from different clinical and environmental sources were identified as *P. aeruginosa* in AL-Hilla Teaching Hospital/ Babylon/ Iraq (Al-Saffar and Jarallah, 2019). A study in Baghdad detected 40 (26.7%) *P. aeruginosa* out of 150 samples (Al-Daraghi and Al- Badrwi, 2020). In the other direction, a study done by reported AL-Fridawy *et al.*, that *P. aeruginosa* isolates were 54 (36%) out of 150 (AL-Fridawy *et al.*, 2020). This rate was higher than the current study and this may be due to differences with regard to the sources of samples and sites of isolation. In earlier research done by other researchers, pointed out that the rate of occurrence of *P. aeruginosa* (51.50%) is the highest among other bacteria isolated from clinical samples included in the study (Hussein *et al.*, 2018).

Prevalence of *Pseudomonas aeruginosa* Regarding Gender and Age Group

In the current study, frequencies of *P. aeruginosa* among different age groups have the highest infection rate in the age group 21-40 years. The age group 41-60 years, comes in the second degree, compared to other age groups, this could be due to the low immunity of this age group and the nature of the samples collected. The emergence of *P. aeruginosa* among females is more than among males 51.34% versus 48.66%, respectively, in the age group 41-60 (19.52%) less than 21-40 (33.18%) and it was statistically significant ($P < 0.0001$). This result was in agreement with Tran and Co-workers (2023) who found that among 19 *P. aeruginosa* isolates, 63.16% (12/19) were females and 36.84% (7/19) were males aging from 2 to 73 years (Tran *et al.*, 2023). Another study nearly correlated with the current results, in Sudan found that the distribution of *P. aeruginosa* was 54% females and 46% from males, ages ranged from 4 to 76 years, with an average of 49 (Abdallah, 2022). In contrast with the current study, Ali, in Pakistan found 59 (31.6%) *P. aeruginosa* isolates obtained from female patients and 128 (68.4%) from male patients (Ali, 2019). Haghighi and Goli, in Iran found that, out of 100 isolates, 60 (60%) of them were obtained from men while 40 (40%) were from women, and the mean age of women was 47.85 and the mean age of men was 44.76 (Haghighi and Goli, 2022). *P. aeruginosa* commonly infected all ages, but the highest frequency of bacterial isolates was in the adults' age and the older patients more than in children, however, the risk of infections in different sites changed significantly with age, changes in hormonal status, a decline in the immune system, and malnutrition (Abdulrahman *et al.*, 2023).

Distribution of *Pseudomonas aeruginosa* among Different Clinical Samples

In the current study, the frequencies of *P. aeruginosa* isolates were detected at most from ear samples 39/131(9.55%), and followed by urine 27/220 (6.61%), whereas the lowest rate was among sputum samples 21/57(5.14%).

A study conducted at Minia University Hospital/ Egypt showed different rates which were urine (15/50), sputum (27/87), ear discharges (32/83), wound exudates (35/85), and stool (16/45) (Ghanem *et al.*, 2023). *P. aeruginosa* isolates were isolated in Turkey, among them 36 (18%) sputum, 34 (17%) urine, and 10 (5%) wound swabs (Cayci, *et al.*, 2022). Other studies showed different rates which were in contrast to this result in all different samples. For example, in Jeddah / Saudi Arabia, the majority (32.5%) of isolated *P. aeruginosa* were from urine, (18.2%) wounds, (13.6%) ear swabs, and (13%) sputum (Abdulrahman *et al.*, 2023). Other researchers have pointed out that the highest rate was in the ear (27.5%), then wound (12.5%), and burn (5%) (Al-Daraghi and Al-Badrwi, 2020).

The variation in the occurrence rate of *P. aeruginosa* isolates which lead to resistance to different antibiotics in a different clinical samples and different regions may be due to the sample size used for the study, co-existence with other resistant pathogen and the uncontrollable use of antibiotics (Mohammed, 2020).



Antimicrobial Susceptibility Patterns

The increased rate of antibiotic resistance was observed in the current study. Isolates of *P. aeruginosa* (87) were subjected to the Kirby Bauer test with 16 antibiotics, and VITEK 2 compact system in order to confirm the bacterial identification.

In this study, the results exhibited that all of the 87 isolates were 100% resistant to chloramphenicol, nitrofurantoin, cefixime, piperacillin, ceftazidime, and cefepime. This is in line with a study by Okezie *et al.*, who reported that a 100 % level of resistance was recorded by all *P. aeruginosa* isolates against ceftazidime, cefuroxime, and nitrofurantoin while a 99.9 % resistance was recorded against cefixime (Okezie *et al.*, 2021). This corroborates the work of Saleh and Co-workers in Baghdad, who found that 100% of *P. aeruginosa* isolates were resistant to ampicillin, cefixime, erythromycin, naldixic acid, chloramphenicol, and bacitracin (Saleh *et al.*, 2021). Another study nearly correlated with the current results, in Qatar found that the resistance rate of *P. aeruginosa* isolates on cefepime was (96.6%) (Ahmed *et al.*, 2019). While in 2023 a study showed intermediate resistance of *P. aeruginosa* to ampicillin (90%), amoxicillin/clavulanic acid (80%), cefixime and erythromycin (80%) (Arbaba *et al.*, 2023). In China, the lowest rate than the current study was reported by Guo and his colleagues, who showed that 41.2% and 47.6 % of the isolates were resistant to ceftazidime, and cefepime, respectively (Guo *et al.*, 2022). Farhan *et al.*, in Egypt, found that the highest resistance rate of ceftazidime against *P. aeruginosa* (60%), which disagrees with the results of the current study (Farhan *et al.*, 2019). Resistance rate differences among the different countries and periods are mainly related to the prescription habits and drug use in each country (Arbaba *et al.*, 2023).

Antibacterial Activity of *Plantago major* Extract

The rise of pathogenic microorganisms and their resistance to a widerange of antibiotics along with the economic and social problems resulting from them have led to the expansion of studies on the production of herbal medicines (Sales and Pashazadeh, 2020). Therefore, the use of plants as a source of novel compounds to combat microbial infections has gained prominence (Demeke, 2014). The necessity to search for plant-based antimicrobial compounds that can inhibit the growth of pathogenic microorganisms or kill them without toxicity to host cells are considered candidates for the production of new antimicrobial drugs is increasing due to high cost, reduced efficacy and increased resistance to conventional medicines (Sales and Pashazadeh, 2020).

P. major is a medicinal plant that is available all around the world, it has several medical benefits like wound healing, anti-inflammatory, antimicrobial, anti-ulcerative, and anti-oxidative agents (Keshavarzi *et al.*, 2022). Leaves of *P. major* have been used for centuries to treat diseases relating to skin, digestive organs, and blood circulation like wounds, inflammation, and hypertension. *P. major* leaves contain biologically active substances and natural compounds, such as essential oils, minerals, and amino acids besides polysaccharides, lipids, flavonoids, and terpenoids (Turgumbayeva *et al.*, 2022). The result obtained from this work indicated that all the extracts showed varying degrees of antimicrobial activity on the tested microorganism. The petroleum ether extract of leaves has an antibacterial activity in the growth of *P. aeruginosa*, and the diameter zones of inhibition were 1.93-9.63 mm. Based on the dose response, the zone of inhibition increased with increasing the concentration of petroleum ether extracts. The lowest concentration (12.5 mg/ml) resulted in weak inhibition of the bacterium, while the higher concentrations of each extract (100, 75, 50, and 25 mg/ml) recorded noticeable inhibition activity against the bacterium.

Data from the present study for petroleum ether extract of *P. major* leaves showed the lowest antibacterial activity (1.93-9.63 mm diameter of inhibition zone) on *P. aeruginosa* isolates, which was in disagreement with earlier researches done by other researchers who reported that the petroleum ether did not show any significant activity against *P. aeruginosa* (Abbasi *et al.*, 2022).



Failure of some of the extracts to exert an antibacterial effect upon the test organism is not enough to conclude that the extract does not contain substances that can exert antibacterial activity against the test organism because the potency of the extract depends on the method used to obtain the extract (Vaghasiya and Chanda, 2007). Research has also shown that the difference in antimicrobial properties of the plant extracts might be attributable to the age of the plant used, freshness of the plant material, physical factor (temperature, light, or water), contamination by field microbes, and incorrect preparation of the plant, etc. (Demeke, 2014).

Conclusions

From the results of the present study, the following are concluded:

- The majority of *P. aeruginosa* isolates were collected from ear, followed by urine, and sputum swabs.
- This study proves that herbal extract is more effective and can also be used as a replacement for chemical drugs and antibiotics in the elimination of bacterial growth, and the antibacterial activity of *P. major* against *P. aeruginosa* gives a clue to the strong potential of the mentioned plant for drug discovery with a broad spectrum of activity.

References

- Abbasi, A., Maddah, S.M., Ali, D.S., & Kamalinejad, M. (2022). Antibacterial Activity of Herbal *Plantago major* and *Plantago lanceolata* extracts on *Pseudomonas aeruginosa* with emphasis on Exotoxin A gene expression and Bioinformatics approach. ResearchSquare. <https://doi.org/10.21203/rs.3.rs-1637465/v1>
- Abd Razik, B.M., Hasan, H.A., & Murtadha, M.K. (2012). The study of antibacterial activity of *Plantago major* and *Cerantonia siliqua*. The Iraqi Postgraduate Medical Journal, 11(1), 130-135.
- Abdallah, A.M.M. (2022). *Antimicrobial Susceptibility and Molecular Detection of Carbapenem-Resistant Genes NDM-1 and IMP-1 of Pseudomonas aeruginosa Isolated from Clinical Specimens in Khartoum State-Sudan*. MSc Thesis. Science and Technology College, Sudan University.
- Abdulrahman, W.M.A., Mohamed, E.B.S., Saidi, F.M.S., & Elhassan, M.M. (2023). Frequency of Metallo-B-Lactamase among *Pseudomonas aeruginosa* Isolated from patients In Intensive Care Units and Operating Rooms. *Egypt. Acad. J. Biolog. Sci.*, 15(1), 1-11.
- Adom, M. B., Taher, M., Mutalabisin, M. F., Amri, M. S., Abdul Kudos, M. B., Wan Sulaiman, M. W. A., Sengupta, P., & Susanti, D. (2017). Chemical constituents and medical benefits of *Plantago major*. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, 96, 348–360. <https://doi.org/10.1016/j.biopha.2017.09.152>
- Aghamollaei, H., Moghaddam, M. M., Kooshki, H., Heiat, M., Mirnejad, R., & Barzi, N. S. (2015). Detection of *Pseudomonas aeruginosa* by a triplex polymerase chain reaction assay based on *lasI/R* and *gyrB* genes. *Journal of infection and public health*, 8(4), 314–322. <https://doi.org/10.1016/j.jiph.2015.03.003>
- Sid Ahmed, M. A., Hassan, A. A. I., Abu Jarir, S., Abdel Hadi, H., Bansal, D., Abdul Wahab, A., Muneer, M., Mohamed, S. F., Zahraldin, K., Hamid, J. M., Alyazidi, M. A., Mohamed, M., Sultan, A. A., Söderquist, B., Ibrahim, E. B., & Jass, J. (2019). Emergence of Multidrug- and Pandrug- Resistant *Pseudomonas aeruginosa* from Five Hospitals in Qatar. *Infection prevention in practice*, 1(3-4), 100027. <https://doi.org/10.1016/j.infpip.2019.100027>



- Al-Daraghi, W.A.H., and Al-Badrwi, M.S.A. (2020). Molecular Detection for Nosocomial *Pseudomonas aeruginosa* and its Relationship with multidrug Resistance, Isolated from Hospitals Environment. *Medico-legal Update*, 20(1). <https://doi.org/10.37506/mlu.v20i1.433>
- AL-Fridawy, R.A.K., Al-Daraghi, W.A.H., & Alkhafaji, M.H. (2020). Isolation and Identification of Multidrug Resistance among Clinical and Environmental *Pseudomonas aeruginosa* Isolates. *Iraqi Journal of Biotechnology*, 19(2), 37-45.
- Ali, A. (2019). *Genetic Diversity and Molecular Mechanisms of Antibiotic Resistance and Virulence in clinical Isolates of Pseudomonas aeruginosa*. PhD Thesis. Centre of Biotchnology and Microbiology, University of Peshawer, Pakistan.
- Al-Saffar, M.F., & Jarallah, E.M (2019). Isolation and Characterization of *Pseudomonas aeruginosa* from Babylon Province. *Biochem. Cell. Arch.* 19(1), 203-209. <http://dx.doi.org/10.13140/RG.2.2.22943.20644>
- Arbab, S., Ullah, H., Wei, X., Wang, W., Ahmad, S. U., & Zhang, J. (2021). Drug resistance and susceptibility testing of Gram negative bacterial isolates from healthy cattle with different β - Lactam resistance Phenotypes from Shandong province China. *Brazilian journal of biology = Revista brasleira de biologia*, 83, e247061. <https://doi.org/10.1590/1519-6984.247061>
- Astuti, A.D., Etikawati, N., Pangastuti, A. (2020). Antibacterial activity of *Plantago major* leaves against *Streptococcus pyogenes* ATCC 19615 as a cause of tonsillitis. *Bioteknologi*, 17(1), 14-21.
- López-Causapé, C., de Dios-Caballero, J., Cobo, M., Escribano, A., Asensio, Ó., Oliver, A., Del Campo, R., Cantón, R., Solé, A., Cortell, I., Asensio, O., García, G., Martínez, M. T., Cols, M., Salcedo, A., Vázquez, C., Baranda, F., Girón, R., Quintana, E., Delgado, I., ... Cantón, R. (2017). Antibiotic resistance and population structure of cystic fibrosis *Pseudomonas aeruginosa* isolates from a Spanish multi-centre study. *International journal of antimicrobial agents*, 50(3), 334–341. <https://doi.org/10.1016/j.ijantimicag.2017.03.034>
- Cayci, Y.T., Biyik, I., & Birinci, A. (2022). VIM, NDM, IMP, GES, SPM, GIM, SIM Metallobetalactamases in Carbapenem-Resistant *Pseudomonas aeruginosa* Isolates from a Turkish University Hospital. Research Article. *J Arch Mil Med.* 10(1), e118712. <http://dx.doi.org/10.5812/jamm-118712>
- Chen, J. W., Lau, Y. Y., Krishnan, T., Chan, K. G., & Chang, C. Y. (2018). Recent Advances in Molecular Diagnosis of *Pseudomonas aeruginosa* Infection by State-of-the-Art Genotyping Techniques. *Frontiers in microbiology*, 9, 1104. <https://doi.org/10.3389/fmicb.2018.01104>
- Chirumamilla, P., Dharavath, S. B., & Taduri, S. (2022). GC-MS profiling and antibacterial activity of *Solanum khasianum* leaf and root extracts. *Bulletin of the National Research Centre*, 46(1), 127. <https://doi.org/10.1186/s42269-022-00818-9>
- Demeke, A. (2014). *Antimicrobial and bacteria cultivative value of Moringa olifera leaf extract in selected animal pathogens*. MSc Thesis. College of Veterinary Medicine and Agriculture, Addis Ababa University, Ethiopia.
- Farhan, S. M., Ibrahim, R. A., Mahran, K. M., Hetta, H. F., & Abd El-Baky, R. M. (2019). Antimicrobial resistance pattern and molecular genetic distribution of metallo- β -lactamases producing *Pseudomonas aeruginosa* isolated from hospitals in Minia, Egypt. *Infection and drug resistance*, 12, 2125–2133. <https://doi.org/10.2147/IDR.S198373>
- Ghanem, S. M., Abd El-Baky, R. M., Abourehab, M. A. S., Fadl, G. F. M., & Gamil, N. G. F. M. (2023). Prevalence of Quorum Sensing and Virulence Factor Genes Among *Pseudomonas aeruginosa* Isolated from Patients Suffering from Different Infections and Their Association with Antimicrobial Resistance. *Infection and drug resistance*, 16, 2371–2385. <https://doi.org/10.2147/IDR.S403441>



- Guo, L., Xu, H., & Yue, Z. (2022). Antibiotic resistance pattern of *Pseudomonas aeruginosa* wound isolates among Chinese burn patients: A systematic review and meta-analysis. *Asian Pacific Journal of Tropical Medicine*, 15(1), 17-25. <https://doi.org/10.4103/1995-7645.335703>
- Haghighi, S., & Reza Goli, H. (2022). High prevalence of *bla*VEB, *bla*GES and *bla*PER genes in beta-lactam resistant clinical isolates of *Pseudomonas aeruginosa*. *AIMS microbiology*, 8(2), 153–166. <https://doi.org/10.3934/microbiol.2022013>
- Hussein, Z.K., Kadhim, H.S., Hassan, J.S. (2018). Detection of New Delhi Metallo-Beta-Lactamase-1 (*bla*NDM-1) in Carbapenem-Resistant *Pseudomonas aeruginosa* Isolated from Clinical Samples in Wasit Hospitals. *Iraqi JMS*, 16(3). <http://dx.doi.org/10.22578/IJMS.16.3.3>
- Kaigongi, M.M. (2014). *AIGONGI, antimicrobial activity, toxicity and phytochemical analysis of four medical plants traditionally used in Msambweni district, Kenya*. MSc Thesis. School of Biological Sciences, University of Nairobi.
- Kartini, S. P., Suchitra, T., Pongpun Siripong, P., & Omboon Vallisuta, O. (2017). Effects of *Plantago major* Extracts and Its Chemical Compounds on Proliferation of Cancer Cells and Cytokines Production of Lipopolysaccharide -activated THP-1 Macrophages. *Pharmacognosy Magazine*, 13(51).
- Keshavarzi, A., Montaseri, H., Akrami, R., Moradi Sarvestani, H., Khosravi, F., Foolad, S., Zardosht, M., Zareie, S., Saharkhiz, M. J., & Shahriarirad, R. (2022). Therapeutic Efficacy of Great Plantain (*Plantago major* L.) in the Treatment of Second-Degree Burn Wounds: A Case-Control Study. *International journal of clinical practice*, 2022, 4923277. <https://doi.org/10.1155/2022/4923277>
- Mirzaei, B., Bazgir, Z. N., Goli, H. R., Iranpour, F., Mohammadi, F., & Babaei, R. (2020). Prevalence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) phenotypes of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated in clinical samples from Northeast of Iran. *BMC research notes*, 13(1), 380. <https://doi.org/10.1186/s13104-020-05224-w>
- Mohammed, H.H. (2020). *Phenotypic and Molecular Detection of Carbapeneme Resistant Klebsiella pneumoniae In Dubok province/ Kurdistan Region/ Iraq*. PhD Thesis, College of Medicine, University of Duhok.
- Najafian, Y., Hamed, S. S., Farshchi, M. K., & Feyzabadi, Z. (2018). *Plantago major* in Traditional Persian Medicine and modern phytotherapy: a narrative review. *Electronic physician*, 10(2), 6390–6399. <https://doi.org/10.19082/6390>
- Okezie, U.M., Okoye, A.C.E., Adione, N.M., Ezejiegu, C.K., Anyanwu, O.O., Ngwoke, K.G. (2021). The AntibioGram of Pyocyanin-Producing Laboratory and Environmental *Pseudomonas aeruginosa* Isolates. *JCBR*, 1(4), 1-12.
- Pereira, G. A., Arruda, H. S., de Moraes, D. R., Peixoto Araujo, N. M., & Pastore, G. M. (2020). Mutamba (*Guazuma ulmifolia* Lam.) fruit as a novel source of dietary fibre and phenolic compounds. *Food chemistry*, 310, 125857. <https://doi.org/10.1016/j.foodchem.2019.125857>
- Sahakyan, N.Z., Ginovyan, M.M., Petrosyan, M.T., Trchounian, A.H. (2019). Antibacterial and Anti-phage activity of *Plantago major* L. raw material. Proc. of the Yerevan State Univ. *Chemistry and Biology*, 53(1), 59-64.
- Saleh, B.H., Ibrahim, R.N., Aal Owai, H.A. (2021). Study the Effect of Ultraviolet (UV) on Mutagenicity of *Pseudomonas aeruginosa* Isolated from Burn Wound Infections. *Indian Journal of Forensic Medicine & Toxicology*, 15(2). <https://doi.org/10.37506/ijfmt.v15i2.14833>
- Sales, A. J., and Pashazadeh, M. (2020). Research article. Antibacterial Effect of Methanolic Extract of Saffron Petal (*Crocus sativus* L.) on Some Standard Gram Positive and Gram Negative Pathogenic Bacteria



In vitro. *Current Perspectives on Medical and Aromatic Plants*, 3(1), 1-7.
<https://doi.org/10.38093/cupmap.692879>

Tanoglu, F. (2019). *Investigation of Antioxidant and Antimicrobial Activity of Ficus sycomorus Fruit and Leaf Extracts*. MSc Thesis, School of Applied Sciences, Near East University, Nicosia, Cyprus.

Tariq, S., and Mehmood, A. (2022). Pharmacognosy and Phytochemical Screening of *Plantago major* L. and *Plantago lenceolata* L. from Rawalakot, Azad Jammu and Kashmir: Research Paper. *Kashmir Journal of Science*, 1(1), 1-11.

Taylor, P. K., Yeung, A. T., & Hancock, R. E. (2014). Antibiotic resistance in *Pseudomonas aeruginosa* biofilms: towards the development of novel anti-biofilm therapies. *Journal of biotechnology*, 191, 121–130.
<https://doi.org/10.1016/j.jbiotec.2014.09.003>

Tran, N. B. V., Truong, Q. M., Nguyen, L. Q. A., Nguyen, N. M. H., Tran, Q. H., Dinh, T. T. P., Hua, V. S., Nguyen, V. D., Lambert, P. A., & Nguyen, T. T. H. (2022). Prevalence and Virulence of Commensal *Pseudomonas Aeruginosa* Isolates from Healthy Individuals in Southern Vietnam (2018-2020). *Biomedicine*, 11(1), 54. <https://doi.org/10.3390/biomedicine11010054>

Turgumbayeva, A., Zhakipbekov, K., Shimirova, Z., Akhelova, S., Amirkhanova, A., & Koilybayeva, M., (2022). Study of phytochemical compounds of *Plantago major* leaves grown in Kazakhstan. *Pharmacia*, 69(4), 1019-1026. <https://doi.org/10.3897/pharmacia.69.e96526>

Vaghasiya, Y., & Chanda, S.V. (2007). Screening of Methanol and Acetone Extracts of Fourteen Indian Medicinal Plants for Antimicrobial Activity. *Turkish Journal of Biology*, 31, 243-248.

Valentine, M. E., Kirby, B. D., Withers, T. R., Johnson, S. L., Long, T. E., Hao, Y., Lam, J. S., Niles, R. M., & Yu, H. D. (2020). Generation of a highly attenuated strain of *Pseudomonas aeruginosa* for commercial production of alginate. *Microbial biotechnology*, 13(1), 162–175. <https://doi.org/10.1111/1751-7915.13411>

Vignesh, A., Selvakumar, S., Krishnan Vasanth, K. (2022). Comparative LC- MS analysis of bioactive compounds, antioxidants and antibacterial activity from leaf and callus extracts of *Saraca asoca*. *Phytomedicine Plus*. <https://doi.org/10.1016/j.phyplu.2021.100167>

World Health Organization. (2017). *Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics*. World Health Organization, Geneva, Switzerland.

