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Journal of Internal Medicine and Health Affairs
info@mediresonline.org

ISSN: 2836-2411 RESEARCH ARTICLE

Effects of *Plantago major* leaf aqueous extracts against *Pseudomonas aeruginosa* isolated from wound infections

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Received Date: 05 April 2023; **Accepted Date:** 17 April 2023; **Published date:** 30 April 2023

Citation: Alaa'T.Monawer, (2023). Effects of Plantago major leaf aqueous extracts against Pseudomonas aeruginosa isolated from wound infections. Journal of Internal Medicine and Health Affairs 2 (2). DOI: 10.58489/2836-5038/016

Abstract

The use of herbal extracts is becoming more popular as a result of the rise in bacterial resistance to conventional antibiotics. Plantago major is a common herbal plant used in conventional medicine. It was well known to have a number of medicinal benefits. The current study aimed to evaluate the antibacterial activity of the aqueous extract of *P. major* leaves against *Pseudomonas aeruginosa* isolated from burn infections. One hundred and thirty-five of *P. aeruginosa* were collected from hospitalized patients in the Emergency hospital in Duhok city. The isolates were identified by routine methods. *P. major* leaves antibacterial activity were performed by using aqueous extract in serial dilutions with the concentrations of (100, 75, 50, 25, and 10 %) by agar well diffusion assay, also antibiotics susceptibility test was done by the disc diffusion method using Muller-Hinton agar medium. Varied aqueous extract of *P. major* leaves concentrations showed different inhibitory zones of *P. aeruginosa.* The inhibitory zone measured by the efficacy of the aqueous extract of *P. major* leaves on *P. aeruginosa* ranges from [6.8- 16.4] mm in diameter. The zone of inhibition grew as extract concentration increased; P. aeruginosa was most inhibited by an extract concentration of [100%] ethanolic extract, which exhibited a [16.4 mm] inhibition zone. Additionally, the bacteria display ed a high level of resistance to the utilized antibiotics. The results of this study show that the replacement of chemical drugs with herbal extract could be effective in the elimination of bacterial growth.

Keywords: *aqueous, plantago major, pseudomonas aeruginosa, wound*

Introduction

Pseudomonas aeruginosa is a multidrug-resistant pathogen, recognized early for its ubiquity, and its mechanism of advanced inherently antibiotic resistance [Abkhoo and Jahani, 2017]. The antibiotics are among the most common methods used to eliminate pathogenic bacteria and of great benefit in maintaining human health. However, the resistance of many pathogenic bacteria to these antibiotics is one of the biggest challenges facing humans let alone their adverse effects in humans' bodies [Al-Wazni et al., 2018; Liu et al., 2017]. During the last several decades, natural products with antimicrobial effects have been investigated to eliminate the use of synthetic antibiotics which cause the resistance of microorganisms and can exhibit side effects on human health [Mahato and Sharma, 2018]. *P. aeruginosa* is an aerobic, rod-shaped Gram-negative bacteria. It is extensively dispersed in nature ada adapts to a variety of situations; in hospitals, it may be isolated from almost any sources [Brooks et al., 2013]. It is a significant contributor to both hospital- and community-acquired illnesses.

When compared to other bacterial pathogens, these bacteria have been linked to significant death and morbidity rates of infections [Brusselaers et al., 2011].

Infections with *P. aeruginosa* are a clinical concern that are challenging to treat due to their high level of antibiotic resistance (multi-

drug resistance) and a high chance of resistance emerging during therapy [Livermore, 2012; Hussein et al., 2018].

The use of plants and seeds has been a widespread practice in folk medicine in urban and rural areas as an alternative or complementary treatment of

conventional medicine [Samuelsen, ,2000; Mello et al., 2015]. The genus Plantago (Plantaginaceae) [Spring, 1989], popularly known as tansagem or “Barhang” in Traditional Persian Medicine, is widely distributed worldwide, including 275 species [Samuelsen, 2000]. *P. major* originate in Northern Europe and Central Asia and adapt well in tropical regions. *P. major* species is easily propagated by seeds that are small, with high roughness and resistance [Hassemer, 2020].

As an anesthetic, antiviral, anti-inflammatory, antihistamine, antitumor, diuretic, and hypotensive, P. major has medicinal value [Samuelsen, 2000] in common treatment [Zubair et al., 2012; Palavicini et al., 2022].

The objective of the present study to determine the in vitro antibacterial effect of different concentrations of the aqueous extract from the leaves of *Plantago major* against pathogenic bacterium *Pseudomonas aeruginosa.*

Materials and Methods 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 135 clinical samples that were collected from burn infections from patients at the emergency hospital in Duhok city; including both sexes & different ages, & transported to Microbiology Laboratory at Department of Basic Sciences, College of Nursing within 1-2 hours for cultures, & bacteriological detections.

Bacterial Identification

All isolates were first cultured using sterile cotton swabs in sterile vials containing Nutrient broth & Amies transport medium swabs that were incubated overnight at 37 °C / 24 h for microbiological identifications. To isolate *P. aeruginosa* each sample was then inoculated onto Nutrient, blood & MacConkey agar. Then Bacterial identification was done by using Gram stain, biochemical tests (catalase test, oxidase test, IMViC test & Triple Sugar Iron [TSI] test] & Cetrimide agar. Confirmation test done by using Automated Vitek 2 system.

Sample collection

Protocol for Obtaining the Extract

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

The plant was taken to Department of Basic Sciences, College of Nursing, University of Duhok.

The plant was transported to the Microbiology Laboratory of the same university, where leaves that were in good condition were selected. Then, the plant leaves were washed with tap water.

After that the leaves left to dry in shade for 5 days. Then ground to fine powder by a mortar & pestle & by mechanical grinder, then stored in containers in dark until extraction.

Preparation of plant leaves extract Soxhlet apparatus Extraction method

(30) grams of *P. major* leaves powder was macerated in (300) ml [i.e. 1:10 ratio] of aqueous solvent by using soxhlet apparatus for 8 hrs. at a temperature not exceeding the boiling point of the solvents [Abd Razik et al., 2012], then the extract was filtered through Whatman NO. 1 filter paper. The extract was stored in screw cup bottles were kept in refrigerator at 4 °C until use. Extraction solvent was evaporated & the extract was concentration at room temperature or by the oven.

A stock solution of the extract was prepared by dissolving 0.1 g of extract with 100 mL of aqueous to produce a final concentration of 100 mg/mL, then the stock solution [100 mg/mL] was diluted to concentrations [doses] [75, 50, 25, and 10 mg/mL of extract] with appropriate volumes of sterile distilled water needed for the study.

Antibacterial activity

The Antibacterial effect of the plant extract was evaluated using the disk inhibition zone method. In this method (Kirby and Bauer) [Ibrahim et al., 2021], 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. After incubation for 24 hrs at 37°C, the antimicrobial activity was measured in diameter of the inhibition zone formed around the disk. At the same time, a comparison antibiotic control test was made using commercial disks (Amoxicillin/clavulanic acid, Cefixime, Chloramphenicol, and Nitrofurantoin), and the diameter of the inhibition zones were measured in mm.

Results

Out of 135 samples only 25 samples showed positive isolates for *P. aeruginosa* as demonstrated in table [1]

Table 1: Number & percentage of P. aeruginosa isolates

Source	Number of samples	Positive	Negative
Wound	135 [100%]	25 [18.5%]	110 [81.5%]

The results of this study showed that Ethanolic *P. major* leaves extract had antimicrobial effects on the growth of *P. aeruginosa* isolates as indicated in table [2]

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

Concentration of extracts %	Average diameter of inhibition zone (mm) by Ethanol extract
100	16.4
75	14.6
50	12.6
25	9.9
10	6.8

Commercial Antibiotic Sensitivity Testing: The bacterial isolates (*P. aeruginosa*) were also tested for their susceptibility against commonly used (commercial) antibiotics by the modified Kirby-Bauer method, as shown in table [3].

Table 3: Antibiotic sensitivity pattern with the inhibition zones of each antibiotic discs used against *P. aeruginosa* isolates

Antibiotics	Symbol	Disc potency [µg]	The average diameter of the inhibition zone (mm)
Amoxicillin / clavulanic acid	AMC	3	R [0]
Cefixime	CFM	10	R [0]
Chloramphenicol	C	5	R [0]
Nitrofurantoin	F	100	R [0]

Discussion

The development of bacterial resistance to antimicrobials is one of the most significant and rapidly spreading health challenges in the world.

As the issue increase, it gets harder to combat; for example, illnesses brought on by these germs are unlikely to be successfully treated.

In this way, if treatment methods must be based on knowledge of the antibiotic that should be provided, without ignoring developments in bacterial resistance profiles, in a more thorough manner, sensible antibiotic usage is advised [Kose A., and Colak C., 2021]. *P. major* is used for different purposes in traditional medicine around the world; therefore, researchers have tested it for different types of biological activities. Most tests have been performed on crude extracts without examining the nature of the active compounds [Abd Razik et al., 2012]. Presently it is not known exactly what phytochemicals in *P. major* are most important in mediating the beneficial effects. Both polysaccharides and polyphenols have been proposed to be bioactive, and the antiviral activity of P. major is reported to derive mainly from its phenolic compounds [Abd Razik et al., 2012]. *P. major* leaves contain a mixture of different polyphenolic antioxidants that may contribute to its wound healing properties [Chiang et al., 2002].

The goal of this study to gather data regarding P. major leaves' potential antibacterial effects on the growth of *P. aeruginosa*. In this investigation, the agar-well diffusion method was chosen as the method of choice. The aqueous extract of this plant had a significant antibacterial activity, according to the findings.

The result of the present study indicates different concentrations of *P. major* extract exhibited different inhibition zone against *P. aeruginosa*, [Table 2]. The potency of *P. major* leaf extract on *P. aeruginosa* ranging from [6.8-16.4 mm] diameter zone of inhibition. According to the dose response, the zone of inhibition was increased with increasing the concentration of ethanol extracts. The Lowest concentration [10 mg/ml] was inhibited the bacteria weakly, while for the high concentrations of aqueous extract [100, 75, 50 and 25 mg/ml], these extracts were recorded noticeable inhibition activity against bacterium. The concentration [100 mg/ml] of aqueous extract had the highest inhibitory effect about [16.4 mm] inhibition zone for *P. aeruginosa*. This supports Abbasi A., et al. 2022 findings that the mean zone of inhibition of Water extract of P. major leaves ranges up to 16mm against *P. aeruginosa* [Abbasi A., et al. 2022].

A study by Behrooz 2020 in Iran revealed that the aqueous *extracts* of *P. major has* inhibitory active on the growth of *P. aeruginosa* [Behrooz et al., 2022].

Conclusion

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

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