



In Vitro Antibacterial Activity of Aloe Barbadensis Millar (Aloe Vera) Against *Pseudomonas aeruginosa* Isolates in Khartoum State, Sudan

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<p>Abstract: Background: It is becoming more widely acknowledged that <i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>) is a significant nosocomial pathogen that can cause serious infections. In general, new therapeutic approaches that prioritize the utilization of extracts and physiologically active chemicals extracted from herbal plants should be used to control <i>P. aeruginosa</i> infections. The World Health Organization (WHO) estimates that in underdeveloped countries, around 80% of the population mostly uses traditional medicine and plant extracts as their primary source of medicine to treat a variety of infectious diseases. Objective: The purpose of this study was to investigate the in vitro antimicrobial activity of <i>A. vera</i> against <i>P. aeruginosa</i> isolated in Khartoum state hospitals. Materials and Methods: The present descriptive study was performed in Khartoum state. Between November 2022 and April 2023, a total of 100 clinical isolates of <i>P. aeruginosa</i> were obtained from patients hospitalized at Khartoum State hospitals. Standard microbiological techniques, such as the Gram stain, catalase test, and oxidase test, were used to identify the isolates. Ciprofloxacin, Ceftriaxone, Meropenem, and Gentamicin. <i>A. vera</i> gel were used in an antimicrobial susceptibility test against the <i>P. aeruginosa</i> strains. Results: Isolates of <i>P. aeruginosa</i> were collected from hospitalized individuals. Of the 100 isolates included in the study, 63 isolates (63%) had been isolated from males and 37 isolates (37%) had been isolated from females. Using the microdilution method, antimicrobial susceptibility testing for 100 <i>P. aeruginosa</i> clinical isolates was conducted for four antibiotics. The results showed that 38% of the isolates were resistant to ciprofloxacin, 94% to ceftriaxone, 82% to gentamicin, and 0% to meropenem. Conclusions: <i>P. aeruginosa</i> demonstrated multidrug resistance to a widely used antibiotic. A different therapeutic agent for the treatment of clinical multi-drug resistance bacteria may be employed in the ethanol extracts of Aloe vera since they have shown antibacterial efficacy against multi-drug resistant <i>P. aeruginosa</i> which is resistant to many drugs and may be treated with a combination of conventional antibacterial discs and the crude extract of Aloe vera. The study's findings validate the traditional wisdom that aloe vera plants may cure microbial infections and suggest that aloe vera may be used to make powerful new antibacterial compounds.</p>	<p align="center">Research Paper</p>
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INTRODUCTION

A collection of medical procedures—whether native or imported—that are provided outside of the official healthcare system is referred to as traditional, complementary, and alternative medicine (TCAM) [1]. African, Arabic, and Islamic cultures are uniquely merged in Sudanese folkloric medicine. Traditional

medicine has continued to be the most practical means of treating a variety of illnesses and infections in underprivileged areas [2]. In many regions of the nation, particularly in rural areas, traditional medicine frequently serves as the population's only source of healthcare services [3]. The antibacterial plant aloe vera is mentioned several times, Herbalists and medical

folklorists have recognized the plant for decades as a medicinal herb [4]. One of the more frequent sources of infections in hospital settings is *Pseudomonas aeruginosa*. Significant morbidity and medical expenses are linked to these infections, particularly if timely administration of antibiotic therapy is not received. Selecting the right antibiotic for patients suffering from *P. aeruginosa* infections can be difficult due to the pathogen's inherent resistance to most commercially available antibiotics. Since multidrug-resistant bacteria are common, treating them with innovative or "last resort" medicines is frequently necessary [5]. Numerous nosocomial infections, such as bacteremia, endocarditis, urinary or wound infections, and in certain cases, fatalities, are caused by this opportunistic and extremely resistant bacteria. *P. aeruginosa* infections are linked to higher rates of death and morbidity in patients with cystic fibrosis, hospitalized burn patients, and immunocompromised, disabled people [6]. It is widely believed that the primary cause of invasive operations is the widespread and unselective use of antibiotics. Thus, the rapid development of various resistances among *P. aeruginosa* isolates in clinical settings has been facilitated by the establishment of inherent or acquired resistance mechanisms [6]. *P. aeruginosa* infections are becoming a serious concern when it comes to hospital-acquired infections, particularly in patients with impaired immune systems. Since the emergence of drug-resistant strains is the main factor contributing to high mortality, numerous strategies for developing innovative anti-infective medications are now being investigated [7]. Many infectious disorders in humans have been treated with extracts or oils of medicinal plants that have antibacterial and anti-inflammatory properties in recent years. Among these well-known medicinal plants is aloe vera (*A. vera*) [8]. *A. vera* is a perennial succulent plant that resembles a cactus and is resistant to dehydration. It is a member of the Liliaceae family, which has over 360 species identified. The plants elongated, pointed leaves yield two unique products: a clear, mucilaginous gel (*A. vera* gel) and a yellow latex known as exudate. The thick outer cuticle is removed to reveal *A. vera* gel [9]. 99.3% of the gel is made up of water, with the remaining 0.7% containing a variety of active substances such as organic acids, polysaccharides, vitamins, amino acids, and phenolic compounds [10]. From the interior gel, more than 75 active compounds have been found overall [11]. In the 1930s, *A. vera* was initially employed to treat radiation burns [2]. Numerous claims of its advantageous effects on humans have led to its use in the culinary, cosmetic, and pharmaceutical industries to this day. Since ancient times, *A. vera* gel has been used to treat wounds, sunburns, and gastrointestinal issues. *A. vera* also has several biological activities, including anti-inflammatory, antioxidant, immune-modulating, and cell growth-stimulating qualities, in addition to antibacterial, antiviral, and antifungal ones, as shown by several *in vitro* and *in vivo* investigations [13]. While *A. vera* gel's antibacterial qualities are widely known, its applications are still little understood, and further research is

necessary. The purpose of this study was to investigate the *in vitro* antimicrobial activity of *A. vera* gel against *P. aeruginosa* isolated from Khartoum state hospitals.

MATERIALS AND METHODS

Study Design: An experimental study to evaluate Aloe vera extract's antibacterial efficacy against *P. aeruginosa*.

Study Area: Khartoum State hospitals.

Study Duration: From November 2022 to April 2023.

Study Population

A clinical examination was used to diagnose 100 patients, both male and female, whose ages ranged from one day to 80 years. The patients exhibited clinical signs of pneumonia, wound infection, otitis media, septicemia, and urinary tract infections (UTIs).

Sample Size: A total of Hundred (n=100) isolates were discovered from 100 patients participating in this study.

Samples Collection:

During the study period from November 2022 to April 2023, samples were taken from every patient who had a wound infection, urinary tract infection, pneumonia, septicemia, otitis media, or vaginitis.

Laboratory Procedures: Isolation of *P. aeruginosa*: Within two hours of collection, the isolates were processed.

Cultivation of Bacteria

Following an overnight aerobic incubation period at 37°C, each isolate was grown on nutrient agar.

Colonial Morphology

Plates were inspected for colony morphology following incubation.

Gram's Stain

The smear was made, let to dry, and then repaired. After being smeared with crystal violet stain for 30 to 60 seconds, it was quickly cleaned with tap water, flooded with iodine for one to two minutes, and then again with tap water. The smear was then decolorized using an alcoholic decolorizer, immediately followed by a water wash. Safranin was then applied as a counter stain for one to three minutes, and after that, the slide was cleaned, allowed to air dry, or examined under a microscope using oil immersion objective lenses [14].

Data Analysis

Data were analyzed using SPSS 25.0, descriptive statistics in terms of frequency, percentages, means and standard deviations, and Chi-square test were calculated. A p-value ≤ 0.05 is considered statistically significant.

Ethical Considerations:

Ethical approval for the study was obtained from the Board of the Faculty of Medical Laboratories Sciences, at Shendi University. The written informed consent form was obtained from each guardian of the participant as well as from the subject himself before

recruitment into the study. All protocols in this study were done according to the Declaration of Helsinki (1964).

RESULTS**Table 1: Number of *P. aeruginosa* isolates detected from different sites of infection**

Infection	Isolates
Septicemia	45 (45%)
Wound Infection	22 (22%)
Otitis Media	13 (13%)
UTI	9 (9%)
Pneumonia	4 (4%)
Vaginitis	4 (4%)
Plural Infection	3 (3%)

Table 2: Frequency of sampling according to patient's age group

Variables	1day-10 y	11-20 y	21-40 y	41-60 y	61-80 y	Total
Septicemia	20	3	5	5	12	45
Wound Infection	2	0	7	10	3	22
Otitis Media	1	2	1	1	8	13
UTI	0	1	2	2	4	9
Pneumonia	1	0	0	0	3	4
Vaginitis	0	0	4	0	0	4
Plural Infection	0	0	0	0	3	3
Total	24	6	19	18	33	100

Table 3: Frequency of *P. aeruginosa* infections according to gender

Gender	Frequency	Percentage
Male	63	63
Female	37	37

Table 4: Antimicrobial activity of different concentrations of Aloe vera extract on *P. aeruginosa* isolates

Extract concentration (mg/ml)	100	75	50	25
Sensitive isolates (NO)	100	98	92	80
Mean of inhibition zone of isolates in (mm)	21	19	17	15
Inhibition zone of the standard in(mm)	20	18	17	14

Table 5: Susceptibility of *P. aeruginosa* to some common used antibiotic

Antibiotics	Sensitivity %	Resistance %	Total
Ciprofloxacin (5 µg)	62	38	100
Ceftriaxone (30 µg)	06	94	100
Meropenem (10 µg)	100	00	100
Gentamicin (10 µg)	18	82	100

DISCUSSION

The present descriptive study was performed in Khartoum state. One hundred clinical isolates of *P. aeruginosa* were collected from patients admitted to Khartoum State hospitals between November 2022 and April 2023. Out of the 100 isolates that were part of the investigation, 37 isolates (37%) from females, and 63 isolates (63%) had come from males. Antimicrobial susceptibility testing for four drugs was done on 100 *P. aeruginosa* clinical isolates using the microdilution method. The study's findings indicate that males were more likely than females to contract *P. aeruginosa* (63%

versus 37%), which is consistent with Maison's findings that 35% of patients were female and 65% of patients were male [3]. Males are more likely than females to come into touch with pollution outside of their homes, which explains the greater frequency. The study found that the adult group (aged 21–80 years) had a higher prevalence of clinical infections caused by *P. aeruginosa* (70%), compared to the younger group (aged 1–20 years) (30%) [15], also found that the prevalence is higher in adults than in younger people and that this is because aging-related changes in immune function, exposure to nosocomial pathogens, and an increasing number of co-

morbidities put older people at higher risk of contracting infections. A significant section of the Iranian populace uses a variety of medicinal plants, A. vera being the most significant, as potential sources of new antibacterial agents for the treatment of burn site infections [16-18]. Also in the study done by Mehdi and his colleagues [29], The antibacterial activity of A. vera gel extracts was found to have varying effects against five bacterial cultures (*Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*) and three fungal cultures (*Aspergillus fumigatus*, *Candida albicans*, and *Penicillium spp.*). These findings are consistent with previous research by Antonisamy *et al.*, The outcomes demonstrated that A. vera DMSO gel extracts had the greatest level of action against the chosen pathogens, and the extent of inhibition varied according to the extract's concentration [19-22]. A. vera gel's stronger antibacterial efficacy against fungal strains was demonstrated in multiple investigations [23]. Agarry *et al.*, carried out a study to assess the antibacterial effectiveness of A. vera gel and leaf against *P. aeruginosa*, *S. aureus*, *Microsporum canis*, *Trichophyton schoenleinii*, *T. mentagrophytes*, and *C. albicans*. The results proved that the gel and the leaf have potent antibacterial properties against *S. aureus*. While the leaf inhibits the growth of *P. aeruginosa* and *C. albicans*, only the gel inhibited the growth of *T. mentagrophytes* [24]. Different A. vera extracts have different antibacterial properties. In comparison to ethanol and distilled water, Irshad *et al.*, demonstrated that the A. vera extract of methanol had the highest level of antibacterial activity [25]. Ibrahim *et al.*, (2011) used three distinct forms (ethanol, acetone, and aqueous extracts) in their investigation of the phytochemical analysis and antibacterial assessment of A. vera gel against certain plant and human infections using the disc diffusion method. It was discovered that the acetone extract's antibacterial and antifungal properties outperformed those of ethanol and aqueous extracts [26]. The absence of antibacterial activity may be influenced by the type and part of A. vera, the method used, and the type of bacteria. Different types of A. vera affected the presence or absence of A. vera extracts for bacteria inhibition. Pandey and Mishra reported that there was no inhibitory effect of aqueous extract on the Gram-negative bacteria and a weak inhibitory effect on Gram-positive bacteria [27]. A different study stated that A. vera flesh extract had no inhibitory activity at all concentrations, carried out four times for *P. aeruginosa* and *S. Pyogenes* bacteria [28].

CONCLUSION

P. aeruginosa demonstrated multidrug resistance to a widely used antibiotic. A different therapeutic agent for the treatment of clinical multi-drug resistance bacteria may be employed in the ethanol extracts of Aloe vera since they have shown antibacterial efficacy against multi-drug resistant *P. aeruginosa* which is resistant to many drugs may be treated with a combination of conventional antibacterial discs and the

crude extract of Aloe vera. The study's findings validate the traditional wisdom that aloe vera plants may cure microbial infections and suggest that aloe vera may be used to make powerful new antibacterial compounds.

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Conflict of Interest: Authors have declared that no competing interests exist.

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