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Antibacterial Activity of *Aloe vera* Gel against Multidrug Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract

Staphylococcus aureus and Pseudomonas aeruginosa have been implicated as important nosocomial pathogens causing severe infections especially in hospitalized patients. The aim of the study was to assess the antibacterial activity of Aloe vera gel against multidrug resistant S. aureus and P. aeruginosa isolated from wound. Clinical isolates of S. aureus and P. aeruginosa from wound infection were collected from Microbiology laboratory of Barau Dikko Teaching Hospital (BDTH), Kaduna and re-confirmed using standard microbiological procedure. Antibiotic susceptibility pattern of the isolates was determined using Kirby Bauer disk diffusion method. Aloe vera gel was obtained fresh matured leaves of Aloe vera plant and was screened for the presence of phytochemical constituents. Antibacterial activity of the Aloe vera gel against Multidrug Resistant (MDR) Staphylococcus aureus and Pseudomonas aeruginosa isolates was determined by agar well diffusion technique. The result revealed that all the two isolates were resistant to more than three classes of antibiotics. The Staphylococcus aureus isolate was resistant to fluoroquinolone (ciprofloxacin), aminoglycoside (gentamicin), cephalosporin (cefaroline), folate pathway antagonist (trimethoprim-sulfamethoxazole), penicillin(cefoxitin) and macrolide (erythromycin) while the Pseudomonas aeruginosa isolate was resistant to B-lactam (ticarcillin-clavulanate), fluoroquinolone (ciprofloxacin and norfloxacin), aminoglycoside (gentamicin) and cephalosporin (ceftazidime)hence regarded as MDR isolates. Phytochemical screening of the gel revealed the presence of saponins, flavonoids, terpenoids and alkaloids. The Aloe vera gel was found to have antibacterial activity against the test isolates with MIC and MBC values of 25µg/mL and 50µg/mL against MDR S. aureus 50µg/mL and 100µg/mL against MDR P. aeruginosa respectively. The study identified that Aloe vera gel possesses antibacterial activity against MDR S. aureus and P. aeruginosa isolated from wound infection.

Keywords: *Aloe vera gel, Staphylococcus aureus, Pseudomonas aeruginosa, antibacterial, MDR*

INTRODUCTION

The development of resistance against convectional antibiotics has led to researchers looking for alternative medicine in many countries today (Ingrida *et al.*, 2012). Approximately 20%of the world's plants have been pharmacologically tested against majority of pathogenic microorganisms (Adnan *et al.*, 2015). This has led to introduction of new antibiotics into the drug market (Radha and Laxmipriya, 2015).

One of the plants that have gained a lot of popularity in medicine is *Aloe barbadensis* Miller (*Aloe vera*) belonging to the family *Liliaceae* (Seyyed *et al.*, 2015). The plant is succulent with a whorl of elongated, pointed leaves with over 360 known species. *Aloe vera* has been shown to have anti-inflammatory activity, immune stimulatory, antioxidant, immune modulating and cell growth stimulating activity (Yebpella *et al.*, 2011). In addition, extracts from *Aloe vera* have reported to possess antimicrobial, antiviral and antifungal properties (Jothi *et al.*, 2014).

Aloe vera has been used widely in the treatment of various skin conditions such as cuts, burns and eczema. It is reported that sap from *Aloe vera* eases pain and reduces inflammation. Hence, the current wide use of the plant in skin care, cosmetics and as nutraceuticals (Arunkumar and Muthuselvam, 2009).

Staphylococcus aureus is one of the important hospital and community acquired pathogen. It is responsible for causing a broad spectrum of diseases ranging from mild superficial skin and soft tissue infections to life threatening infections such as infective endocarditis, septicaemia, deep-seated abscess, pneumonia and toxic-shock syndrome (Onemuand Ophori, 2013). *S. aureus* has been naturally susceptible to most of the antibiotics developed so far (Chambers and DeLeo, 2009).

However, it has been developing increased resistance to antimicrobial agents (Foster, 2017). It is of greatest concern because of its intrinsic virulence and its ability to adapt to different antibiotics (Lowy, 2013). The two most remarkable antibiotic resistance achieved by *S. aureus* is methicillin (MRSA) and vancomycin resistance (VRSA and VISA). Methicillin resistance was achieved by interspecies transfer of *mecA* gene from an ancestral *Staphylococcus* species to *S. aureus*. Vancomycin resistance was achieved by gene mutations (VISA) and horizontal genes transfer (*van A*-gene) (Hiramatsu *et al.*, 2014).

Pseudomonas aeruginosa is increasingly recognized as important nosocomial pathogens causing severe infections especially in hospitalized patients in burn wards (Japoni *et al.*, 2014). This opportunistic and highly resistant pathogen is responsible for a variety of nosocomial infections, including wound and urinary tract infections, bacteremia, endocarditis, and in some conditions death. *P. aeruginosa* infections in immunocompromised, debilitated, cystic fibrosis, and burn patients are associated with increased rates of mortality and morbidity (Japoni *et al.*, 2014).

Development of antibiotic resistance mechanisms eitherintrinsic or acquired has promoted the rapid developmentof multiple resistances among *P. aeruginosa* isolates in the clinical settings. The rapid increase of drug resistance inclinical isolates of *P. aeruginosa* is a growing concern among hospitalized patients. The widespread multidrug-resistant (MDR) *P. aeruginosa* strains not only lead to increased economicburden, but also can directly threaten the life of the patient (Goudarzi *et al.*, 2015).

The aim ofthis study was to evaluate the antibacterial activity f *Aloe vera* gel against MDR *S. aureus* and *P. aeruginosa* isolated from wound infections.

MATERIALS AND METHODS

Sample Collection and processing

Matured and fresh leaves of *Aloe vera* were collected from Faculty of Arts, Kaduna State University, Kaduna in a clean container. Leaves were washed thoroughly with a sterile water to remove dirt and their thick epidermidis was then dissected longitudinally into pieces. The colourless parenchymatous tissue (*Aloe vera* gel) was collected in a sterile container. Two hundred grams (200g) of the gel was mixed in one liter (1L) of 2.0% Dimethyl Sulfoxide (DMSO) to make 200µg/mL stock solution and kept at 4°C for further analysis. This stock solution was serially diluted to achieve 100µg/mL, 50µg/mL, 25µg/mL and 12.5µg/mL

Phytochemical screening of *Aloe vera* gel

The *Aloe vera* gel was screenedfor the presence of the phytochemicals: alkaloids, saponin, tannins, terpenoids, steroids, flavonoids, cardiac glycosides and phlobatannins as follows:

i. Test for alkaloids

This test was carried out by adding 1.5 mL of 1% HCl to 2 mL of the gel. The solution was then heated in a water bath before adding 6 drops of Wagner'sreagent. Formation of orange precipitated indicated the presence of alkaloid (Abdurohman, 2015).

ii. Tests for saponins

The gel was diluted with distilled water and shaken ingraduated cylinder for 15 minutes. The formation of foam layer indicated the presence of saponins (Abdurohman, 2015).

iii. Test for tannins

The gel was dissolved in distilled water to which 2 mL of 5% ferric chloride solution was added.Formation of blue green indicated presence of tannins (Abdurohman, 2015).

iv. Test for terpeniods

The gel was in treated in chloroform with few drops of concentrated sulphuric acid, shaken properly and allowed to stand for some time; formation of yellow colored lower layer indicated the presence of terpenoids (Abdurohman, 2015).

v. Test for steroids

To 2 mL of gel, 5 mL of chloroform and 2 mL of aceticanhydride was added followed by concentrated H_2SO_4. Reddish brown coloration at interface indicated the presence of steroids (Abdurohman, 2015).

vi. Test for flavonoids

Few drops of ferric chloride solution were added to the gel. Formation of intense green color indicated thepresence of flavonoid (Abdurohman, 2015).

vii. Test for cardiac glycosides

To 2 mL of the gel, 3 mL of glacial acetic acid and 1drop of 5% ferric chloride were added in a test tube. Carefully 0.5 mL of concentrated sulphuric acid was added by the side of the test tube. Formation of blue color in the acetic acid layer indicated the presence of cardiac glycosides (Abdurohman, 2015).

viii. Test for phlobatannins

The gel was boiled with 1% aqueous HCL. No deposition of red precipitate indicated the absence of phlobatannins (Ezeonu and Ejikeme, 2016).

Test bacterial isolates

Isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were collected from Microbiology laboratory of Barau Dikko Teaching Hospital, Kaduna. The isolates were collected on nutrient agar slants and

transported to the laboratory, Department of Microbiology, Kaduna State University, Zaria for further assay.

Re-confirmation of Test bacterial Isolates

The isolates were re-confirmed based on their colonial morphology, Gram reaction and Biochemical reactions as described by Cheesbrough(2012).

Determination of Antibiotic Susceptibility Pattern of the Test Isolates

McFarland standard (0.5) was prepared by adding 0.05 mL of 1% Barium Chloride solution ($BaCl_2.2H_2O$) to 99.4 mL of 1% (v/v) sulphuric acid solution (H_2SO_4) and mixed thoroughly. Inocula of the test isolates were standardized by emulsifying discrete colonies of the isolates into sterile normal saline and comparing the turbidity with that of 0.5 McFarland standard (Andrews, 2001).

The antibiotic susceptibility pattern of the isolates was determined by the Kirby Bauer disk diffusion method. Briefly, about 0.1 mL of each of the standardized bacterial suspension was inoculated evenly on to Mueller-Hinton agar plates with the aid of sterile swab sticks. The antibiotic discs were then placed on the surface of the media and allowed to diffuse for 10 minutes. Then the plates were incubated at 37^{o}C for 18hours. The diameters of the zones of inhibition were measured, recorded and interpreted as susceptible, intermediate or resistant using the Clinical Laboratory Standards Institute guideline (CLSI, 2018). Susceptibility of *S. aureus* to cefoxitin (30µg), chloramphenicol (30µg), gentamicin (30µg), ciprofloxacin (5µg), erythromycin (15µg), ceftaroline (30µg), sparfloxacin (5µg), clindamycin (2µg), nitrofurantoin (300µg) and trimethoprim/ sulfamethoxazole (1.25/23.75µg) as well as the susceptibility of *P. aeruginosa* to piperacillin(100µg), ticarcillin-clavulanate (75/10µg), norfloxacin (10µg),ceftazidime(30µg), amikacin (30µg) chloramphenicol (30µg), gentamicin (30µg) and ciprofloxacin (5µg) were determined.

Antibacterial activity of *Aloe vera* gel against the test isolates

Antibacterial activity of *Aloe vera* gel against the test isolates was determined using agar-well diffusion method. About 0.1 mL of each of the standardized (0.5 McFarland) bacterial suspension were inoculated evenly on to Mueller-Hinton agar plates using sterile swab sticks. With the aid of a sterilized cork borer, wells of 6mm diameter were bored in the MHA. A 0.1 mL aliquot of 100µg/mL, 50µg/mL, 25µg/mL and 12.5µg/mL concentrations of *Aloe vera* gel were added aseptically into the respective wells and the plates were then held for 1 hour at room temperature for the gel to diffuse before incubation for 24 hour at 37^{o}C. After incubation, the diameters of the zones of inhibition were measured to the nearest mm (Kingsbury and Wagner, 2013).

Determination of Minimum Inhibitory Concentration (MIC)

The lowest concentration of *Aloe vera* gel capable of preventing growth (Minimum Inhibitory Concentrations (MIC) was determined as follows; 2 mL of Muller-Hinton broth was transferred in to four sets of test tubes for each of the test isolates. Serial doubling dilution was carried out using 2 mL of the 200µg/mL concentrations of the gel to achieve final concentrations of 100µg/mL, 50µg/mL, 25µg/mL and 12.5µg/mL respectively. One loopful each of the standardized inocula was inoculated into the tubes labelled appropriately and incubated at 37^{o}C for 24 hours. The least concentration (highest dilution) that shows no sign of visible growth (turbidity) was recorded as the Minimum Inhibitory Concentrations (MIC) of the *Aloe vera* gel (Bandaru *et al.,* 2017).

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentrations were determined by sub-culturing the contents of the Mueller-Hinton broth that showed no visible growth onto Mueller-Hinton agar plates by streaking using sterile wire loop, after which the plates were incubated at 37^{o}C for 24 hours. The lowest concentration with no growth was considered as the Minimum Bactericidal Concentrations (MBCs) of the *Aloe vera* gel (Bandaru *et al.,* 2017).

RESULTS

The phytochemical screening of *Aloe vera* gel for bioactive secondary metabolites revealed the presence of saponins, alkaloids, flavonoids, and terpenoids in the gel and the absence of steroids, phlobatannins, tannins and cardiac glycosides (Table 1).

Table 1: Phytochemical constituents of *Aloe vera* Gel

Phytochemical constituents	**Results**
Saponins	+
Flavonoids	+
Terpenoids	+
Alkaloids	+
Phlobatannins	-
Steroids	-
Tannins	-
Cardiac glycosides	-

Keys: + = present, - = absent

The study revealed that *Staphylococcus aureus* was resistant to 6 antibiotics of different classes namely: fluoroquinolone (ciprofloxacin), aminoglycoside (gentamicin), cephalosporin (cefaroline), folate pathway antagonist (trimethoprim-sulfamethoxazole), penicillin (cefoxitin) and macrolide (erythromycin) while *Pseudomonas aeruginosa* was resistant to 5 antibiotics of different classes namely: ß-lactam (ticarcillin-clavulanate), fluoroquinolone (ciprofloxacin and norfloxacin), aminoglycoside (gentamicin) and cephalosporin (ceftazidime). The multiple antibiotic resistance (MAR) index of *Staphylococcus aureus* and *Pseudomonas aeruginosa* found to be was 0.60 and 0.63 (Table 2).

Table 2: Susceptibility patterns and Multiple Antibiotic Resistance (MAR) indices of the test isolates

Isolates	No. of antibiotic tested	Antibiotics susceptible to	Antibiotics resisted	*MAR indices
S.aureus	10	C, SPX, CC, F/M	CIP,CN,SXT,CPT,FOX,ERY	0.60
P.aeruginosa	8	PIP, AM, C	TIM,CIP,NOR,CN,CAZ	0.63

Key: MAR=Multiple Antibiotic Resistance; CIP=ciprofloxacin; CN=gentamicin; SXT= trimethoprim/sulphamethoxazole; CPT=ceftaroline; FOX=cefoxitin; ERY=erythromycin;TIM =ticarcillin-clavulanate; NOR=norfloxacin; CAZ=ceftazidime; C= hloramphenicol; SPX=sparfloxacin; CC=clindamycin; F/M =nitrofurantoin; PIP=piperacillin; AM=amikacin

* MAR index was calculated as the ratio of the number of antibiotic resisted to the total number of antibiotic tested.

Table 3 illustrates the antibacterial activity of *Aloe vera* gel on the MDR isolates. It revealed that the gel exhibited its highest activity at the concentration of 100 µg/mL and produces zones of inhibition of 25±1.16mm and 21±0.58mm against MDR *S. aureus* and *P. aeruginosa* respectively. At the lowest concentration of 12.5µg/mL, the gel produces mean zones of inhibition of 09±0.44mm and 07±0.88mm against MDR *S. aureus* and *P. aeruginosa* respectively.

Table 3: Antibacterial activity of *Aloe vera* gel against the test isolates

Concentrations (µg/mL)	**Mean zones of inhibition ± SE (mm)**	
	S. aureus	***P. aeruginosa***
100	25±1.16	21±0.58
50	20±0.58	17±0.33
25	15±0.45	12±0.17
12.5	09±0.44	07±0.88

Table 4 revealed that the Minimum Inhibitory Concentration (MIC) of *Aloe vera* gel was recorded at 25µg/mL and 50µg/mL against MDR *S.aureus* and *P. aeruginosa* respectively. While the Minimum Bactericidal Concentration (MBC) was recorded at 50µg/mL and 100µg/mL against MDR *S. aureus* and *P. aeruginosa* respectively.

Table 4: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the *Aloe vera* gel against the test isolates

Multidrug resistant isolates	***Aloe vera***	
	MIC (µg/mL)	**MBC (µg/mL)**
Staphylococcus aureus	25	50
Pseudomonas aeruginosa	50	100

DISCUSSION

The findings of this study revealed the presence of presence of phytoconstituents such as saponins, alkaloids, terpenoids and flavonoids in the *Aloe vera* plant. The presence of these phytochemicals in *Aloevera* gel is in accordance with the report of Karpagam and Devaraj (2018). Saponins, alkaloids, terpenoids and flavonoids were previously reported to have broad spectrum of antimicrobial activity against bacteria, fungi and viruses (Abdurohman, 2015). Hence medicinal plants are regarded as synthetic laboratory where bioactive chemical compounds are produced (Jothi *et al.*, 2014).

Susceptibility profile of the test isolates in this study shows that all the test isolates were resistant to more than 3 different classes of antimicrobial agents hence considered MDR isolates. So also the MAR indices of the isolates were higher than 0.2 suggesting that they originated from high risk sources where antibiotics are often used.The MAR index gives an insight on the number of isolates showing antibiotic resistance and the consequence risk zone in routine susceptibility testing (Olonitola *et al.*, 2007).

Increase in the number of multi-drug resistant pathogenic microorganisms as well as undesirable side effects of some antimicrobials have led to the search for new effective antimicrobial drugs of plant origin. The findings of this study indicated that *Aloe vera* gel exhibited antibacterial activity against the test isolates at all the concentrations tested. This activity could be attributed to the presence of bioactive phytochemicals present in the *A. vera* gel. The study observed that the degree of inhibition varied directly with varying concentrations of the gel. Higher concentrations of the gel produce wider zones of inhibition against both the MDR *S. aureus* and MDR *P. aeruginosa*. These observations corroborate with those of Goudarzi *et al.* (2015).

Among the two isolates, higher antibacterial activity by the gel was observed against MDR *S. aureus* compared to MDR *P. aeruginosa*. This may be attributed to the higher MAR index of the MDR *P. aeruginosa* and the presence of outer membrane comprising of phospholipids and lipopolysaccharides covering the peptidoglycan of layer of *P. aeruginosa* (and other Gram negative bacteria) (Dahiya and Purkayastha, 2012).This outer membrane acts as barrier to entrance of most antimicrobials. This is in agreement with the report of Masoumian and Zandi (2017) and those of Antonisamy *et al.* (2012). However, it is in contradiction with the result of Goudarzi *et al.* (2015) who reported that none of the MDR isolates of *Pseudomonas aeruginosa* screened in their study weresensitive to *A. vera* gel at concentrations lower than 25 µg/mL. Likewise, Anderl *et al.* (2010) reported that *Aloe vera* gel inhibited the growth of *S. aureus* isolated from wound infection but has no effect on *P. aeruginosa* isolates.

The MIC of *Aloe vera* gel was recorded at the concentration of 25µg/mL against MDR *Staphylococcus aureus* and 50µg/mL against MDR *Pseudomonas aeruginosa* while the MBC of *Aloe vera* gel was recorded at the concentration of 50µg/mL against *Staphylococcus aureus* and 100µg/mL against MDR *Pseudomonas aeruginosa* respectively. The higher MIC and MBC values for MDR *Pseudomonas aeruginosa* is an indication that the gel is less effective against the organism.This result is similar to the result of Takon *et al.* (2015). However, higher MIC values of ≤ 400 µg/mL was reported by Goudarzi *et al.* (2015) for most (89.4%) of the MDR *P. aeruginosa* strains screened and 800 µg/mL MIC values for the remaining 10.6%of the isolates.

CONCLUSION

The study revealed that *Aloe vera* gel exhibited antibacterial activity against the test bacteria (MDR *S. aureus* and MDR *Pseudomonas aeruginosa*) with the gel having higher activity against MDR *S. aureus* (zone of inhibition at 100 µg/mL = 25±1.16 mm and MIC = 25 µg/mL) than MDR *Pseudomonas aeruginosa* (zone of inhibition at 100 µg/mL = 21±0.58 mm and MIC = 50 µg/mL). The study identifies that *Aloe vera* gel has shown great potential as an effective antibacterial agent for medicinal purposes and should be categorized among the agents considered in the search for novel antimicrobials.

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