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Phytochemical analysis and Antibacterial activities of *Aloe vera* leaves extracts on bacteria isolated from diabetic foot ulcer patients from Hospital Environment

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Abstract
Infected leg ulcers resulting from diabetic mellitus are a major health problem resulting in morbidity and disability. Longstanding leg ulcers are frequently colonized by microorganisms and are usually multi-drug resistant and refractory to treatment. This study was aimed at determining the phytochemical analysis and Antibacterial activities of ethanol and methanol extract of *Aloe vera* leaves on bacteria isolated from diabetic foot ulcer patients. 150 swabs were obtained from diabetic foot ulcer patients in the hospital. The obtained swabs were cultured on the appropriate media and incubated at 37^{o}C for 24 hours for bacterial growth and identified through appropriate biochemical test. Solvents such as ethanol and methanol were used to extract the leaves of *Aloe vera* and screened for their phytochemical and antibacterial activities using standard methods. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC) of the plant extracts were also determined by agar dilution methods. Standard antibiotic (Gentamicin) and DMSO (Dimethyl Sulfoxide) were used as positive and negative controls respectively. Tannins, Saponins, terpenoids, Flavonoids and reducing sugar are the phytochemical compounds present in *Aloe vera leaves.* The maximum antibacterial activitity was observed in ethanolic extract of *Aloe vera* (22mm and 24mm). The efficacy of the extracts towards inhibition of the bacteria increased with concentrations. The activity of the plant extract compared with conventional antibiotics, such as gentamicin confirmed that plant extracts are more active than constitutional antibiotics. The MIC value ranged from 0 to 100mg/ml and the MBC ranged from 0 to 200mg/ml. Extract of *Aloe vera* leave is cheap and was effective even against multi-drug resistant organisms as compared to the routinely used topical anti-bacterial agents. This leaf extracts could be used as a broad spectrum antibiotics in the treatment of diabetic foot infections

Keywords: *Aloe vera* leaves, antibacterial activities, phytochemical analysis, diabetic foot ulcers

1. Introduction

Aloe vera plant is an important medicinal plant belongs to the family Liliaceae and is also called the magical plant. It is not a cactus; it has thick, tapered, spiny leaves growing from a short stalk near ground level [1]. There are over 250 species of aloe grown around the world. However, only two species are grown today commercially, with *Aloe barbadensis* Miller and *Aloe aborescens* being the most popular [1]. Concentrated extracts of Aloe leaves are used as laxative and as a haemorrhoid treatment [2]. *Aloe* vera gel can help to stimulate the body's immune system [1].

Aloe vera or *Aloe barbadensis* Miller, is most biologically active among 400 species [1, 3-4]. According to World Health Organization, medicinal plants would be the best source for obtaining a variety of drugs [5]. The plant is native to southern and eastern Africa along the upper Nile in the Sudan, and it was subsequently introduced into northern Africa and naturalized in the Mediterranean region and other countries across the globe. The plant is commercially cultivated in Aruba, Bonaire, Haiti, India, South Africa, the United States of America, and Venezuela [6-7], while the finest quality of Aloe is grown in desert of Southern California. The plant can survive in hot temperatures of 104°F and with stand in below freezing temperatures until root is not damaged [7].

Aloe plant contains 25 percent of solid fraction that contain sugars [8]. Sugar acts as immune modulators capable of enhancing and retarding the immune response [8-10]. Aloe contains saponins which are soapy substances form 3 per cent of the gel and are general cleansers, having antiseptic and anticarcinogen properties [13].

A. vera is a very versatile plant that has many different uses. Numerous scientific studies on *A. vera* are demonstrating its analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, anti-bacterial, and antifungal properties [14]. Infected leg ulcers resulting from diabetic mellitus is a major health problem resulting in morbidity and disability [15]. Leg ulceration affects about 1% of the middle aged and elderly population (Vishwanathan *et al.*, 2011). It commonly occurs after a minor injury in association with chronic venous insufficiency,

chronic arterial insufficiency and diabetes [16]. There are also many less common causes of ulcers including skin cancer, systemic sclerosis, vasculitis and various skin conditions [16]. Longstanding leg ulcers are frequently colonized by microorganisms in a biofilm [17]. They are usually multi-drug resistant and refractory to treatment. Topical, as well as systemic antibiotics and agents have been used, solely and in combination, to eradicate the resistant infections. Moreover, these agents have led to the emergence and subsequent rapid overgrowth of resistant bacterial strains, drug side effects like allergy and organ specific toxicity [17]. In the present context where there is evolution of super bugs (antibiotics resistant organism), it is very important to find alternatives. Various herbal products have been used in the management and treatment of wounds over the years. Many substances like tissue extracts, vitamins and minerals and a number of plant products have been reported to possess pro healing effects [18]. In this context, *Aloe vera,* which is cheap, cost effective and easily available, would open up a reasonable solution. Medicinal properties of *Aloe vera* have been recognized for a long time [19]. The antiseptic and antimicrobial agents present in *Aloe vera* provide the ability to attack, reduce, control, or even eliminate infections as the gel penetrates directly into the deeper layers of the skin [19]. The analgesic property helps to be a fast and effective painkiller. The polysaccharides in *Aloe vera* are an important stimulus to the immune system, and also act as a catalyst for the healing properties of *Aloe vera* [18]. Although there are many studies to prove its effectiveness in cosmetology, studies done to evaluate the efficacy of topical *Aloe vera* in decreasing the bacterial count and bringing about clinical improvement in chronic ulcers are few and far between. Therefore the present study is aimed at determining the Phytochemical analysis and Antibacterial effect of ethanolic and methanolic extracts of *Aloe vera* leaves on bacteria isolated from diabetic foot ulcer patients from hospital environment.

2. Materials and methods

Study Area

This study was conducted at Braith-whyte Memorial Hospital, Port-Harcourt, Rivers State. A total of one hundred and fifty (150) swabs were obtained from diabetic foot ulcer patients in the hospital and the subject's age were between 30 – 79 years. Socio-demographic data were collected with the help of a questionnaire.

Cultivation and Isolation of Microorganisms

The obtained swabs taken to the laboratory and cultured on the appropriate media (Nutrient agar, Macconkey agar and blood agar); and incubated at 37ºC for 24 hours for bacterial growth. Discrete colonies were further sub-cultured onto fresh prepared plates of nutrient agar plates to obtain pure cultures. The purified cultures were gram stained and stored on nutrient agar slants for biochemical tests and identification according to defined methods [20].

Ethical approval:

This study was approved by the hospital management board of Braith-whyte memorial hospital, Port-Harcourt, Rivers State.

Statistical Analysis

The results were subjected to statistical analysis using SPSS version 20. Socio-demographic data was analyzed using Chi-square. Level of significant was also considered at $P < 0.005$.

Source and maintenance of test organisms

The wound isolates of *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae*, were obtained from patient's samples in the laboratory section of the hospital.

Reference Strains

Escherichia coli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATTC 27853) were used as reference strains for culture and sensitivity testing and obtained from Nigerian Institute of Medical Research (NIMR) Yaba, Lagos State.

Collection and identification of plant sample

Fresh leaves of *Aloe vera was* harvested from farms in Federal Research Institute Umudike, Abia State, Nigeria.

Preparation of Crude Extract

Fresh *A. vera* whole leaves were washed with distilled water to remove impurities followed by water with 0.5% chlorine, chopped into small pieces, air dried and grinded into powder. The dried powder was extracted with 95% ethanol and methanol for one week. Then it was filtrated through filter paper and the entire extract of *Aloe. vera* then evaporated at 90°C in oven to get a paste form. This concentrated leaf extract was used for further experiments [21].

Phytochemical Analysis

The phytochemical analysis of methanol and ethanol extract of *Aloe vera* leaves was carried out using standard methods as described by [22].

Screening of Phytochemical Components

The freshly prepared extracts were subjected to standard phytochemical analyses for different constituents such as tannins, alkaloids, flavonoids, sterols, glycosides, saponins, terpenoids and reducing sugars as described by [22].

In-vitro screening of antibacterial activities of the plant leaf extracts.

The agar well diffusion assay method described by [23] was used to evaluate the antibacterial activities of the crude extracts of *Aloe vera* leaves against the test microorganisms. Dilutions of 100, 50, 25, 12.5, and 6.25mg/mL were prepared from the stock solution of the plant extracts in a 2-fold dilution process. Twenty (20) mL of molten Mueller Hinton Agar (MHA) were poured into sterile Petri dishes (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates and holes (6mm) were made in the agar plates using a sterile metal cork-borer. Twenty (20µl) of the various dilutions of the plant extract and control were put in each hole under aseptic condition, kept at room

temperature for one hour to allow the agents to diffuse into the agar medium and incubated accordingly. Gentamicin (5µg/mL) was used as positive control while Di-Methyl sulfoxide (DMSO) was used as the negative control. The MHA plates were then incubated at 37°C for 24 hours. The inhibition zones diameters (IZDs) were measured and recorded. The size of the cork borer (6mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in triplicate and the mean IZDs calculated and recorded.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The estimation of MIC of the crude extracts was carried out using the method [24]. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism. To 0.5 ml of varying concentrations of the extracts (100, 50, 25, 12.5, and 6.25mg/mL), 2ml of Nutrient Broth was added in the test tubes and then a loopful of the test organism was introduced. The procedure was repeated on the test organisms using the standard antibiotic streptomycin. A tube containing Nutrient broth only was seeded with the test organisms as described above to serve as controls. The culture tubes were then incubated at 37ºC for 24hrs. After incubation the tubes were then examined for microbial growth by observing for turbidity. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile Nutrient agar plates by streaking. Nutrient agar plates were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37ºC for 24 h. After incubation, the concentration at which no visible growth was seen was noted as the Minimum Bactericidal Concentration (MBC) [25].

3. Results and Discussion

Phytochemical Screening

The result revealed the presence of medicinal active constituents of *Aloe vera*. In analysis of tannin compounds, brownish green colour was developed to indicate the presence of tannin. Similarly, based on the presence or absence of colour change indicate positive or negative results. In the screening process, Tannins, Saponins, Alkaloids, Flavonoids, Glycosides gave positive results while steroids and phenols gave negative results. Phyto-constituents have been found to inhibit bacteria, fungi, viruses and pests [26]. The presence of Phyto-constituents in the root extracts may be responsible for the antibacterial and antioxidant activity of the plant [26].

Table 1: Socio-demographic characteristics of Patients Parameters Number Percentages (%)

Parameters	Number	Percentages (%)
Sex		
Male	106	70.7
Female	44	29.3
Age In Years		
30-39	5	3.3
40-49	45	30.3
50-59	59	39.3
60-69	31	20.7
70-79	10	6.7
Educational Status		
Tertiary	31	20.7
Secondary	10	6.7
Primary	59	39.3
No formal Education	50	33.3
Occupational Status		
Farmers	65	43.3
Civil servants	11	7.3
Traders	42	28.0
Professionals	10	6.6
Students/Unemployed	5	3.3
Artisans (welders, Carpenters, etc)	17	33.3

Table 2: Number and percentage of bacterial isolates from foot ulcers

No of Patients Screened	No of Patients infected	Bacterial Isolates	No of Isolates	Percentage of No of Isolates (%)
150	92	*E coli*	20	17.7
		P. aeruginosa	37	32.7
		S. aureus	29	25.7
		K. pneumoniae	13	15.5
		Proteus vulgaris	3	2.7
		Bacillus subtilis	11	9.7

Table 3: Phytochemical Analysis of Aloe vera Extracts

Phytochemical analysis	Ethanolextract	Methanol extract
Tannins	+	+
Saponins	+	+
Alkaloids	+	-
Glycosides	+	-
Terpenoids	+	+
Flavonoids	+	+
Steroids	-	-
Reducing sugar	+	+
Phenols	-	-

Key: + = detected: - = Not detected

Anti-Bacterial Activity

All the extracts of the plant showed varying degree of antibacterial activities against the test bacterial species. The ethanol extract exerted highest activity on bacterial agents tested compared to the methanol extract. The ethanol extract at the concentration of 100 mg / ml showed 22 mm and 24 mm diameter zone of inhibition against *E. coli and klebsiella pneumoniae respectively* This was followed by 20 mm, 18 mm and 17 mm zone of inhibition *against Pseudomonas aeruginosa, E coli* and *S. aureus* by the methanolic extract at 100 mg/ml concentration. The results of this study showed that the ethanolic extract was more effective than methanolic

extract. This may be due to the better solubility of the active components in organic solvents [27].

Several other reports are available regarding the antibacterial properties of ethanol extract of *Aloe vera leave* gel [28], identified aromatic or saturated organic compound which is obtained through initial ethanol or methanol extraction. This explains the higher antibacterial activity of ethanol extract. Ascorbic acid was reported from *Aloe vera* leave gel [29-30].

The comparsion of the activity of the plant extract with conventional antibiotics, such as gentamicin (positive control) confirmed reports by other researchers [31] that plant extracts are more active than constitutional antibiotics. The above findings pointed out that the higher the concentrations of the extracts, the higher the sensitivities of bacterial to the extracts as showed by the increased size of inhibition zones diameter and this is in conformity with [32]. The negative control (DMSO) showed no effect against the bacteria, and the result of the positive control (gentamicin).

Table 4: Anti-bacterial Activity of *Aloe vera* leave**s**

	Concentration of Extracts/Zone of Inhibition in mm**									
	Ethanol (mg/ml)					**Methanol (mm/mg)**				
Bacterial Isolates	100	50	25	12.5	6.25	100	50	25	12.5	6.25
E coli P. aeruginosa	22	20	16	13	12	8	14	11	9	6
S. aureus K. pneumoniae	16	12	9	8	8	20	10	7	5	ND
Proteus vulgaris	19	18	16	11	9	17	11	8	6	6
Bacillus subtilis	24	17	14	8	ND	11	8	6	5	5
DMSO	11	8	7	7	ND	10	7	5	ND	ND
Gentamicin	10	8	8	7	ND	9	6	6	5	N
	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	15	14	14	13	15	15	14	14	13	15

Key: DMSO = Dimethyl Sulfoxide. ** All the values are average values of the antimicrobial activities obtained from all the isolates ND = Not detected

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The minimum inhibitory concentration (MIC) of the extracts against the bacteria ranged from 50 -100 mg/ml and the minimum bactericidal concentration (MBC) ranged from 50 – 200 mg/ml. The comparsion of MIC and MCB of the ethanol and methanol extract of the plant leaf observed that ethanol extract showed greater antimicrobial activity compared to its corresponding extract in the methanolic extract. The ethanolic extract showed the highest activity against the clinical isolates of *Klebsiella pneumoniae, Escherichia coli, Staphylococcus auerus and Pseudomonas aeruginosa.* This is in line with the work of [33], whose work showed that the highest ethanolic extract activity was against the clinical isolates of *Staphylococcus auerus* and *Escherichia coli* then, *C. albicans*, followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Table 5: MIC and MBC of the extracts against bacterial isolates

Isolates	**E**		**M**	
	MIC	MBC	MIC	MBC
E coli	100	100	100	100
P. aeruginosa	50	100	50	100
S. aureus	50	50	50	50
K. pneumoniae	100	200	100	200
Proteus vulgaris	50	50	100	100
Bacillus subtilis	ND	ND	ND	ND

E = Ethanolic extract of *Aloe vera* leaves
M = Methanolic extract of *Aloe vera* leaves
MIC = Minimium Inhibitory concentration
MBC = Minimium Bactericidal Concentration
ND = Not detected

4. Conclusion

This study has revealed that *Aloe vera* leave extract possesses compounds with antibacterial properties which can be used as antibacterial agents in new drugs for the treatment of diabetic foot ulcers in patients. The result of the present study thus explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve microbial infections and underline the importance of ethno botanical approach for the selection of *Aloe vera* in the discovery of new bioactive compounds. This plant could be a source of new antibiotic compounds being nontoxic and less expensive than the allopathic drugs.

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