Phytology

# Evaluation of antibacterial activity of aloe vera extract on some bacterial pathogens

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**Abstract**

The antibacterial activity of Aloe Vera extract was tested on pathogenic bacteria *(Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus)* at different concentration of 40, 80, 120mg/ml using agar well diffusion method. Two aqueous extracts and ethanol were used. The ethanol extracts shows a high inhibition activity which revealed maximum inhibition *on E. coli* at the average of (11.3 ± 1.52 to 21.6 ± 2.08) *Pseudomonas aeruginosa* (15.6 ±1.15 to 23.6 ±0.58) and *Bacillus cereus* (15.6 ± 1.52 to 19.3 ± 1.52) compared with the average of the pathogenic organism on aqueous extract which show the minimum inhibition on *E. coli* (8.6 ±1.24 to 13.3 ±l.53), *Bacillus cereus* (12.6 ± 0.41 to 16.3 ±0.59), and *Pseudomonas aeruginosa* (11.51 ±1.00 to 17.7 ±1.52). Ethanol extract of aloe vera has a better inhibition than the aqueous extract. The minimum inhibitory concentration (MIC) of the aqueous extracts on the test organism was between 12.5 to 25mg /ml and ethanol extract between 6.25 to 12.5mg/ml. The minimum bactericidal concentration (MBC) of the aqueous extract on the test organisms ranged between 25 to 50mg/ml and on ethanol extract between 12.5 to 25mg/ml. These results suggest that aloe Vera gel extract with ethanol can be used as antibactericidal agent on human pathogens for medication, cosmetic and food purposes.

**Keywords:** aloe vera, concentration, inhibition, bactericidal

## Introduction

Traditional medicine is a practice for many centuries and plant extracts represents a continuous effort to find new compound against pathogens (Reynold and Dweck 1999) [14]. Approximately 20% of the plants found in the world have been sub milted to pharmacological or biological test, and a substantial number of new antibiotics introduced in the market are obtained from nurtured or semi-synthetic resources (Mothana *et al.*, 2005) [6]. The use of plant extracts with known antibacterial properties can of great significance in the treatment of numerous microbial infections (Saroj *et al.*, 2004) [16]. The use of plant product for pharmaceutical purpose has been gradually increased. According to World Health Organization, medicinal plants would be the best source for obtaining a variety of drugs (Alaebo and Onuoha 2022) [1].

*Abu- barbadensis miller* (aloe vera) belongs to lialiaceal family of which there are about 360 species. It is a cactuc-likc plant that grows readily in hot, dry climate and currently because of demand- it is cultivated in large quantities (Sulcyman and Scma 2009) [20]. When the leaf is cut, an orange-yellow sap drips from the open end. When the green skin of a leaf is removed, a clear mucilaginous substance appears that contains fibres, water and the ingredients to retain the water in the leaf (ShamimSumbul *et al.*, 2004) [17]. The aloe Vera gel contains about 99.3% of water, the remaining 0.07% is made up of solids with carbohydrates constituting for a large components (Onuoha and Alaebo 2022) [10]. The gel of Aloe Vera is contained in the leaves that were used to treat stomach ailments, gastrointestinal problems, skin diseases, constipations, radiation injury, inflammatory effects, healing wounds and burns, ulcer (Onuoha *et al.*, 2022a) [9]. The gel stimulates cell growth and enhances the restoration of damaged skin (Pugh *et al.*, 2001) [12]. It moisturizes the skin because it has water holding capacity. As a drink, it protects the mucous membrane of the stomach especially when irritated or damaged (Rcbc, 1997) [13].

Aloe Vera plant contains different nutrients including vitamins, minerals, enzymes, sugars, phenolic compounds and amino acids (Rodriquez *et al.*, 2005) [15]. Aloe Vera contains many vitamins including vitamin A, C, E and vitamins $b_1$(thiamine), $B_2$ (Riboflavin), $B_3$ (Niacin), and excluding vitamin D, and a number of therapeutic effects viz; anti-inflammatory, antibacterial, antifungal, antimicrobial and cell growth stimulatory activity (Alaebo *et al.*, 2022) [1].

**Fig 1:** Aloe Vera Plant (Sliarma *et al.*, 2022) [18]

## Materials and method

### Materials

The fresh, mature and healthy Aloe Vera leaves was collected at Chief Erasmus Kalu garden located at Ntiri new layout along Achara secondary school road, Ihechiowa community, Arochukwu L.G.A, Abia State. The Aloe Vera leaves were identified by the Botany department of Michael Okpara University of Agriculture Umudike. All the laboratory equipments used for this work were gotten from the Microbiology Department MOUAU. The test organisms were collected from the Microbiology Department of Federal Medical Centre, Umuahia (FMC) Abia State.

### Ethanol extraction method

A healthy, mature and fresh Aloe Vera leaves collected were washed with clean water and rinsed with distilled water. It was placed on a clean plastic to air dry, then it was dissected longitudinally and colourless parenchymatous tissue which is the Aloe Vera gel was scrapped out carefully using a sterile scalpel blade without the green fibers. 400g of Aloe Vera gel was weighed out using an electric weighing balance and it was ground using sterile mortar and pestle. 400ml of the aloe Vera gel was mixed with 100ml of ethanol in a beaker and covered with aluminum foil, it was allowed to stand for 24hours, then filtered through Watman filter paper and it was evaporated in the oven at 50°c for 24hours.

### Hot water extraction method

A healthy, mature and fresh Aloe Vera leaves collected were washed with clean water and rinsed with distilled water. It was placed on a clean plastic to air dry, then it was dissected longitudinally and colourless parenchymatous tissue which is the Aloe Vera gel was scrapped out carefully using a sterile scalpel blade without the green fibers. 400g was weighed out using electric weighing balance and it was ground using a sterile mortar and pestle. 400ml of the Aloe Vera was mixed with 100ml of hot water in a beaker and covered with aluminum foil, then left for 24hours and filtered with Watman filter paper and it was allowed to evaporate in the oven at 50°e for 24hours.

### Preparation of media

The media used were Nutrient agar and Mueller Hinton Agar (MHA). The required amount was measured and prepared according to manufacturer's instruction and poured into conical flask and covered with aluminum foil respectively, then sterilized by autoclaving at 121°c for 15minutcs and was poured in the Petri dishes, it was allowed to solidified.

### Test organisms used

1. *Escherichia Coli*
2. *Bacillus cereus*
3. *Pseudomonas aeruginosa*

### Determination of the antibacterial activity of Aloe Vera gel extract using agar well diffusion method

The laboratory bench was cleaned with cotton wool soaked in ethanol, the respective media (Mueller Hinton Agar and nutrient agar) were prepared according to the manufactures instruction into conical flask and auto-cleaved at 121°c for 15 minutes, it was allowed to cool and poured into labeled Petri dishes using pour plate method of dispensing and allowed to solidify according to Onuoha *et al*, (2022b) [11]. The test organisms *E. coli*, *Pseudomonas aeruginosa* and *Bacillus* were used to carry out the practical for three different days. A sterile wire loop was used to pick a loopful of the test organism and streaked on the appropriate solidified medium respectively. A sterile cork borer was used to make well on the surface of the dishes used (Diameter 5mm) different concentration of the extracts were prepared by dissolving 0.2g, 0.4g and 0.6g of the various extracts in 5ml of normal saline to obtain 40g/ml, 80mg/ml and 120mg/ml respectively. The extract was injected into the well made and was used as the positive control and normal saline as negative control. The dishes were incubated at 37° for 24hours.

### Determination of the Minimum Inhibitory Concentration (MIC)

The MIC of ethanol and water extracts were obtained by dissolving 0.5g of each of the extracts in 5ml sterile normal saline to obtain an initial concentration of 100mg/ml which was diluted to 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.12mg/ml and 1.6mg/ml respectively.

### Determination of Minimum Bactericidal Concentration (MBC)

One Milliliter (1ml) was pipetted from the mixture obtained in the determination of minimum inhibitory concentration (MIC) tubes which did not show any growth and inoculated into sterile nutrient agar by streaking and incubated for 24hours, the least concentration of the extract with no visible growth after incubation was taken as the minimum bactericidal concentration (MBC).

## Result

The 400ml of the Aloe Vera gel prepared after mixing with aqueous and ethanol in separate conical flask with 100ml of each solvent was filtered with Watman paper after 24 hours. The standard ethanol extracts obtained weighed 32g and the aqueous extract was 28g.

The antibacterial activities of Aloe Vera gel extracts using agar well diffusion method on a gram positive bacteria *(Bacillus cereus)* and gram negative *(E. coli* and *Pseudomonas aeruginosa* showed maximum zone of inhibition on ethanol extract in test organism 24mm on *E. coli*, 21mm on *Bacillus cereus* and 25mm on *Pseudomonas aeruginosa.* The aqueous maximum zone of inhibition on *E. coli is* 15mm, *Bacillus cereus* 7mm and *Pseudomonas aeruginosa* 18mm.

The three different concentration of both aqueous and ethanol extracts of Aloe Vera gel showed inhibitory effect on the three bacteria organism with maximum zone of inhibition in the highest concentration (120mg/ml).

The test organisms caused inhibition with significant difference. Ethanol extract has the highest one of inhibition on the three organisms as showed in the table 2.

Antibacterial activity of both aqueous and ethanol extract of Aloe average (The data represents average of three replicates i.e. mean and standard deviation. The diameter are in mm and the concentration are 40, 80, and I20mg/ml of both aqueous and ethanol extracts (mean $\pm$ SD) (n-3).

**Table 1:** Diameter zones of Inhibition (mm) produced by aqueous extracts on the test organisms

| Test organisms | 40 | 80 | 120mg/ml | Gentamycin (10mg) |
|---|---|---|---|---|
| *E.coli* | 8.6 ± 1.24 | 10.3 ± 0.58 | 13.3 ± 1.53 | 27.3 ± 3.21 |
| *Bacillus cereus* | 12.6 ± 0.41 | 14.3 ± 0.37 | 16.3 ± 0.59 | 26.3 ± 2.51 |
| *Pseudomonas Aeruginosa* | 11.5 ± 1.00 | 14.3 ± 1.49 | 17.7 ± 1.52 | 29.6 ± 4.66 |

**Table 2:** Diameter zones of Inhibition (mm) produced by ethanol extracts on the test organisms

| Test organisms | 40 | 80 | 120mg/ml | Gentamycin (10mg) |
|---|---|---|---|---|
| *E.coli* | 11.3 ± 1.52 | 19.3 ± 1.52 | 21.6 ± 2.08 | 29.6± 3.78 |
| *Bacillus cereus* | 15.6 ± 1.52 | 17.0± 1.00 | 19.3 ± 1.52 | 28.3 ± 3.51 |
| *Pseudomonas Aeruginosa* | 15.6± 1.52 | 19.0± 1.00 | 13.6± 0.58 | 30.6 ± 4.14 |

**Table 3:** The Minimum Inhibition Concentration (MIC) of the ethanol and aqueous extracts of the Aloe Vera on the test organisms

| Test organisms | Aqueous | Ethanol |
|---|---|---|
| *E.coli* | 12.5 | 6.25 |
| *Bacillus cereus* | 12.5 | 6.25 |
| *Pseudomonas Aeruginosa* | 25 | 12.5 |

**Table 4:** The Minimum Bactericidal Concentration (MBC) of both ethanol and aqueous extracts of Aloe Vera on the test organisms

| Test organisms | Aqueous | Ethanol |
|---|---|---|
| *E.coli* | 25 | 12.5 |
| *Bacillus cereus* | 25 | 12.5 |
| *Pseudomonas aeruginosa* | 50 | 25 |

The Minimum Inhibitory Concentration (MIC) of the aqueous for different organism ranged from 12.5 to 25mg/ml with that of ethanol which ranged between 6.25 to 12.5mg/ml in table 3. The Minimum Bactericidal Concentration (MBC) of the extract for different bacteria ranged between 25 to 50mg/ml for aqueous and ranged between 12.5 to 25mg/ml for ethanol in table 4.

## Discussion and conclusion

The antibacterial activity of Aloe Vera gel extract used on the test organism using agar well diffusion method with their varying zones of inhibition have shown the effect of the gel of Aloe Vera depending on the concentration of the extract used. The result reveals that the plant gel inhibits growth of the bacteria organism tested. The results confirmed the recent similar studies of Melini *et al.*, 2013 [5], that worked on a similar bacteria organism such as *Klebsilla* specie, *Staphylococcus* species observed inhibition using ethanol extract of Aloe Vera gel. This study investigated the antibacterial effect of Aloe Vera on some bacteria pathogens. The aqueous and ethanol extract of Aloe Vera was used in this study. The ethanol extract gave a better antibacterial activity than the aqueous extract, this may be due to better solubility of the active components in organic solvent (De Boer *et al.*, 2005) [4] and when compared the zone of inhibition with standard antibiotic drug (Gentamycin) the zone of inhibition of the drug was higher than the plant extract. The average range of inhibition of the test organism on the three concentration of 40, 80 and 120mg/ml on *Escherichia coli* with aqueous extract are 8.6 ± 1.24, 10.3 ±0.58,13.3 + 1.53 and for ethanol extract was 21.3 ± 1.52, 19.00 ±1.00, 21.6 ± 2.08.

*Pseudomonas aeruginosa* zone of inhibition on ethanol extract were 15.6± 1.15, I7.0 + 1.00and 23.6 ± 0.58 and on aqueous were 11.5 ± 1.00, 14.3 ± 1.49, 17.7 ± 1.52. *Bacillus cereus* zone of inhibition on aqueous extract were 12.6 ±0.41, 14.3 ±0.37, 16.3+0.59 and on ethanol extract were 15.6 + 1.52, 17.0 + 1.00, 19.3 +1.52. The bacteria isolates were sensitive to both extract used. In recent years, development of multidrug resistance in the pathogenic bacteria has created major clinical problems in the treatment of infectious disease (Davies, 1997) [3]. The problems such as toxicity of certain drugs on the host tissues triggered interest in search of new antibacterial substances drugs of plant origin. Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their antibacterial activity may provide new antibacterial drugs (Ni and Tizard 2004) [8]. Hence, the present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant (Aloe Vera) could be exploited in the management of diseases caused by both gram positive and gram negative organisms such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus* (Ncall, 2004) [7]. Previous studies revealed the antimicrobial activities of Aloe vera gel to kill or to greatly reduce the growth *Staphylococcus auerus, Samonella typhi, Staphylococcus pyogenes* (Allan and Lawless 2000) [2] also reported the capability of Aloe Vera gel extract in inhibiting the growth *of Streptococcus* species and *Klebsiella* species (Singh *et al.*, 1995) [19]. The result of their study is almost in accordance with the observation of present study. The bacterial isolates used in this study were sensitive to the extracts of ethanol and aqueous from Aloe Vera gel between the two extracts used. Ethanol showed higher zone of inhibition over aqueous extract. The study has revealed the importance of natural products to control antibiotic resistant bacteria which have a threat to human health. The result of this study support that plant extracts possesses compounds with antibacterial properties that can be used as antibacterial agents in drugs for the treatment of bacteria disease. The antibacterial activity of the extracts and their potency was assessed by the presence of inhibition zone. The ethanol extract was found to be a better solvent for inhibition of antibacterial organism compared to aqueous extracts.

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