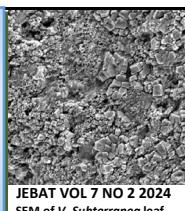


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SEM of *V. Subterranea* leaf

Antibacterial Activity of Aloe Vera Extracts on Some Clinical Bacterial Isolates

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ABSTRACT

Aloe is a plant that is used against skin irritation, skin exposure to UV and gamma radiation, scalds, sunburn wounds, eczema, psoriasis, acne, dermatitis, ulcers, and to stimulate cell regeneration. This study aimed to determine the antibacterial activity of ethanolic and gel extracts of Aloe vera against certain clinical bacterial isolates. The extracts were screened for phytochemicals, and their antibacterial activities were determined using the well diffusion method at concentrations of 50, 100, 200, and 400 mg/mL. The result of phytochemical screening indicates the presence of alkaloids, flavonoids, tannins, anthraquinones, and steroids in both extracts. The result of the antibacterial assay revealed that both ethanolic and gel extracts of Aloe vera were active against *E. coli* at different concentrations, with zones of inhibition ranging from 25.5 mm to 20 mm. However, *S. aureus* shows resistance to the different concentrations of the extracts. Findings show that *S. aureus* was sensitive to ciprofloxacin, while resistant to Augmentin. Thus, the observed antibacterial resistance confirms *S. aureus* as a multi-drug-resistant strain, thereby justifying its resistance to the Aloe vera plant. This study suggests that the Aloe vera plant contains antibacterial properties.

INTRODUCTION

Multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate usage of antimicrobial drugs for the treatment of diseases. This scenario prompted scientists to search for new antimicrobial products derived from medicinal plants, which are considered better sources of novel antimicrobial chemotherapeutic agents. The search for new sources of antibiotics is a global challenge for preoccupying research institutions, pharmaceutical companies, and academia, as many infectious agents are becoming resistant to synthetic drugs. Plants are the cheapest and safer alternative sources of antimicrobials [1]. Aloe vera (*Aloe barbadensis miller*) is a plant, which belongs to the family of Liliaceae and is mostly succulent with a whorl of elongated, pointed leaves [2]. The term ALOE refers to a solid residue obtained by evaporating the latex derived from the outer layers of the plant leaf. Taxonomists now refer to *Aloe barbadensis* as Aloe vera. The central bulk of the leaf contains colorless mucilaginous pulp, made up of large, thin-walled mesophyll cells containing the aloe vera gel itself. Despite its widespread use as a folk remedy over a long period, the biochemical details of its constituents and antibacterial activities have not been investigated [3].

Staphylococcus aureus is commonly a part of the body's microflora, found on the skin, in the nose, and in the mouth. About 20% of the human population is long-term carriers of *Staphylococcus aureus* [4]. The corotenoid pigment staphyloxaqthin is responsible for its characteristic golden color, which may be seen in the colonies of the microorganism. *S. aureus* can cause a range of illnesses from minor skin infections, such as impetigo, boils (furuncles), cellulitis, and also cause intestinal tract infection mostly through food poisoning [5].

Escherichia coli is a facultative anaerobic Gram-negative bacterium from the *Enterobacteriaceae* family that colonizes the gastrointestinal tract of warm-blooded animals shortly after birth, and it is a lifelong colonizer of adults. This species persists as a harmless commensal in the mucous layer, interacting with the host in a mutualistic manner. However, certain strains of *E. coli* with pathogenic properties can cause disease. In turn, nonpathogenic intestinal *E. coli* can eventually cause or contribute to disease in compromised hosts [6].

MATERIALS AND METHODS

Collection and Processing of the Aloe vera plant

The Aloe vera plant was purchased from Gombe main market and was brought to Gombe State University herbarium for identification. It was washed to remove the dirt. The gel from the freshly harvested leaves was extracted using a sterile knife and blender, then transferred into a sterile container and stored in the refrigerator at 4 °C prior to use. Some of the Aloe vera leaves were dissected longitudinally into three different parts using a Razor blade. The dissected leaves were air-dried for two weeks under low light intensity to expel water, then crushed in a mortar and further ground into fine powder using a mortar and pestle. The powder was sieved to remove residue, it was then stored in a polythene bag and kept in a dry place before extraction [7].

Method of Extraction of Aloe vera

Gel extraction

Mature, healthy, and freshly collected leaves of Aloe vera were washed with clean water, and then dissected longitudinally, and colorless parenchymatous tissue (aloe gel) was scraped out carefully using a sterile knife without the green fibers. The collected gel, weighing 100g, was ground and mixed with 100ml of hot water, and then allowed to stand for 24 hours. The extract was then filtered through Whatman's No. 1 filter paper and evaporated. 19.0g of the aqueous extract was obtained and stored in the refrigerator at 4 °C until required [12].

Ethanol extraction

About 20 g of Aloe vera powder was measured carefully using a digital weighing balance. The measured powder was soaked in 200 mL of ethanol in a conical flask and allowed to stand for two weeks with regular shaking at room temperature. It was then filtered using filter paper. The collected filtrate was evaporated using a rotary evaporator, leaving behind a thick, semi-solid extract, and stored at 4 °C [12].

Phytochemical Tests

The ethanolic and gel extracts of Aloe vera were subjected to phytochemical screening to detect the presence of bioactive constituents, such as alkaloids, flavonoids, tannins, steroids, and anthraquinones, using standard methods [9].

Collection of test organisms

The test isolates, which were *Escherichia coli* and *Staphylococcus aureus*, were collected from Specialist Hospital Gombe in a slant and then transported to the Microbiology Laboratory.

Identification and confirmation of the test organism

The bacterial isolates of *E. coli* and *S. aureus* obtained from Specialist Hospital, Gombe, were identified and confirmed using Gram staining and biochemical tests [10].

Standardization of inocula

Colonies of *E. coli* and *S. aureus* were picked and emulsified in 5 mL of normal saline in separate test tubes, and the turbidities were matched with a 0.5 MacFarland standard [11].

Antibacterial activities of Aloe vera extracts on *E. coli* and *S. aureus*

Mueller-Hinton agar was swabbed with the standardized inoculum of the test organisms using a sterile swab stick. A sterile cork borer was used to drill wells of 6 mm in the agar. 0.1 mL of the different concentrations (50, 100, 200, and 400 mg/mL) of

aloe vera gel and ethanolic extracts were poured into the wells, and the plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured and recorded [11].

RESULTS

Physical properties of Aloe vera Extracts

Table 1 shows the results of the extraction of aloe vera. Two different types of Aloe vera extract were obtained. The ethanolic extract appeared dark brown in color and gummy in texture. The gel extract was gray and gummy as well.

Table 1. Physical properties of Aloe vera extracts.

Extract Code	Weight of Material Used (g)	Weight of Extract Obtained (g)	Colour	Texture
EEA	20	10	Dark brown	Gummy
GEA	100	19	Gray	Gummy

Keys:

EEA: Ethanolic extract of Aloe vera

GEA: Gel extract of Aloe vera

Phytochemical Composition of Aloe vera

Table 2 presents the results of the phytochemical screening conducted on both the ethanolic extract and the gel extract of *Aloe vera*. The results show that both types of extracts contain several important classes of bioactive compounds. Specifically, alkaloids, flavonoids, tannins, anthraquinones, and steroids were all detected in both extracts. The presence of these compounds in both extracts suggests that *Aloe vera*, whether in gel or ethanolic form, has potential for use in traditional medicine and pharmaceutical applications.

Table 2. Phytochemical composition of Aloe vera gel and ethanolic extract.

Extract Code	Alkaloid	Flavonoid	Tannin	Anthraquinone	Steroid
EEA	+	+	+	+	+
GEA	+	+	+	+	+

Keys:

EEA: Ethanolic extract of Aloe vera

GEA: Gel extract of aloe vera

Antibacterial Activity of Aloe vera Extracts

Table 3 presents the antibacterial effects of *Aloe vera* gel and ethanolic extracts tested against the pathogen *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) at the following concentrations: 50, 100, 200, and 400 mg/mL. The results show that *E. coli* was sensitive to both preparation of *Aloe vera* extracts. As an increase in the concentration of the extracts was carried out, the diameter of the inhibition zones also increased, indicating a concentration-dependent antibacterial activity. The gel extract of *Aloe vera* (G.E.A) exhibited the strongest effect, reaching a maximum inhibition zone of 25 mm at the highest concentration (400 mg/mL). In comparison, the ethanolic extract (E.E.A) produced a maximum inhibition zone of 20 mm at the same concentration.

At the lowest concentration (50 mg/mL), the gel extract inhibited *E. coli* with a zone of 16 mm, while the ethanolic extract produced a smaller zone of 12 mm. These findings suggest that the gel extract has stronger antibacterial properties than the ethanolic extract, particularly against *E. coli*. Instead, *S. aureus* was completely resistant to both extracts at all tested concentrations. No inhibition zones were observed for *S. aureus*. This means that neither the gel nor the ethanolic extract of *Aloe vera* was effective against this bacterium under the conditions tested. As a comparison, standard antibiotics were also included in the test. Ciprofloxacin (CIP) and Augmentin (AUG) showed

clear inhibition zones of 24 mm and 20 mm, respectively, confirming the susceptibility of *S. aureus* to conventional antibiotics and validating the experimental setup.

Table 3. Antibacterial activity of gel and ethanolic extracts of aloe vera plant on *S. aureus* and *E. coli*.

Extracts	<i>E. coli</i>				<i>S. aureus</i>				CIP (μ g)	AUG (μ g)
	50	100	200	400	50	100	200	400		
G.E.A	16	19	21	25	0.0	0.0	0.0	0.0		
E.E.A	12	14	17	20	0.0	0.0	0.0	0.0	24	20

Key:

E.E.A = Ethanolic Extract of *Aloe vera*, G

E.A = Gel Extract of *Aloe vera*,

CIP = Ciprofloxacin, AUG = Augmentin.

DISCUSSION

The phytochemical analysis (**Table 2**) of the extracts revealed the presence of alkaloid, flavonoids, tannins, anthraquinones and steroid. The presence of these bioactive compounds in the extracts has made the plant known for its medicinal usage, especially for antibacterial activity against disease-causing organisms. Tannin has been reported to interfere with bacterial cell protein synthesis and is important in the treatment of ulcerated or inflamed tissues and also in the treatment of intestinal disorders [12]. Alkaloid has also been reported to have analgesic properties, and saponin exhibits a moderating effect against inflammation [13]. Flavonoids are also important in combating inflammation and microorganisms. This result is in agreement with the work of Gary et al. [14] who reported the presence of tannins, alkaloids, steroids, and flavonoids in *Aloe vera* extracts.

The antibacterial activities of *Aloe vera* extracts against *E. coli* showed zones of inhibition in different variations, ranging from 12 to 25 mm. The gel and ethanolic extracts were active against *E. coli* at different concentrations, with the gel extract exhibiting higher activity. This is in line with the work of Gary et al. [14] who work on the phytochemical screening and a comparative study of the antibacterial activity of *Aloe vera* green rind, gel, and leaf pulp extracts. His results show that all the extracts were active against *E. coli* at different concentrations. It is also in agreement with a previous work [15] that reported that *aloe vera* gel was active on *Escherichia coli* at 10^{-1} , 11.5 mm and 12.5 mm for 1st and 2nd agar well, respectively and a lower inhibitory growth of 10 mm at 10^{-6} .

S. aureus was not sensitive to either the gel and the ethanolic extract of the *Aloe vera*. This may be due to resistance acquired by the organism, which can occur through mutation, transformation, or the misuse of antibiotics. This result contrasts with a previous report [15], who investigated the antibacterial effect of *Aloe vera* gel extract against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* isolated from the gastrointestinal tract of poultry birds. The result shows that *Aloe vera* gel extract at different concentrations inhibited the growth of the microorganisms. Though there was a higher inhibitory effect on *Staphylococcus aureus* at 10^{-1} , 13.5 mm and 14.5 mm for 1st and 2nd agar well, respectively, and a lower inhibitory growth of 8 mm and 8.3 mm at 10^{-8} .

CONCLUSION

Conclusively, this study revealed that the *Aloe vera* plant has an antibacterial effect on *Escherichia coli*, but is resistant to *S. aureus*. The gel of *Aloe vera* has higher antibacterial activity on

the isolates than the ethanolic extract. Therefore, the gel and the ethanolic extracts have potential for the development of drugs to treat diseases caused by *E. coli*. Several studies have attributed the inhibitory effect of the plant to the presence of bioactive constituents such as alkaloids, flavonoids, steroids, terpenoids, carbohydrates, tannins, and anthraquinones. This illustrates that *Aloe vera* can be used for treating infections.

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