



## Preliminary Investigation Associated with Antibacterial Potency of Both Juice Extract and an Isolate from *Aloe vera* Gel

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### Authors' contributions

This work was carried out in collaboration between all authors. Author AMD designed the study, wrote the protocol and wrote the first draft of the manuscript. Author RONJ managed the literature searches, analyses of the study performed the spectroscopy analysis, authors AMD and MB managed the experimental process and author RONJ identified the species of plant. All authors read and approved the final manuscript.

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### ABSTRACT

**Objective:** The aim of this study was to investigate the antibacterial activity of both the juice extract and an isolate of *Aloe vera* leaves of gel from Ghana on clinical isolate bacteria found to prolong wound infections.

**Methods:** Using sharp knife, the rind (outer cover) of the leaf and the layer immediately below the rind were removed to obtain the internal gel matrix portion (fillet). The isolate was precipitated from propylene glycol of the Aloe juice extract and was used to inhibit *Escherichia coli* and *Staphylococcus aureus*. Agar diffusion bioassay was used for inhibitory test of both the whole aloe juice and the isolate against *E. coli* and *S. aureus*.

**Results:** Our findings exhibited that there was no significant difference in activity of both the isolate

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and the whole juice on *E. coli* at all concentrations used. *S. aureus* was resistant to both the isolate and the whole juice at all concentrations used, compared with the positive control, chloramphenicol which showed an intermediary zone of inhibition of 13.5 mm at a concentration of 10 g/L. A one-way analysis of variance (ANOVA) at ( $P \leq 0.05$ ) of the diameter zone of inhibition values compared with the activity of Chloramphenicol (positive control) indicated significant inhibitory activity by both the isolate and the whole juice extract against *E. coli* thus suggesting their efficacy in treating *E. coli* infections within the concentration used in this inhibitory studies.

**Conclusion:** Both the *A. vera* juice extract and the isolate showed high activity against *E. coli*. These seem to justify the widespread use of the plant in the treatment of gastrointestinal diseases and its application in many pharmaceutical products.

**Keywords:** *Aloe vera*; Acemannan; *Staphylococcus aureus*; *Escherichia coli*; Zone of inhibition; Isolate.

## 1. INTRODUCTION

There is a growing demand for medicinal plant and plants products as an option to orthodox medicines principally in developing countries. The potential of higher plants as sources for new drugs is still chiefly uncharted. Medicinal plants are valuable antimicrobial agents and source of many potent and powerful drugs [1]. Over the ages right up to these modern era, the *A. vera* plant has been used to treat a broad catalog of diseases as well as its application in a host of cosmetic purposes. The inner gel has been used to sooth flesh wounds such as burns, cuts, scrapes and stings; it has also been used for headaches, gum sores, hemorrhoids, skin disorders, acne, dandruff and even baldness [2]. Aloe's role on healing process and its ability to penetrate target areas and stimulate cell regeneration has also been scientifically documented along with a multitude of other properties that buttress *A. vera*'s role as a healer, a restorer and a protector [3]. Research work by Tanka et al. [4] in Phytotherapy Research has found *A. vera* to contain two anti-diabetic active compounds and therefore have shown the plant's ability to stabilize blood sugar in diabetics.

Scientific research has shown that the polysaccharides within *A. vera* have strong immune boosting and cancerous fighting capabilities [5,6]. The polysaccharides are believed to exhibit potent macrophage activators and produce increase volume of nitric acid, a compound that has tumor fighting potential [7]. One major polysaccharide, acemannan, a phytonutrient found in *A. vera* has been established to improve wound healing [8], it has also proven to exhibit antiviral activity, inhibiting reproduction of herpes [9,10], measles and the HIV virus *In vitro* [11]. Acemannan, which is the active ingredient in *A. vera* plant has been found not only important in sealing wounds but also

keeping out bacteria agents. In this research we obtained the crude extract from the leaves of the *A. Vera* plant and further isolated a crude product from the extract. We then investigated the effects of both the isolate and the juice extract on clinically isolated bacteria, *E. coli* and *S. aureus*.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The *A. vera* plant were collected from Bolgatanga Parks and Gardens, in the Upper East Region of Ghana in the month of November, 2014 and authenticated as *Aloe barbadensis miller* by Dr. Isaac Sackey of the Department of Applied Biology in the Faculty of Applied Sciences of the University for Development Studies, Navrongo Campus

### 2.2 Source of Test Bacteria

Clinical isolates of *E. coli* and *S. aureus* were obtained from the Medical Microbiology Laboratory of the Bolgatanga Regional Hospital Ghana. Pure cultures of each of the bacterial isolates were obtained by sub-culturing the isolates on Chocolate agar (Oxoid, Basingstoke, Hampshire, England)

### 2.3 Preparation of *Aloe vera* Juice Extract

The leaves, 8.8 kg of *A. vera* were cut at the base without breaking or damaging any part of the leaves using a sharp knife. The leaves were washed thoroughly under running water to remove surface dirt and traces of any contaminants. The lower end portions of the leaves were cut using sharp knife in other to drain the yellow sap in the leaves and the remaining portions of the leaves were cut into segments and placed uprightly in an aqueous solution to drain all remaining yellow sap for about thirty minutes. Using sharp knife, the rind

(outer cover) of the leaf and the layer immediately below the rind were removed to obtain the internal gel matrix portion (fillet). The fillets were washed under running water to remove any yellow sap and rinds that might have adhered to the fillet. The internal gel matrixes were drained for approximately an hour by placing the fillets on a mesh to obtain a gel matrix strip. The drained gel matrix strips was weighed and recorded as 600 g.

#### **2.4 Isolation of the Crude Product ('isolate') from the *A. vera* Juice**

The gel matrix strips, 500 g were transferred into a blender and rotated at a high speed for two minutes. The blended fillets were then cooled in a refrigerator to allow the foam formed to settle and the cooled fillets were transferred into a Pyrex measuring cylinder and the volume was found to be 500 ml. The pH of the blended fillets was adjusted to 3.1 by addition of 5 ml 30% hydrochloric acid. Ethanol 70% (v/v) was then added gently to the measured volume in the ratio 3:1 ethanol/fillets mixture while stirring. This was done to precipitate most of the polysaccharides in the mixture. The flocculent residue floating on the ethanol layer was removed using a stirrer. The mixture was allowed to settle for four hours at room temperature and the ethanol layer was decanted gently without disturbing the precipitate at the bottom of the mixture. The precipitate was then transferred into a test tube and centrifuged at 5000 rpm for two minutes. The supernatant was decanted and the precipitate transferred into a VWR brand test tube for use in the next experiment.

Propylene glycol, 80%, 20 mL, was added to the precipitate recovered from the ethanol mixture and the mixture was vigorously shaken and then centrifuged at 5000 rpm for two minutes. The thick smooth gel forming the supernatant, 15 mL, was collected and diluted with 28 mL distilled water to obtain a 30% (v/v) propylene glycol mixture. The solution was kept at -30°C for 24 hours and then mounted on a freeze-dryer overnight to afford a thick gel of 1.5 g.

#### **2.5 Preparation of the Aloe Juice Extract and the Isolated Product**

Five different concentrations 2.0, 4.0, 6.0, 8.0 and 10.0 g/L each of both the thick gel (isolate) and the juice extract were prepared using buffer obtain a thick gel of 1.5 g yield. The gel was characterized by applying simple physical

solution, pH 7.2. These concentrations were used for the antibacterial activity studies.

#### **2.6 Agar Diffusion Bioassay**

The modified agar well diffusion method was employed. Colonies of a pure culture of each test organism was suspended in 100 mL of sterile peptone water to get a turbidity of about 2 McFarland Standard. The suspended test organism, 1 mL was surface-spread on Mueller Hinton Agar (OXOID) until complete diffusion. Five-millimeter diameter wells were made on the culture plates with a sterile cork-borer at wide enough intervals and corresponding with the number of concentrations of each of both the juice extract and the isolate, and that of the positive and negative controls. For each juice extract and the isolate, 100  $\mu$ L of each concentration was drawn into a labeled well with a sterile micropipette taking care to avoid spillage onto the surface of the culture plate. Same quantity of the negative control (buffer, pH 7.2) and positive control (2.0, 4.0, 6.0, 8.0 and 10.0 g/L concentrations of chloramphenicol) were also introduced into a well each on the same plate. Plates were left to stand until complete diffusion of the formulations into the medium. Plates were incubated in inverted positions at 37°C for 48 hours after which they were observed for inhibitory activity depicted by zones of inhibition around the wells. Inhibition zone diameters were measured using a ruler and recorded in millimeters (mm). The experiments were repeated three times to check for reproducibility.

#### **2.7 Statistical Analysis**

Means and standard mean errors were calculated for the zones of inhibition measured for the three sets of experiments in each case. These means were statistically scrutinized using the one – way ANOVA to determine if they were significantly different at  $P \leq 0.05$ .

### **3. RESULTS**

#### **3.1 Extraction**

The juice, a jelly-like residue was extracted from 8.8 kg of *A. vera*. An isolate was precipitated from 500 mL of the aloe juice extract to obtain a light yellowish residue. The precipitate was dissolved in propylene glycol and freeze dried to properties such as pH, solubility and alkaline test. The pH of the gel was found to be 3.1. The

gel formed less viscous homogenous solution in propylene glycol and alkali test indicated a non-viscous gel, an indicative of the cleavage of an o-acetyl bond in a polysaccharide, which suggested the presence of acemannan.

### 3.2 Inhibitory Studies of the Isolate and Juice Extract on *E. coli*

*E. coli* were resistant at lower concentrations of 2 g/L and 4 g/L of the *A. vera* juice. The bacteria became susceptible to the juice extract when the concentrations were increased from 6, 8 and 10 g/L with corresponding zone of inhibition, 17.5, 19.0, and 23.0 mm respectively (Fig. 1). This means that a study increase in concentration increases the activity of the *A. vera* juice with no significant toxicity. *E. coli* were resistant to the *A. vera* gel isolate at a concentration of 2.0 g/L but showed intermediate sensitivity at 4.0 g/L concentration. The bacteria became more susceptible at a concentration of 10.0 g/L with zone of inhibition of 25.5±0.3 mm (Fig. 1). Comparing the two extracts on *E. coli*, the isolate was potent against the bacterium at higher concentrations except at the concentrations of 2.0 g/L, zone of inhibition of 11.5 mm, which did not show any inhibition, but gave intermediate inhibitory effect at 4.0 g/L, zone of inhibition of 14.5 mm. The juice extract also exhibited no inhibitory effect on *E. coli* at a concentration of 2.0 g/L but gave intermediate potent effect at 4.0 and 6.0 g/L, zone of inhibition of 12.0 and 17.5 mm respectively. At higher concentrations of 8.0 and 10.0 g/l, the juice extracts were susceptible

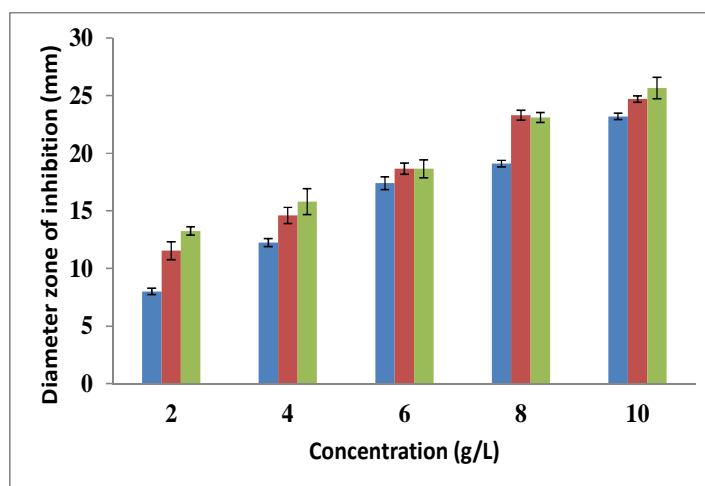
to *E. coli*, zone of inhibition of 19.0 and 23.0 mm correspondingly (Fig. 1). The propylene glycol used in isolating the active compound showed no inhibitory activity when tested. The positive control, that is, chloramphenicol showed only intermediate susceptible zones of inhibition at all concentrations.

### 3.3 Inhibitory Studies of an Isolate and Juice Extract of *A. vera* on *S. aureus*

*S. aureus* was resistant all the concentrations of the *A. vera* juice. However, at concentration of 10 g/L the juice extract exhibited zone of inhibition although it fell in the resistant zone. Similarly, the isolate also inhibited the growth of *S. aureus* at 10 g/L concentration, showing a zone of inhibition within the resistant zone. Negative control showed no zone of inhibition. However, four concentrations of the positive control showed zones of inhibition with three of them appearing in the resisted zone (Fig. 2).

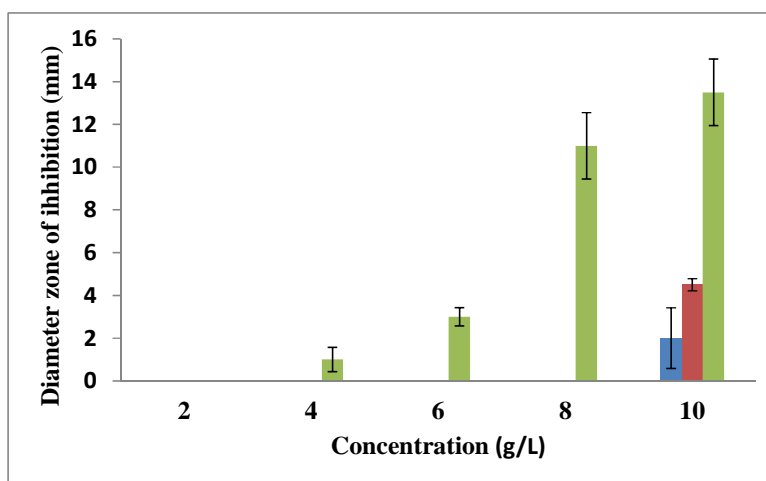
## 4. DISCUSSION

Wound healing is affected by innumerable wound healing factors and the deficiency of any of these factors can result in the retardation of the healing process. Several mechanisms have been proposed for the wound healing effects of acemannan, which include keeping the wound moist, increase epithelial cell migration, more rapid maturation of collagen and reduction in inflammation [12]. In the wound healing process,



**Fig. 1. Representative antibacterial activity of *A. vera* juice against *E. coli*. The data shown represent the average of three wells treated on the same day. The experiment was repeated 3 times and day-to-day variation was found to be within one fold of the presented data**

(■) An Isolate from *Aloe vera* juice; (■) *Aloe vera* juice; (■) Standard antibiotic



**Fig. 2. Representative antibacterial activity of an Isolate from Aloe barbadensis juice against *S. aureus*. The data shown represent the average of three wells treated on the same day. The experiment was repeated 3 times and day-to-day variation was found to be within one fold of the presented data**

(■) An Isolate from Aloe vera juice; (■) Aloe vera juice; (■) Standard antibiotic

acemannan stimulates the release of growth factor-beta, platelet-rich plasma, activated macrophage supernatant and growth hormone mediators, components responsible for wound healing. The potent pharmacological component, acemannan, is known to inhibit bacterial growth by inducing macrophages to release cytokines that help fight bacteria.

In this current research, we intended to extract pure beta-(1,4)-linked acetylated mannan (acemannan) from the aloe juice and physico-chemically and spectroscopically characterize using  $^1\text{H}$  and  $^{13}\text{C}$  NMR, IR spectroscopy, GC/MS, after HPLC purification to homogeneity. We further aimed at comparing the antibacterial activity of the pure mannan to that of the crude juice extract on wound infecting microbes. Since there was not enough physicochemical characterization to elucidate the structure of the isolated compound, we termed it an "isolate". Even though the solubility of the isolate in propylene glycol, the pH and alkali test gave positive indication for the presence of acemannan it was inconclusive without other vital spectroscopic characterization. Alkali treatment of the isolate displayed less jelly-like forming property, indicating the disruption of an o-acetyl bond and resulting in decreasing entirely its viscosity. The de-acetylated product was not soluble in water due to perceptibly increased stronger hydrogen bonding. Moreover, a 0.2% (w/v) of the isolate gave a pH  $6.31 \pm 0.33$  which

was approximately similar to the value determined by a scientific research group [13].

The zones of inhibition of both *A. vera* juice and the isolate on *E. coli* showed linear correlation. The activities were dose dependent with increasing zones of inhibition with a corresponding increase in concentration. Although the activity of the isolate indicated a higher potency against *E. coli*, it was not statistically significant compared with that of the juice extract. The probability of accepting or rejecting the null hypothesis ( $p_r$ ) was greater than the  $\alpha$ -value at 95% confidence interval, that is, ( $p_r > 0.05$ ). This meant that the difference in their activity was statistically insignificant. The p-value for the extracts together with chloramphenicol on *E. coli* was also greater than the  $\alpha$ -value at a 95% confidence interval, that is, ( $p_r > 0.05$ ). Similarly the p-value for the extracts on *S. aureus* was also greater than the  $\alpha$ -value. This statistics showed that the efficacy of the aloe isolate was independent on other compounds present in the juice.

## 5. CONCLUSION

The comparatively high activities shown by both the *A. vera* juice extract and the isolate against *E. coli* seem to justify the widespread use of the plant in the treatment of gastrointestinal diseases and its application in many pharmaceutical products. The high activity exhibited also seems

to favour aloe's ability to penetrate target areas and stimulate cell regeneration. Isolation, purification, characterization and structure elucidation of the isolate could lead to the development of novel drugs from *A. Vera* and this is the future work in our laboratory.

### CONSENT

It is not applicable.

### ETHICAL APPROVAL

It is not applicable.

### ACKNOWLEDGEMENT

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### COMPETING INTERESTS

It is hereby declared that the authors have no competing financial interests whatsoever in relation to the work described here. It is purely for academic and intellectual purposes.

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