The in vitro susceptibilities of *Escherichia coli* and *Staphylococcus aureus* were evaluated and the two organisms were susceptible to the inner gel of *Aloe barbadensis*, though it was more effective against *Staphylococcus aureus* than *Escherichia coli*. The reduction for *Aloe Vera* (AV) needed to suppress the growth of the gram-positive bacterium was attributed to the structural differences between the two organisms.

**Key Words:** *Aloe barbadensis*, *Escherichia coli*, *Staphylococcus aureus*.

The fresh whole leaves of the succulent are fleshy and removal of the thick outer cuticle reveals a mucilaginous inner gel. This is the major component in many reputable commercial products, found in a preserved but otherwise untreated form. More than 75 active ingredients have been identified from the inner gel, each of which may have a range of mechanisms of action, acting synergistically, or individually to explain the numerous curative properties. This has led to a trend to isolate and analyze individual ingredients in an attempt to attribute therapeutic potential to each ingredient. This has not necessarily shed light on the issue, and in particular, in vivo studies have not been able to distinguish between the antimicrobial effects of the plant and stimulation of the immune system. Whole leaf components proposed to have direct antibacterial properties include anthraquinones and saponins, while polysaccharides have been attributed with indirect bactericidal activity through stimulation of phagocytic leukocytes to destroy bacteria.

Since there has been limited research on the inner gel, the aim of this study was to provide evidence for the antibacterial effectiveness of this component prior to extensive experimentation. The ultimate goal would be to attempt to characterize the factors that provide biologic activity. The main organism used in this study was *Escherichia coli*, motile bacilli, belonging to the Enterobacteriaceae family. They are an important cause of gastrointestinal illness, diarrhea and urinary tract infection in adults and children. The increasing multiple antibiotic resistance, particularly resistance to ampicillin, chloramphenicol, tetracycline, nalidixic acid, carbenicillin, neomycin is of major concern. There is also increasing evidence that antibiotic therapy can exacerbate the symptoms of gastrointestinal diseases, and an alternative treatment would be beneficial. In addition, susceptibility of the gram-
positive *Staphylococcus aureus* to AV was briefly investigated, as this is another organism which shows antibiotic resistance.16

### Materials and Methods

*Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538-p) were used. Stock cultures were subcultured and maintained in Tryptic soy broth, at 37°C. Freeze dried *Aloe Vera* was obtained that consisted of inner gel from plants that were two and half years old at the time of harvest. The powder of freeze dried powder was reconstituted in phosphate buffered saline (pH 7.5) at 37°C for 30 min and then sterile filtered through a 0.22 μm pore size filter. For comparative studies *Aloe Vera* gel consisting of undiluted, unfiltered inner leaf gel was also used. This was filtered through sterile Whatman no. 54 filter paper. It was not possible to accurately determine the amount of active material lost by this process. Ampicillin (AMP) and/or nalidixic acid (NAL) (Sigma – Aldrich Ltd, St Louis, MO, USA), also used for comparison, were dissolved in phosphate-buffered saline and filtered through a 0.22-pore size filter. Reagents used in this study were obtained from the following sources: Muller-Hinton broth and agar were from Himedia, Mumbai, India. 96 well tissue culture plates from Becton Dickinson, Bradford, MA, USA.

#### Disc-Diffusion Assay

The zones of inhibition were calculated of by agar diffusion method. Wells were cut into nutrient agar plates, and 75 per the test agent, AV (7 to 450 mg/ml) or antibiotic (2 to 512 mg/ml) was added to each well. The plates were seeded with *Escherichia coli* lawn was seeded on the agar and incubated at 37°C for 24 hrs. The diameters of the inhibition zones were measured (Table I) concentrations greater than 115 mg of AV per ml 34 per of AMP per ml, and 126 mg of NAL per ml showed significant (P<0.001) growth inhibition compared with untreated control walls. It was not possible to make a comparison with *Aloe Vera* gel due to difficulties encountered with diffusion through the agar. The agar failed to set in an attempt to mix the *Aloe Vera* Gel with the agar prior to seeding the *Escherichia coli* on the surface to obtain a total viable count, which was attributed to the presence of pectinase in the preparation.

#### Growth Curve

Growth curves were generated as follows. A 96 well micro titer plate containing 100 μl of TSB per well was inoculated with 10 μl of the organism (10^3 CFU) at 37°C for 30 hours (this was done in triplicate). The plate was agitated, and A620 readings were were taken at hourly intervals on a Trizertek Multiscan MCC/340 (Midland, ON, Canada) plate reader. The number of bacteria per milliliter was calculated from the following equation (A620 × 2 × 10^8 )/0.2. Growth curves were also set up with 100 μl of test agent: AV (6 to 450 mg/ml), *Aloe Vera* gel (7 to 90%), AMP (2 to 512 μg/ml), NAL (2 to 512 μg/ml).

#### Methods

The growth inhibition of AV was further quantified by incubating 100 μl of both with 100 ml

<table>
<thead>
<tr>
<th>Dilution</th>
<th>AV (mg/ml)</th>
<th>Mean zone of inhibition (diam) ± SD (cm) (n = 3)</th>
<th>AMP (μg/ml)</th>
<th>NAL (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>2.6 ± 0.2</td>
<td>–</td>
<td>3.2 ± 0.1</td>
<td>3.7 ± 1.0</td>
</tr>
<tr>
<td>1:4</td>
<td>2.1 ± 0.1</td>
<td>–</td>
<td>2.0 ± 0.2</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>1:8</td>
<td>1.6 ± 0.1</td>
<td>–</td>
<td>1.7 ± 0.2</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>1:16</td>
<td>1.4 ± 0.2</td>
<td>–</td>
<td>–</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>1:32</td>
<td>1.1 ± 0.1</td>
<td>–</td>
<td>–</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>1:64</td>
<td>1.1 ± 0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Wells were cut in nutrient agar; test agent was added to each well and overlaid with a lawn of *Escherichia coli*. The starting concentrations of the test agents were as following AV (*Aloe Vera*), 450 mg/ml; AVG (*Aloe Vera* gel); 90% (Vol/ Vol); AMP and NAL, 515 mg/ml. c. Significant incubation compared with untreated wells (P<0.001); d. Significant incubation compared with untreated wells (P<0.05). No incubation detected.
of two fold dilutions of AV (in triplicate) and 10 μl of Escherichia coli (10^2 CFU) at 37°C for 6 hours. Control wells contained bacteria (positive) or broth (negative) only. Alamar blue (10 μl) was added to each well and incubated at 37°C for a further 2 hours. The plate was centrifuged for 10 min at 12,000 × g to pellet the bacteria, and the supernatant was transferred to a fresh microtiter plate. A_{570} and A_{600} readings were taken on a Spectra max 190 spectrometer, and the percentage growth calculated using the following equation: (mean A_{570} – A_{600} of test agent/mean A_{570} – A_{600} of the positive control) × 100. Figure 1 shows the dose response of Escherichia coli to AV after 8 hours. Since Alamar blue assay incorporates a colorimetric growth indicator to detect metabolic activity, it is useful in cytotoxicity experiments. These assays were designed on a small scale in microtiter plates to reduce the amount of material used. However the same results were obtained from large scale experiments using an inoculum size of >5 × 10^5 CFU/ml. It was important to optimize the assay for microtiter plates, since preliminary follow up experiments have shown that extraction of the plant material can produce a low yield depending on the solvents used.

**Statistical Analysis**

Tests and analysis were run in triplicates. Mean values ±SD of triplicates were calculated. Statistical analysis were performed using Student’s t test. The values were considered significant when P<0.001 and P<0.05.

**Results**

The untreated bacteria reached log phase between 3 to 10 hours, with maximum growth after 22 hours. All the test agents suppressed bacterial growth for up to 24 hours, to various degrees. The 50% inhibition doses were 32 mg of AV per ml, 15% (vol/vol) Aloe Vera gel, 4 μg of NAL per ml and 5 μg of Amp per ml.

To compare the effect of AV against a gram positive bacterium growth curves were set up with Escherichia coli and Staphylococcus aureus. Effective growth inhibition, up to 24 hrs, was achieved with concentrations of more than 100 mg of AV per ml for Escherichia coli and 30 mg of AV per ml for Staphylococcus aureus (Figure 2). The reduction for AV needed to suppress the growth of the gram-positive bacterium was attributed to the structural differences between the two organisms.
**Discussion**

In view of the complexities of examining the pharmacology of *Aloe barbadensis*, simple assays, which can be easily replicated to test multiple fractions, are essential to establish antimicrobial activity. The assays described in this report enable easy multi parameter comparison and allow a range of bacterial species to be examined. This preliminary study established the susceptibilities of *Escherichia coli* and *Staphylococcus aureus* to the inner gel of *Aloe barbadensis* Miller or *Aloe Vera*. The next stage will involve solvents and aqueous extraction of the inner gel to isolate and identify molecules for further research. Though the activities of the AV and Aloe Vera Gel appear to be low in comparison to those of NAL, and AMP, individual components may have greater activity. Glycosides of anthraquinones and dihydroxy anthraquinones, acemannan and saponins are active antibacterial components found in the whole plant and known to be constituents of the inner gel⁹. Further researches on these and other molecules will provide further evidence of the therapeutic potential of the inner gel. The direct effect of the inner gel on bacteria, which are found in accessible areas of the body, enables further development of antibacterial products, which can protect the mucosa.

**References**


3) **CROSSWHITE FS, CROSSWHITE CD.** Aloe vera, plant symbolism and the threshing floor. Desert Plants 1984; 6: 43-50.


