Aloe in bloom
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KODACHROME 64 Professional Film

Investigation of the Potential Antibacterial Properties of *Aloe vera* Gel

Senior Honors Thesis

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I. Introduction

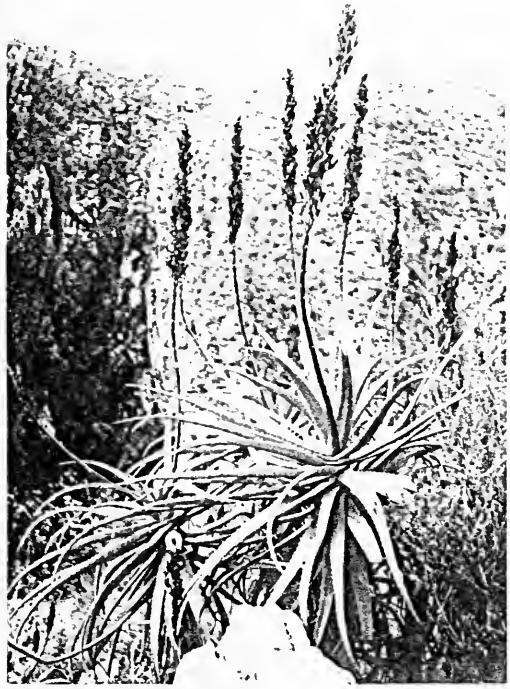
For literally thousands of years, people have taken special interest in the *Aloe vera* plant. It has gained a reputation the world over as the "medicine plant" with the ability to heal just about everything, from the common cold to peptic ulcers. The long-term folk use of *Aloe vera* in nearly every society of people on earth, including our own, has attracted the attention of the scientific community. As a result, an enormous body of data has been gathered concerning its burn and wound healing properties particularly. Much less research, however, has been conducted to investigate its potential antibacterial properties. The extensive commercialism surrounding *Aloe vera* gel has increased the need for research into this and other aspects of its potential medicinal character.

Classification and origin

Aloe vera (a k a *Aloe barbadensis*) is one of about two hundred species of *Aloe*, a perennial succulent (Graf, 1982). Although it resembles a cactus because of its lance-shaped, spiny leaves, it is classified instead along with tulips and onions in the Lily family (*Liliaceae*). *Aloe*, indigenous to southern Africa (Figure 1), spread quickly to other parts of the world once travelers learned of its therapeutic use in the treatment of wounds and various other ailments. Its employment as a drug dates as far back as the 4th century B.C. (Morton, 1961). British sailors brought home *Aloe* plants with tarred cloths tied around the cut stem as early as the 16th century (Lindley and Moore, 1896, as cited in Morton, 1961).

Two medicinal components: exudate and gel

Medicinal properties have been attributed to two distinct components of the *Aloe* leaf, the exudate and the gel. The yellow latex exudate taken from the outer rind of *Aloe vera* leaves has been widely accepted as a cathartic and a laxative for both humans and horses. It is the only part of the plant which is recognized in pharmacopoeias as a drug (Monmaney, 1990). The gel of the plant comes from parenchyma cells that fill the vast central portion of the leaf (Figure 2). Use of the parenchymous gel is far more extensive and controversial. One of the difficulties in evaluating experimental work done on *Aloe vera* is that several scientists fail to mention *which* extract, whether that of the rind, that of the central portion of the leaf, or both, was used in the testing procedure described (e.g. Davis, 1988). Several authors only refer to *Aloe* or *Aloe vera* when they probably mean to indicate use of only the gel.



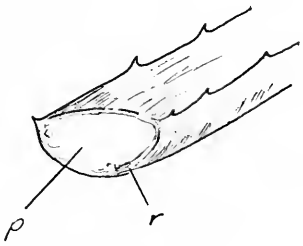


Figure 2: *Aloe vera* leaf cross-section.
p: parenchyma cell and gel;
r: rind and exudate.

Figure 1: This photograph of *Aloe vera* was taken in South Africa its estimated place of origin.

Controversy surrounding Aloe vera *gel*

Claims as to the clinical efficacy of the gel are vast and far-reaching. A plethora of bogus medical claims have been made for *Aloe vera* gel-based concoctions for treatment of everything from stomach cancer to blindness (Hecht, 1981; Grindlay, 1986; Morton, 1961). Exaggerated claims made in the past for amazing, but unsubstantiated cures in a wide variety of conditions have given the whole subject a folksy, less-than-respectable air. One physician this author interviewed dismissed the gel as an "unction" with very little true medicinal value as far as he knew (Glew, 1990).

In light of the commercialism that has made *Aloe* a household word, the FDA became concerned about the enormous number of claims being made about the healing power of *Aloe vera* gel. In 1981, the **FDA Consumer** featured an article titled "The Overselling of *Aloe vera*" which stated that two expert advisory panels had investigated aloe, aloin (a derivative), and *Aloe vera* gel as active ingredients in over-the-counter drugs and both concluded that not enough scientific evidence existed for the FDA to endorse or condemn *Aloe* as a safe and effective treatment for minor burns, cuts, and abrasions, or minor vaginal irritations. The panels recommended further tests be performed. Subsequently, much research *has* been conducted which provides more conclusive evidence. Since 1981, several researchers have shown that *Aloe vera* does possess antiinflammatory, burn, and wound healing properties. However, other claims regarding its medicinal potential are clearly lacking in scientific backing.

Folk use of Aloe vera *gel*

A summary of the use of *Aloe vera* gel would be incomplete if it did not include a section on folk use, especially considering its extraordinarily long, well-documented history as a home remedy. Alexander the Great was advised to seize territory abundant in *Aloe* plants for the sake of his wounded soldiers (Hecht, 1981) and Cleopatra was said to

have used *Aloe* as a cosmetic. Earliest references to medical use of the gel of *Aloe vera* were made in the 1st century A.D. by the Roman physician, Dioscorides. He reported using *Aloe* as a purge, for treating wounds and infections in the mouth, and for soothing itching and curing sores (Gunther, 1934, as cited in Grindlay, 1986). Liu Yu-hsi of China, in the 8th century A.D., healed dermatitis with whole fresh *Aloe* leaf pulp (Cole and Chen, 1943, as cited in Morton, 1961). .

Aloe continues to play an important role in the traditional medicine of many contemporary cultures including our own. In China, *A. vera* has been used for centuries, and is still an important home remedy (Cole and Chen, 1943, as cited in Grindlay, 1986). In Java, the macerated leaves are used on burns to prevent blistering. The crushed leaves are also made into a syrup with rose water to be taken internally to fight early stages of tuberculosis and gonorrhea (Heyne, 1950, as cited in Morton, 1961). Some native South Africans split *Aloe* leaves and place them on wounds (Watt, 1932, as cited in Morton, 1961). Filipinos use the leaves to cool edema and treat beriberi (Quisumbing, 1952 as cited in Morton, 1961). In Mexico, the leaves are used to treat burns, bruises, skin irritations, and even leprosy (Diez-Martinez, 1981 as cited in Grindlay, 1986). The most common ailments treated with *Aloe vera* in an immense variety of traditional medical communities around the world seem to be burns, wounds, and sores.

Currently in the United States, *Aloe vera* is used in herbal medicine and homeopathy. It is grown on kitchen windowsills for immediate treatment of domestic burns and cuts. In 1984, Madis Laboratories reported a list of over one hundred medical disorders that have been treated with *Aloe* including gout, acne, dermatitis, cuts, hair loss, peptic ulcers, and burns (Grindlay, 1986). Faith in the healing power of *Aloe vera* among the general public abounds in this country. As B. C. Anderson (1983) put it, "Judging from the purported skyrocketing sales of *Aloe vera* cosmetics, many people truly believe that they have found the fountain of youth in the aloe vera plant" (Grindlay, 1986).

Scientific investigation of Aloe vera *gel*

The 1930's marked the beginning of modern scientific investigation of *Aloe vera* gel, particularly regarding its burn healing potential. In 1935, Collins and Collins reported a case study in which they successfully used *Aloe* in salves for treating x-ray dermatitis (Collins, 1935 as cited in Grindlay, 1986). This provided the impetus for publication of a series of similar case studies by other physicians.

Burn wound treatment

Since then, burn wound treatment has received far more attention by the scientific community than any of the many other potential medicinal effects of *Aloe vera* gel. Most authors conclude that *Aloe vera* gel *is* valuable as a topical antiinflammatory agent and *is* useful for the treatment of burns. A few scientists, however, disagree.

In 1941, Rowe *et al.* tested different parts of the *A. vera* leaf in an experiment using a total of 44 rats. Each rat had two third-degree burns. Animals in the experimental group were treated with *Aloe vera* gel on one lesion and saline solution on the other. A control group was left untreated. They found that 64% of the rats treated with the gel showed increased healing, 9.5 times the number treated with saline. In an earlier experiment by the same team, 50% of the lesions treated with the gel showed an increased rate of healing as opposed to 25% of the lesions treated with saline solution. Rowe concluded that "The probability that there is benefit with the jell is 9/10. This is not considered certainty."

Rowe's group also found that fresh *rind* from one of their shipments of *Aloe vera* plants gave 100% complete healing in 8 rats within 6 days, but the rind from two other shipments gave negative results. These results are important for two reasons. First, since different shipments of *Aloe* gave completely opposite results, perhaps time of collection or the conditions under which the leaves grow plays a significant role in the composition or medicinal properties of the plant. Variability in leaf shipments has been reported in other

studies as well (Leung, 1977, as cited in Grindlay, 1986). Second, Rowe's findings indicate that the rind, as well as the gel, has burn healing properties.

Recent research has shown that *Aloe vera* gel is effective in treating frostbite and burn wounds by acting as an antiinflammatory agent and by inhibiting the production of thromboxanes, vasoconstrictors normally produced by damaged tissue. It has been postulated that by reducing heat and swelling and increasing the flow of blood and oxygen to the damaged tissues, the gel helps to minimize excessive damage to the burned or frostbitten area (Heggers and Robson, 1985).

In contrast, one researcher hypothesized that part of *Aloe vera* gel's effectiveness as a burn healing agent is due to the fact that it is about 96% water. Morton (1961) suggested that the gel simply provides a means of making water available to injured animal tissue without sealing it off from oxygen in the air. This is supported by information presented by Davis *et al.* (1988) in an article on *Aloe* gel wound treatment potential. They make the point that reepithelialization of wounds depends in part on the oxygen and moisture level at the surface of the wound, and that *Aloe* gel provides both these crucial elements while protecting the wound from bacteria in the air and the body's own necrotizing chemicals. However, there has been no research performed to differentiate between the moisturizing, protecting and thromboxane-inhibiting effects of the gel.

Antibacterial studies on Aloe vera *gel*

Evidence regarding use of *Aloe vera* as an antibacterial ointment is far more sparse and less conclusive than evidence for its burn and wound healing properties. In fact, there are only five scientific papers to date that expressly deal with the issue of whether or not *Aloe vera* has antibacterial properties, and results vary. Figure 3 below is a summary of this work including my own testing of Aloe vera gel for antibacterial activity.

RESULTS: ANTIBACTERIAL TESTS			
Authors	Extract Used	Extract Preparation	Bacteria Used / Results
Fly and Kiem	gel only	grinding, blending, sterilization via filters	*S. aureus* - *E. coli* -
Lorenzetti *et al* .	drained juice	80°C for 15 min	*S. aureus* + *E. coli* - *S. pyogenes* + *C. xerose* + *S. paradysenteriae* - *S. schotmuelleri* - *S. paratyphi* + *S.typhosa* -
Gottshall	unknown	unknown	*E. coli* - *S. aureus* - *M. tuberculosis* -
Robson & Heggers	Dermaide Aloe (commercial)	none	*E. coli* + *S. aureus* + *S. marcescens* + *E. cloacae* + *K. pneumoniae* + *P. aeruginosa* + *S. pyogenes* + *S. galactiae* + *C. albicans* + *S. faecalis* + *B. subtilis* + *E. aerogenes* + *Citrobacter sp.* +
Rodriguez-Bigas	Carrington Aloe (commercial)	none	unknown ++++
Fisher	gel	filter-sterilized, 80°C for 15 min or none	*E. aerogenes* - *S. marcescens* -

Figure 3: Summary of the results of antibacterial tests which have been performed to date on *Aloe vera* extracts.

In 1963, Fly and Kiem concluded that *Aloe vera* gel is ineffective as an antibacterial agent against *Staphylococcus aureus* and *Escherichia coli*, representative gram-positive and gram-negative bacteria, when tested using both agar diffusion and turbidimetric methods.

Earlier work with the same two bacteria as well as *Mycobacterium tuberculosis* using turbidimetric techniques supported these findings (Gottshall *et al.*, 1949).

Lorenzetti and her colleagues (1964) found that the growth of four types of bacteria, *Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium xerose,* and *Salmonella paratyphi* tested by agar diffusion *was* inhibited by a freeze-dried preparation of *Aloe* juice drained from cut leaves and previously heated for 15 minutes at 80°C. The growth of *Escherichia coli, Shigella paradysenteriae, Salmonella typhosa,* and *Salmonella schottmuelleri* was not inhibited when tested in the same way. Lorenzetti *et al.* observed that while heating appeared to stabilize the active factor, once the juice became dark during heating, usually after 20 minutes, the inhibitory property was lost. This evidence may explain the findings of Fly, Kiem, and Gottshall who neither freeze-dried their *Aloe* gel to concentrate it, nor heat-treated it for stabilization of the active factor.

Heggers and Robson (1982) added a "99.5% pure" commercial *Aloe vera* product to cultures of a dozen types of bacteria that are known to cause wound infection, including *Escherichia coli* and *Staphylococcus. aureus.* Growth of nine of the microorganisms: *S. aureus, Serratia marcescens, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus pyogenes, Streptococcus agalactiae,* and *Candida albicans,* was significantly inhibited at 60% *Aloe* concentrations. The remaining three bacteria, *E. coli, Streptococcus faecalis,* and *Bacillus subtilis,* were inhibited at higher concentrations (80 and 90%) of *Aloe* extract. Heggers and Robson concluded that *Aloe vera* has a broad-spectrum antimicrobial effect, especially against agents frequently responsible for burn wound sepsis. The *Aloe vera* extract used in this study was made from a commercial product (Dermaide Aloe) and antibacterial effects were evaluated by minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) techniques. The Nathan agar well diffusion technique (Heggers, 1987) was employed to further test the susceptibility of some of the bacteria to Dermaide Aloe cream. It is important to note that

the authors of this study did not report how the Dermaide Aloe (their "*Aloe* extract") was prepared, from what part of the plant the extract came, how sterilization of the extract was achieved, or what other ingredients were used to make the commercial product. This is important information when comparing Hegger's results with those of other scientists evaluating the potential antibacterial properties of *Aloe vera*, and in consideration of the value of the *Aloe vera* plant itself as a medicinal, antibacterial agent. Commercial *Aloe vera* products obviously differ in both their content and in the way they are prepared. Furthermore, unless more information is provided by the scientists who use them in their research, they should not be used as the basis for broad extrapolations or generalizations about the plant itself.

Although disagreement with respect to the antibacterial effects of *Aloe vera* gel is apparent, several general conclusions can be drawn from the few studies performed to date. Antibacterial properties have been reported only for heat-treated, freeze-dried gel or commercial extracts, not for fresh plant extracts (Gottshall, Fly and Kiem, Lorenzetti, Robson and Heggers). Increasingly in *Aloe* research, extracts used come in the form of commercial products like Dermaide Aloe (used by Robson *et al.*) and Carrington Dermal Wound Gel (used by Rodriguez-Bigas *et al.*). Results of these experiments led researchers to the broader conclusion that the *Aloe vera* plant itself has strong antibacterial qualities (e. g. Rodriguez-Bigas, 1988; Heggers *et al.*, 1979; Cera *et al.*, 1980, as cited in Grindlay, 1986). However, other factors must be considered, like how the commercial *Aloe* product was prepared, from what part of the plant it came, and what other ingredients were used to make it.

The idea for this project was conceived in light of my own interest in medicine and my advisor, Dr. Joanne Rosinski's expertise in botany. *Aloe vera* was an obvious topic for study because of its reputation as a medicinal plant. It turns out that the commercialism which has grown up around the plant has encouraged a flood of unsubstantiated claims

regarding medicinal use of the gel. Furthermore, I feel there is a very real need for more scientific evidence regarding its potential medicinal properties. Whether or not the plant has unusually strong antibacterial properties is especially interesting to me because information on this subject may result in a better understanding of how the gel hastens the healing of burns and wounds.

In the research presented here, liquid culture and agar diffusion methods were utilized to determine whether 80°C heat-treated and unheated fresh, filter-sterilized *Aloe vera* gel has antibacterial effects. Turbidimetric measurements of the growth of test bacteria with and without *Aloe vera* gel and unaided visual observations of agar diffusion tests were used to gather data. Two different bacterial microorganisms were tested. Results indicate that *Aloe vera* gel extract does not inhibit the growth of *Serratia marcescens* and *Enterobacter aerogenes* by any method used.

II. Materials and Methods

Microorganisms

The test bacteria used in the experiments were 24-48 hour cultures of *Serratia marcescens* and *Enterobacter aerogenes* in Luria broth (5 g/l tryptone, 2.5 g/l yeast extract, 5 g/l NaCl) . The original, lyophilized cultures were obtained from Carolina Biological Supply Company. Both types of bacteria are gram-negative, peritrichously flagellated, facultatively anaerobic, straight rods with simple nutritional requirements. They were chosen for inclusion in this study because previous experimentation by Robson *et al.* (1982) resulted in the conclusion that *Aloe vera* gel did exhibit antibacterial properties against one *Enterobacter* species and *S. marcescens*. These bacteria are frequently associated with burn wound sepsis (Robson *et al.*, 1982). *S. marcescens* normally occurs in soil, water, and on plant surfaces. *E. aerogenes* is found in human and animal feces. Both types of bacteria are opportunistic pathogens. (Krieg *et al.*, 1987)

Plants

Aloe vera plants obtained from Carolina Biological Supply Company were approximately three to six months old and leaves were an average of 18 to 25 cm long. Plants were maintained at room temperature with constant fluorescent light. They were watered every two to three days or whenever the potting material (a mixture of soil and perlite) looked dry.

Aloe vera gel extraction and preparation

The longest leaves on the plant (usually about 22 cm long) were cut at the base and peeled using a clean razor blade. Rinds of the leaves were removed and discarded. The leaf interior (comprised of parenchyma cells) was pulverized with a Pyrex Porter-Elvejhem

homogenizer. The gel extract was filter-sterilized using 22 mm, 0.2 μm (pore size) Corning cellulose acetate sterile, membrane filters. In some cases, *Aloe* extract was heat-treated in an 80°C hot water bath for 10-15 minutes. All procedures subsequent to the filtering of the gel were carried out using sterile technique.

Liquid Culture tests

Bacterial growth in liquid cultures with and without gel extract was measured using a Spectronic 20 (Bausch & Lomb) set at a wavelength of 580 nm. Sterilized Luria broth was used as a nutrient medium. One tenth milliliter of a 24-hour L. broth culture of bacteria was used to inoculate control and experimental flasks containing 50 ml of culture media. Two milliliters of *Aloe vera* gel extract were added to experimental flasks. Cultures were swirled at 1500 rpm between readings throughout the experiment using a Lab-Line Orbit Environ-Shaker. The swirling chamber was kept at a constant temperature of 25°C. Aliquots were removed at half hour intervals, assayed and returned to the flask over a total test period of approximately eight hours. Clean, sterilized pipettes were used to remove aliquots from their respective flasks. These samples were immediately reintroduced to their flasks after each measurement in order to maintain the level of nutrient medium, bacteria, and *Aloe* extract in each flask throughout the testing period. Growth curves displayed and discussed in the Results section were plotted using Cricket Graph on an Apple Macintosh SE computer.

Agar diffusion tests

These tests involved use of agar inoculated with bacteria before solidification. In all three types of agar diffusion tests to be described below, Luria broth with 15 g/l Bacto-agar was autoclaved and maintained at 45°C in a water bath for one hour before addition of 2 mL bacteria for every 500 ml medium. 100 x 15 mm Falcon Petri dishes were filled with

approximately 20 ml of bacteria-inoculated agar medium. Control dishes containing bacteria but no test material (*Aloe*) were prepared during each of the following experiments in order to observe normal bacterial growth. Experimental dishes were exposed to *Aloe vera* gel extract in various ways. These will be described in detail below.

A. Trench Technique

After solidification of the agar, 5 mm diameter wells were cut in the agar in approximately 10 experimental plates using the wide ends of sterilized Pasteur pipets. The 4 mm deep plugs of agar created in this manner were aspirated with sterile Pasteur pipets such that only a thin layer of agar (1 mm) remained at the bottom of each well. Control wells on each plate were filled with water or left empty (Figure 4). Filter-sterilized *Aloe* gel extracts, heat-treated and unheated, were introduced to experimental trenches using a syringe with a 20 gauge needle attached. Two to three drops (approximately 0.05 g) of the *Aloe* gel extract filled experimental wells. Plates were stored upright at room temperature and observations were recorded after 10, 24, and 48 hours of incubation.

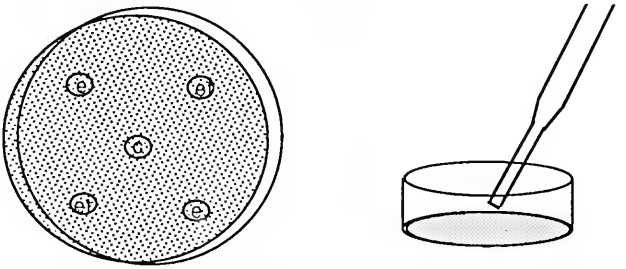


Figure 4: (Left) Drawing of Petri dish with agar containing wells filled with heat-treated (e_t) *Aloe vera* gel, unheated (e) *Aloe vera* gel extract, and water (c). (Right) Close-up side view of a well being evacuated by a Pasteur pipet.

B. Nathan Agar Well Technique

This technique involved preparing agar plates exactly as in the trench assay specified above. After the test material was added to the well, however, another layer of

uninoculated agar (just enough to cover the first layer completely) was poured over the top of the trenches and allowed to solidify. This top layer flowed into, around, and on top of the wells before solidifying.

C. Disk Preparations

After solidification of the agar, 5 mm diameter autoclaved paper disks (punched from E D Qualitative laboratory filter papers (grade 636) were saturated with test materials: heat-treated *Aloe* gel extract, unheated gel extract, and distilled water. These saturated disks and dry commercially-prepared antibiotic control disks were placed on the surface of the agar using sterilized forceps (Figure 5). Five evenly-spaced disks were placed on each plate. Plates were incubated at room temperature and observations were recorded after 10, 24, and 48 hours of incubation.

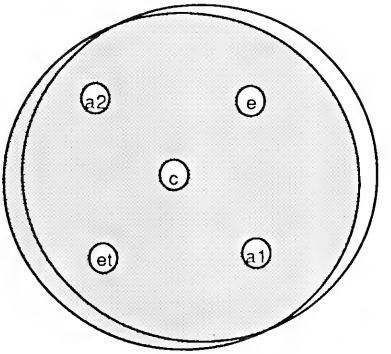


Figure 5: drawing showing Petri dish with disks saturated with heat-treated *Aloe vera* gel (et), unheated *Aloe vera* gel (e), water (c), and antibiotics (a1 and a2) on agar inoculated with bacteria.

III. Results

Liquid Culture Experiments

The rates of growth of *Serratia marcescens* and *Enterobacter aerogenes* in liquid culture were not affected by the presence of either unheated or heat-treated *Aloe vera* gel extract. Liquid culture experiments were performed nine times with basically the same results. In all cases, growth curves exhibited the typical lag and log phases of bacterial growth. Growth curves from one experiment show that the growth of the bacteria in the presence of heat-treated and unheated Aloe *vera* gel extract is nearly identical to growth in its absence (Figure 6, 7, 8, and 9).

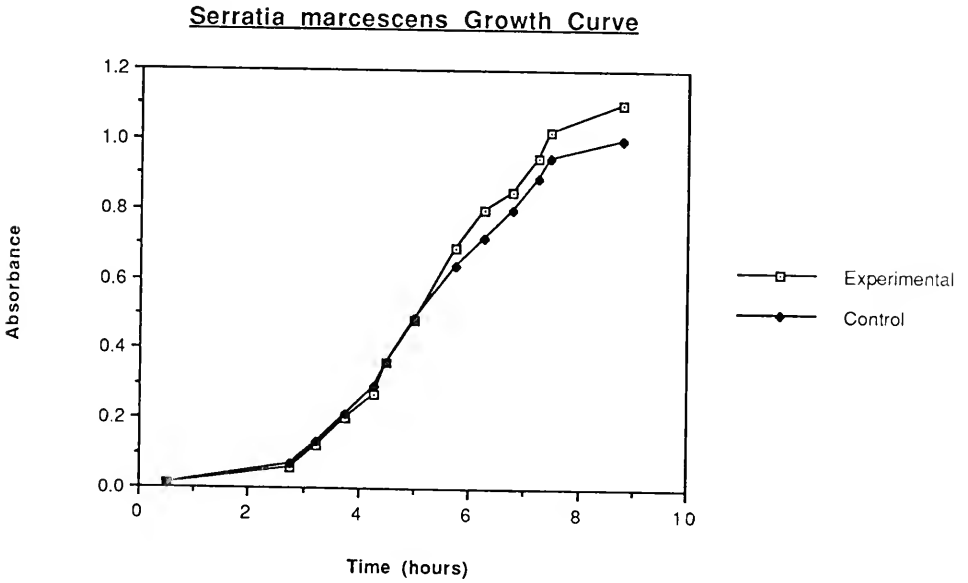


Figure 6: Effect of heat-treated *Aloe vera* gel on the growth of *S. marcescens* in liquid culture.

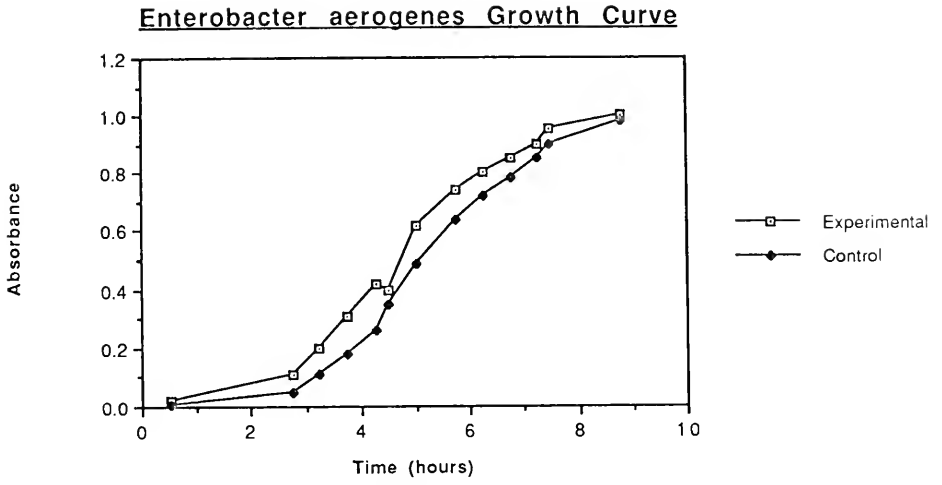


Figure 7: Effect of heat-treated *Aloe vera* gel on the growth of *E. aerogenes* in liquid culture.

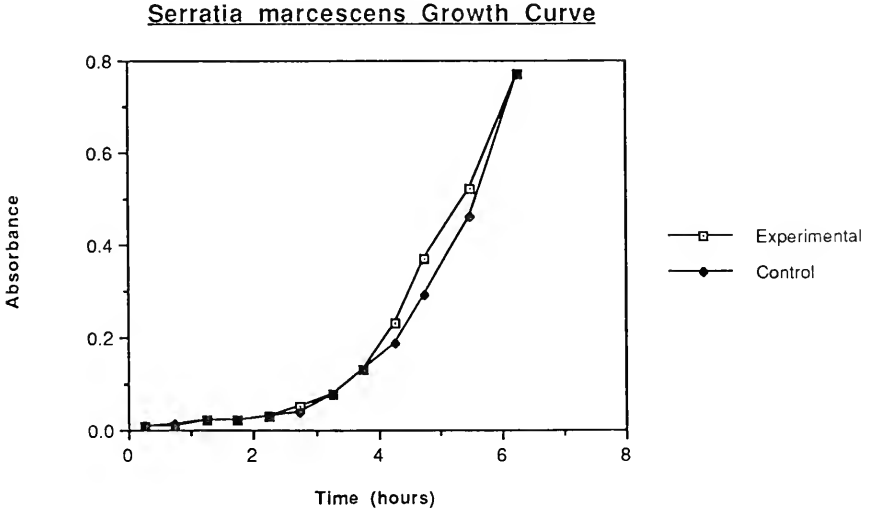


Figure 8: Effect of unheated *Aloe vera* gel on the growth of *S. marcescens* in liquid culture.

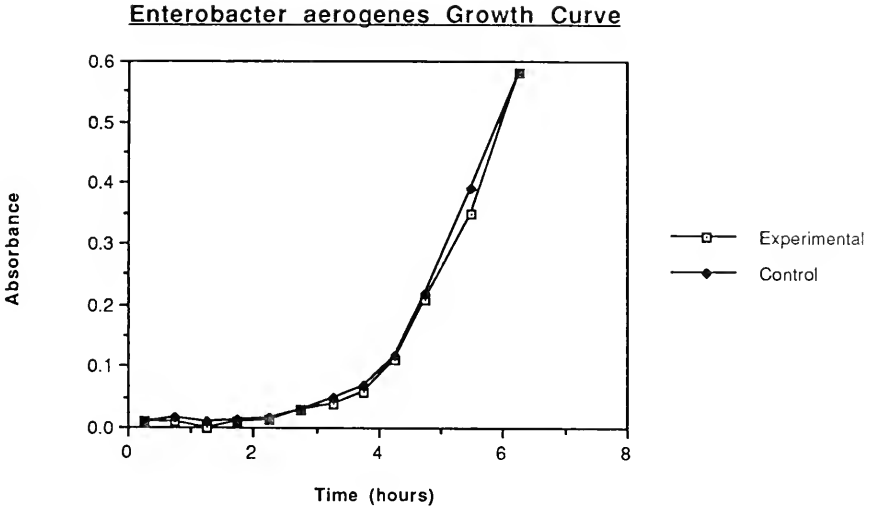


Figure 9: Effect of unheated *Aloe vera* gel on the growth of *E. aerogenes* in liquid culture.

Agar Diffusion Tests

Visual observation of the results of three types of agar diffusion tests revealed that heat-treated and unheated *Aloe vera* gel extracts did not inhibit the growth of bacteria tested. Growth of bacteria on control plates lacking test material (e.g. *A. vera* extract) was identical to growth on experimental plates.

A. Trench Assays

The results of repeated trench assays showed that heat-treated and unheated *Aloe vera* gel extracts did not inhibit significantly the growth of bacteria in agar. The growth of bacteria in each plate was as thick immediately around the trenches filled with *Aloe vera* gel extract as it was in control plates lacking the trenches filled with *Aloe*. After 10 hours, a very sparse lawn of bacteria had grown on the surface of both experimental and control

plates of both species of bacteria. No zones of inhibition were visible at this point. Individual colonies of bacteria could be identified. Growth within the agar was also apparent.

After 24 hours, a smooth, relatively thin lawn covered the surfaces of plates in every case. No distinct zones of inhibition surrounded either the control trenches (filled with water or left empty) or the experimental ones (filled with *Aloe* extracts). By 48 hours, the entire uncut surface of the agar on experimental plates was completely covered with bacteria. Growth on experimental plates matched growth on control plates in color, texture, and degree at every stage of testing (Figure 10).

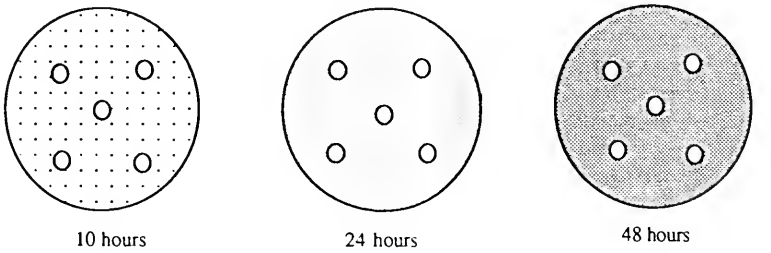


Figure 10: drawing of the results after 10, 24 and 48 hours of incubation.

B. Nathan Agar Well Experiments

The results of two experiments using the Nathan Agar Well Technique with heat-treated and unheated *Aloe vera* gel extract were identical. The growth around wells filled with the gel extract in six experimental plates was identical to growth around control trenches filled with water. Growth on control and experimental plates was invisible or slight after 10-hours of incubation, and occurred in a randomly swirled pattern after 24 and 48 hours of incubation. By 48 hours, growth had progressed such that the plates were quite heavily imbued with bacteria (Figure 11).

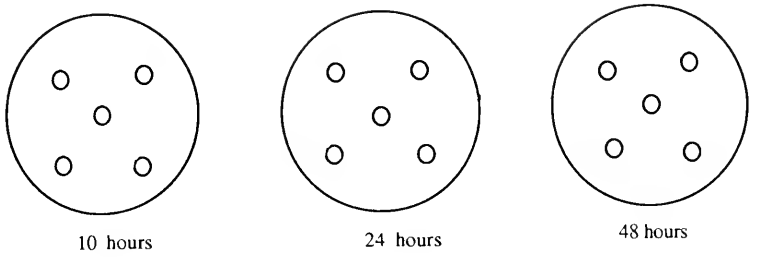


Figure 11: drawing of the results at 10, 24 and 48-hour incubation periods.

C. Saturated Disk Assays

The inhibition of bacteria by novobiocin, erythromycin, penicillin, and gentomycin antibiotic control disks was clearly visible on twelve experimental plates. Zones extended approximately 3.5 mm from each antibiotic disk. No zones of inhibition could be seen around disks saturated with heat-treated or unheated *Aloe vera* gel extract, or around disks saturated with distilled water. Growth around experimental disks and outside the zones of antibiotic disks was smooth and consistent with control plate growth.

IV. Discussion

The results of this experiment lead to the conclusion that freshly extracted *Aloe vera* gel extract, heat-treated and unheated, did not affect the growth of *Serratia marcescens* or *Enterobacter aerogenes* in liquid culture or on agar. The following is a discussion of this experiment including the methods used to extract, filter, and test the gel, the results, and sources of discrepancy between this work and that of other scientists.

Extraction of the gel

Perhaps one of the most difficult and crucial aspects of studying the properties of *Aloe vera* gel is obtaining the extract itself. Few teams of researchers who have investigated the gel have reported performing this task the same way. One method involved cutting the leaves at the base, and standing them upright so that the juice could drain into receptacles (Lorenzetti *et al.*, 1964). I tried duplicating this method, but the cut end dried before any gel could be captured in the container placed beneath. Fly and Kiem (1963) prepared homogenates of the green, vascular portion of the leaf, the gelatinous material, and the entire leaf by grinding or blending. They did not report antibacterial properties with their extract. Gottshall *et al.* (1949) used cold water, boiling water, and ethanol for extractions from a wide variety of plants including *Aloe vera*. Their extract did not inhibit the growth of *Mycobacterium tuberculosis*, the only microorganism tested. Others, particularly clinicians (e.g. Collins and Collins, 1935 as cited in Grindlay and Reynolds, 1986), split the leaf to remove the rind from one side, and macerated the gel, before binding it in place on people with waxed paper and a bandage. Collins and Collins were the first to report to the scientific community the successful treatment of wounds with any preparation of the gel. More recently, with the widespread commercialization of the *Aloe vera* plant, commercial preparations have been used for testing, and the whole issue of

how the gel was extracted to make the preparation is conveniently avoided in the scientific literature (e.g. Robson *et al.*, 1982). In my study, the method of extraction chosen was meant to specifically examine the *gel* of the plant. I first peeled the leaves and then pulverized the gel. In the reports in the literature, many different extraction methods were used, and, furthermore, the extracts being tested may have been different in every case. In future studies it would be wise to consider how extraction methods affect the activity of the material tested.

Another subject of consideration lies with the ultimate source of the extract. The age and subsequent physiological condition of the plants from which the gel is extracted may play a major role in the the outcome of experimentation. In other words, the young plants used in my experiment may be drastically different chemically compared to plants used to make the commercial cream-based extracts used by Rodriguez-Bigas (1988), Robson *et al.* (1982), and others. This could understandably account for discrepancies between the results of our experimentation. Furthermore, it is interesting to note that none of the scientists whose work I examined specified the age of the plants from which they obtained their extract.

Sterilization of the gel extract

Historically, in order to ensure the sterility of the gel in laboratory settings, several methods have been used. Fly and Kiem (1963) autoclaved and filter-sterilized their extracts but they were unable to detect antibacterial effects. Gottshall *et al.* (1949) found that the gel extracts of two species of *Aloe: chinesis* and *littoralis* were not affected by autoclaving. Although they did not mention whether they autoclaved the *Aloe vera* gel extract, they did not find that it had antibacterial activity. The results of these two experiments might suggest that autoclaving could destroy an active principle.

In my experience, heat-treating the gel brought the cellular material to the surface of the vessel so that it could be removed with a sterile pipet. The rest of the *Aloe* material was able to be easily sterilized with disposable screw-on 0.2µm pore size filters. Admittedly, another variable is introduced by removal of the parenchymous tissue from the gel. That is, it may be that the active ingredient is locked in the cell walls or is somehow closely associated with them. Hence, removal of these parenchymous cells in the treatment and the filtration stages incurs removal of a portion of what is considered "the gel". A component of fresh *Aloe vera* leaf gel was removed in this filtration process.

Another danger involved with filter-sterilizing the gel is that certain materials tend to adhere to cellulose filters. Exclusion of the potential active ingredient from the extract collected may be due to its adhesion to the filters. Since Lorenzetti did not filter-sterilize her extract, the discrepancy between my results and Lorenzetti's could conceivably be due to this difference in our techniques.

Heat-treatment of the gel

Treatment of the gel is an equally important aspect of its study. This may explain why Fly and Kiem (1963), Gottshall *et al.* (1949), and others, did not achieve positive results, while Lorenzetti *et al.* (1964) did. The Lorenzetti team claimed that "the principle responsible for the inhibitory activity was found to be unstable." They observed that the active principle could be preserved temporarily by refrigeration and for an even longer period by heating the juice for 15 minutes at 80°C. When the gel was heated, it eventually turned dark and concomitantly lost its antibacterial properties. Hence, heat-treatment of the gel appears to be crucial in preserving antibacterial activity.

In my own experiment, perhaps a different sort of treatment (e.g. heating for a shorter period of time) would have maintained the proposed "active factor" in this substance. I tried to reproduce the stabilizing method used by *Lorenzetti et al.*, but was

unsuccessful. It may be that the active factor *was* stabilized, but was present in too small amounts. Perhaps the fact that the Lorenzetti team lyophilized (i.e. concentrated) their extract accounts for the difference in results.

Methods

The advantage of the turbidimetric method used in the majority of my experiments is that the material to be tested is brought in direct contact with the test organisms in the broth. The diffusion method, on the other hand, is effective only in evaluating the activity of a substance which is diffusible through the medium used (in this case, agar). Since the active factor of *Aloe vera* gel is not as yet unanimously determined to exist, let alone characterized in terms of its diffusibility, it is uncertain that this test method is the most effective. In this experiment, agar diffusion tests were performed in light of the fact that they are standard methods of testing for antibacterial properties and several other researchers have used it specifically to test *Aloe vera* extract. Lorenzetti *et al.* (1964), for instance, used this test method and found that their extract did inhibit the growth of bacteria. Fly and Kiem (1963) also used this method of testing but their results disagreed.

The Nathan Agar Well Diffusion test was used successfully by several researchers. In fact, Heggers and Robson (1987) even performed a study which resulted in the conclusion that, for burns treated with topical antimicrobials, the Nathan Agar Well Diffusion test is an extremely reliable method of testing antimicrobial agents. Perhaps the description of how they performed this test was unclear or incomplete, or I misinterpreted it. It may also be that this particular extract is unsuited to testing by this method because of its very liquid consistency. The mechanics of this method lend themselves to testing more viscous, cream-based, commercial *Aloe vera* products, or chunks of *Aloe vera* gel.

Conclusion

According to the results of my testing, *Aloe vera* gel does not possess antibacterial properties against *Serratia marcescens* or *Enterobacter aerogenes*. In contrast, the work of Lorenzetti, Heggers and Robson, Rodriguez-Bigas, and others, have resulted in the conclusion that the plant does have antibacterial activity.

As this discussion acknowledges, there are a great number of sources for discrepancy between my results and those of other scientists. Testing procedure, age and condition of plants, gel extraction, sterilization, filtration, and treatment techniques all may play a significant role in what conclusions about this substance are made. The use of commercial products as opposed to the use of fresh *Aloe vera* gel extracts probably affects the outcome of tests for antibacterial activity. Furthermore, attention should be paid to the ingredients added to these commercial products since they may be attributable to the inhibition detected. Regarding discrepancies between conclusions of scientists who used fresh *Aloe vera* gel extracts rather than commercially prepared extracts, it appears that lyophilizing the substance may concentrate the active factor to the extent that antibacterial activity can be detected upon testing.

Clinically speaking, solid evidence suggests that *Aloe vera* gel actually does have medicinal value, particularly regarding its ability to accelerate wound healing and reduce inflammation. Whether or not the *Aloe*-enhanced treatment of these wounds is in part due to its antibacterial properties remains to be seen, although this author is justifiably skeptical. Based on my own experimentation, contrary to the findings of Rodriguez-Bigas (1988) and others, antibacterial activity of *Aloe vera* gel is nonexistent and it would appear that the gel's other properties are more appropriately credited with its burn healing success.

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