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Comparative analysis of *Aloe vera* gel, epidermis, and whole leaf extracts for antimicrobial activity

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Abstract
The study examines the antimicrobial potential of ethanolic extracts from Aloe vera gel, epidermis, and whole leaf, using Thin Layer Chromatography. Aloe vera, a plant with medicinal and cosmetic properties, is gaining popularity due to its anti-inflammatory, antimicrobial, and wound healing properties. Its natural health benefits are particularly important in the context of antibiotic infections. The study extracted Aloe vera gel, epidermis, and leaf from 90% ethanol and air-dried for analysis. The extracts were screened for alkaloids, flavonoids, saponins, and terpenoids, and tested for antimicrobial activity. The gel extract showed no significant inhibition zones against all organisms, while the epidermis extract was most effective against Escherichia coli. This supports the therapeutic potential of Aloe vera epidermis as a natural antimicrobial agent. Further research is needed to determine Aloe vera's antimicrobial properties as a single active component, isolate and purify it, evaluate its activity in live creatures, and compare dried extracts.

Keywords: *Aloe vera*, antimicrobial, extract, *Escherichia coli*, *Staphylococcus aureus*, Thin Layer Chromatography

1. Introduction
Antibiotic resistance is a worldwide problem with new varieties of antibiotic resistance spreading rapidly across continent. The leaders of World Health have described antibiotic-resistance microorganisms as "Nightmare bacteria" that pose a disastrous threat to people in every part of the world [1]. The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. For several decades, antibiotics have played a key role in the fight against infectious disease caused by bacteria and other microbes. Antimicrobial chemotherapy has been a top cause for the dramatic rise of average life expectancy in the twenty first century [2]. However, disease causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Antimicrobial resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. The main cause of antibiotic resistance is gene mutation [3]. In 2013, CDC published their first antimicrobial resistant threat report, which stated that each year in the United States at least two (2) million people got an antimicrobial infection, and at least 23,000 people died as a result to this antimicrobial infection. In 2019, CDC went further and reported that there were more than 2.6 million antibiotic-resistance infections and nearly 44,000 deaths occurred each year after the 2013 report was published. WHO also reported that Common bacterial infections are becoming increasingly resistance to treatments. Ciprofloxacin which is one of the most used oral antibacterial is becoming inactive to *Neisseria gonorrhoea*. Over 60% of *Neisseria gonorrhoea* isolates, a common sexually transmitted disease, have shown resistance to Ciprofloxacin. Over 20% of *E. coli* isolates, a common pathogen in urinary tract infections, were resistant to ampicillin, co-trimoxazole and fluoroquinolones. The World Health Organization in 2021 [4], stated that antibiotics are becoming increasingly ineffective, as drug-resistance spreads globally leading to more difficult-to-treat infections and death. Antimicrobial Resistance is a serious threat globally that date years back of medical progress by rendering antibiotics in effective against bacterial infection. In 2023, [5] reported 10 million deaths annually by 2050, while [6] estimated a potential global cost up to $100 trillion. The consequences are severe: routine medical procedures like surgery could become life-threatening, and common infections might become untreatable [7]. Experts warn we may be entering a "post-antibiotic era" without coordinated global intervention to reduce antimicrobial usage and develop alternative treatments. New antibacterials are urgently needed, to treat current and emerging infections.

Medicinal plants are now being considered as alternatives for the treatment of diverse infections [8]. In recent years, multiple-drug resistance in human pathogenic organisms is on the increase, and there is a need for use of effective and more economical new drugs from traditional plants like *Aloe vera* plant. The preliminary review showed that there is little in terms of published literature on the use of *Aloe vera* in Sierra Leone to fight microbes.

Traditional medicine and global ethnomedicine have traditionally used medicinal plants [9]. Historically, medicinal plants have been essential as sources of pharmacological lead compounds. Because early people used plants to heal their ailments as a result of instinct, taste, and experience, the history of medicinal plants predates that of humans. The categorization of medicinal plants is one difficulty in their evolution [10]. The use of therapeutic herbs for healing is as old as humanity itself. There is substantial proof that man and his hunt for remedies in nature have a long history together, including written records, surviving monuments, and even the first plant medicines. The knowledge of using medicinal plants came about as a result of man's long-standing battles with disease, which taught him to look for pharmaceuticals in the barks, seeds, fruit bodies, and other parts of plants [11]. Modern pharmacology today includes a variety of plant-based medications that have been used for millennia and were known to ancient cultures. Modern science has recognized their active effect. The capacity of pharmacists and doctors to respond to difficulties that have occurred with the proliferation of professional services in the facilitation of man's life has improved as a result of their knowledge of the development of concepts linked to the use of medicinal plants as well as the evolution of consciousness [12]. *Aloe vera* gel is recognized for its capacity to soothe burns, wounds, and other skin irritations, while chamomile can aid with skin inflammation and promote healing [13]. Blueberries, green tea, and grapes are a few examples of plants with high antioxidant content. Antioxidants are substances found in plants that help protect the body from the damaging effects of free radicals and oxidative stress [14].

Aloe vera is a succulent plant that belongs to the Aloe genus. It typically has, thick, green, fleshy leaves that store water, allowing the plant to survive in arid conditions. It has been widely used for centuries for its medicinal and cosmetic properties. It is sometime referred to as “miraculous” plant, and has been used by mankind during centuries, for the treatment of mainly skin conditions but also for different disorders, constipation, stomach disease, kidney disease, hair loss and many more. The plant has been traditionally used to treat skin injuries (burns, cuts, insect bites, and eczemas) and digestive problems because of its anti-inflammatory, antimicrobial, and wound healing properties [15]. It contains a gel-like substance that is rich in bioactive compounds, including vitamins, minerals, enzymes, antioxidants, and amino acids. This gel is used typically to treat various skin conditions such as sunburns, rashes, wounds, and inflammation [16]. It is known for its soothing and healing properties. *Aloe vera* gel is a popular ingredient in skincare products such as lotions, creams, and gels. Its moisturizing and nourishing properties make it beneficial for hydrating the skin, reducing redness and irritation, and promoting healing of minor cuts and burns. The plant is believed to help support the immune system due to its high content on antioxidants [17]. These compounds are known to neutralize harmful free radicals in the body, reducing oxidative stress and promoting overall health. It is low in calories but high in nutrients. It contains vitamins A, C, and E, along with minerals like calcium, magnesium, and potassium [16]. *Aloe vera* also contains various beneficial compounds, including polysaccharides, which are thought to contribute to its health benefits. Apart from its medicinal and cosmetic applications, it is sometimes consumed as a dietary supplement [18]. Over time, *Aloe vera* has gained a lot of popularity as a houseplant, as well as with reference to its medicinal uses. *Aloe vera* was formerly a wild plant years back, but it is now extensively cultivated. Overall, *Aloe vera* is a versatile plant that offers a range of potential health benefits when used appropriately. With the above mentioned crises of antibiotic infection which is calling for alternative antibiotics, *Aloe vera* is one of the best options to help contribute to this crises because of its high medicinal value. For thousands of years, the plant has been used as source of health and wellness. Whether it was used as a tropical gel or being ingested for bodily benefits, *Aloe vera* has been highly regarded as a powerful plants to naturally help the human body [19].

Thin Layer Chromatography (TLC) test was done to identify the medically active and some bioactive substances in the plant parts that potentially had effects on the selected microbes. Microbe based infections are common all over the world, and chemical antibiotics can be used to treat them. However according to World Health Organization [20], overuse of antibiotics without a doctor’s prescription has resulted in widespread antibiotic resistance. Multiple drug resistance in human pathogenic microbes has developed in recent years, necessitating the use of new, more affordable, and effective medications from traditional plants like *Aloe vera* as well as the validation of these plants’ antimicrobial activity. This study did not only focuses on confirming *Aloe vera*’s antimicrobial capability, it also explores in finding out which phytochemicals that were responsible for this capability and comparison was also done to highlight among the three plants parts, which had the best potential and why.

This study aims to evaluate the antimicrobial potential of ethanolic extracts from *Aloe vera* gel, epidermis, and whole leaf. Using Thin Layer Chromatography (TLC), we identified bioactive compounds potentially responsible for antimicrobial activity. The study also compares the efficacy of each plant part to determine which extract offers the highest therapeutic potential against selected microbes.

2. Materials and Methods

This research was conducted at Fourah Bay College, the Biological Sciences Laboratory, and the Sierra Leone Pharmacy Board Laboratory.

2.1 Materials

Aloe vera plant extracts, conical flask, electronics scale, measuring cylinder, aluminum foil, beaker, distil water, linen cloth, funnel, knife, spatula, electric blender, stirring rod, 90% ethanol, petri dish, cotton wool, gloves, methanol, vortex machine, silica gel plate, glacial acetic acid, benzene, chloroform, spotter, ammonium solution, filter paper, petroleum ether, ethyl acetate, iodine, ruler, distilled water, Nutrient agar, Muller Hinton agar (OXOID Ltd, England).

2.2 Plant collection and identification

In the morning hours, *Aloe vera* plants were purchased from a domestic garden. At Fourah Bay College’s Biological Science laboratory, the leaves were identified. The plants were pest and disease free and in good health. The leaves were washed under a clean running tap, spread out in the laboratory for 14

days to air dry at 27°C, and then ground into a powder using an electric blender. The powder was then stored in an air-dried container in a cool, dry space.

2.3 Preparation of extracts

2.3.1 Preparation of whole leaf extract

Aloe vera leaves were weighed and air dried in the laboratory. The dried leaves were weighed, crushed using an electronics blender and stored in a sterilized container. 41g of crushed *Aloe vera* leaves was poured into a 2L conical flask washed with distil water and ethanol. 500mL of 90% ethanol was measured using a measuring cylinder and was poured into the conical flask which contain the crushed leaves. The conical flask was sealed with an aluminum foil and the mixture was shaken thoroughly. After every two days the mixture was shaken vigorously, until day 7. Two 500mL of beakers, a filter funnel, and a linen cloth, was washed with distil water and rinsed with ethanol. The linen cloth was placed in the filter funnel, and the filter funnel was then placed on top the beaker, the mixture of the *Aloe vera* leaves and the ethanol which was left for 7 days was then poured into the filter funnel. After filtration, the residue was reserved and the filtrate in the beaker was sealed with an aluminum foil. After a day, the filtrate was allowed to sediment and put into a desiccator until the extract was dried. The ethanolic supernatant and dried extracts were stored into separate sterilized containers [21].

2.3.2 Preparation of epidermis extract

After the gel been removed from the epidermal layers of the *Aloe vera* leaves, the epidermis was weighed and air dried. The dried epidermis was weighed, crushed using an electronics blender, and stored in a sterilized container. 40g of crushed *Aloe vera* epidermis was poured into a 2L conical flask washed with distil water and ethanol. 500mL of 90% ethanol was measured using a measuring cylinder and was poured into the conical flask which contain the crushed epidermis. The conical flask was sealed with an aluminum foil and the mixture was shaken thoroughly. After every two day the mixture is shaken vigorously, until day 7. Two 500mL of beakers, a filter funnel, and a linen cloth, was washed with distil water and rinsed with ethanol. The linen cloth was placed in the filter funnel, and the filter funnel was then placed on top the beaker, the mixture of the *Aloe vera* epidermis and the ethanol which was left for 7 days was then poured into the filter funnel. After filtration, the residue was reserved and the filtrate in the beaker was sealed with an aluminum foil. After a day, the filtrate was allowed to sediment and put into a desiccator until the extract was dried. The ethanolic supernatant and dried extracts were stored into separate sterilized containers.

2.3.3 Preparation of Gel extracts

The gel was removed from the leaves of the *Aloe vera* by scraping in between of the epidermal layers of the leaf with a sterilize spatula and knife. The gel was then weighed stored in a sterilized container. 643g of *Aloe vera* gel was poured into a 2L conical flask washed with distil water and ethanol. 500mL of 90% ethanol was measured using a measuring cylinder and was poured into the conical flask which contain the gel. The conical flask was sealed with an aluminum foil and the mixture was shaken thoroughly. After every two day the mixture is shaken vigorously, until day 7. Two 500mL of beakers, a filter funnel, and a linen cloth, was washed with distil water and rinsed with ethanol. The linen cloth was placed in the filter funnel, and the filter funnel was then placed on top the beaker, the mixture of the *Aloe vera* gel and the ethanol which was left for 7 days was then poured into the filter funnel. After filtration, the residue was reserved and the filtrate in the beaker was sealed with an aluminum foil. After a day, the filtrate was allowed to sediment and put into a desiccator until the extract was dried. The ethanolic supernatant and dried extracts were stored into separate sterilized containers. The ethanolic supernatant was used directly for antimicrobial and phytochemical assays, following similar protocols reported in recent studies [22].

2.4 Qualitative phytochemical analysis of plant extracts using TLC

The primary focus of the qualitative chemotaxonomic analyses of the different *Aloe vera* extracts were alkaloids, flavonoids, saponins, and terpenoids.

2.4.1 Preparation of Silica gel plates

Two lines were drawn at opposite ends of the silica gel plates with a sharp pencil and ruler. At one end was a straight line, above it where initials were written and the other end was 5 broken lines with 4 spaces in between. The silica gel plates were then demarcated, the different ethanolic supernatant extracts were spotted on different plates using a spotter of 4ml and were left to air dry.

2.4.2 Preparation of solvent Solutions

Various solvent solutions with various polarities were created to choose a solvent system that can produce a superior resolution. These solvents were poured into different sterilized bottles for 15 minutes, so that it is saturated. The spotted silica gel plates were placed in their respective solvent solutions. The plates absorb the solvent solution until it reaches the straight demarcated line. The plates were then removed and left to dry.

2.4.2.1 TLC Alkaloid

A solvent phase of 85 ml of Methanol and 15ml of Ammonium hydroxide (17:3) was used [23].

2.4.2.2 TLC Flavonoid

A solvent phase of 90ml of chloroform and 10ml of ethanol (18:2) was used [23].

2.4.2.3 TLC Saponins

A solvent phase consisting of 60ml of chloroform, 20ml of glacial acetic acid, 10 ml of methanol, and 10ml of water (6:2:1:1) was used [24].

2.4.2.4 TLC Terpenoids

A solvent phase of 50ml of Petroleum ether and 50 ml of Ethyl acetate (1: 1) was used [23].

2.4.3 Preparation of Iodine staining

Iodine was heated in an ovum at 70°C for 10 minutes, the sample plates were expose to the iodine vapor to clearly show the components available. After staining the sample plates were traced for spot, and circle the spot to calculate the retention factor (Rf), by measuring the distance of the solute (spot) upon the distance by solvent. The Rf were then compared to literature-reported standards for compound identification and interpretation. For alkaloids, flavonoids, and terpenoids, reference Rf values were drawn from [23], and for saponins reference Rf values were drawn from [24].

Identification was based on literature Rf ranges alone even solvent solutions were the same.

$$Rf = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent}$$

2.5 Preparation of Nutrient Agar
Nutrient agar was made according to the instructions provided by the manufacturer. In a conical flask, 7g of agar powder was dissolved in 25 ml of distil water. The flask was covered with aluminum foil and autoclave tape. After thoroughly mixing, the mixture was sterilized in an autoclave for 15 minutes at 121°C. The mixture was then allowed to cool in a 45°C water bath. Each sterilized petri dish plate received 20ml of Nutrient agar and was allowed to solidify.

2.6 Collection, growth, and maintenance of test organism
The test organisms used in this study were obtained from the Pharmacy Board of Sierra Leone's Microbiology laboratory. Bacterial cultures of two distinct strains, such as *Staphylococcus aureus* and *Escherichia coli*, were included. Solidified nutrient agar was used to subculture the bacteria. The bacteria were suspended when the bacterial culture tubes were retrieved from the refrigerator and stirred. To avoid contamination, the loop was then torched to a red-hot flame while being conducted within a biosafety cabinet. Once the bacteria have been removed via the loop, they are streaked on the nutrient agar plate slightly using a quadrant streak that moves 90° while the disc remains closed, and then placed in an incubator for 24 hours at 37°C to allow bacteria to grow.

2.7 Preparation of Muller Hilton Agar
9.5g of Muller Hilton agar powder was suspended in 25ml of distil water. The mixture was sterilized in an autoclave at 121°C for 15 minutes. A 45°C water bath was used to cool it. The cooled Muller Hilton agar was then placed on other petri plates to solidify.

2.8 Antibacterial susceptibility testing of plant extract
The organisms were collected using a sterile loop from the nutrient agar plates and suspended in 4ml of normal saline. The mixes were then vortexed. Using cotton wool, test organisms were evenly seeded onto Mueller-Hinton Agar surface plates. Both test organisms were replicated twice form the same cultures. Each well's position was noted on the Mueller Hilton agar plates. A cock borer with a diameter of 5mm was used to create a well in the Mueller Hilton agar plates. Using 1ml pipettes, the ethanolic supernatant extracts were put into their corresponding wells, with the same batch of extracts put into the replicates of Mueller-Hinton Agar surface plates, with deionized water acting as the negative control and black seed oil serving as the positive control as demonstrated in a study done by [25]. The plates were incubated for 24 hours at 37°C. A ruler was used to calculate the zone of inhibition. The outcomes were then documented.

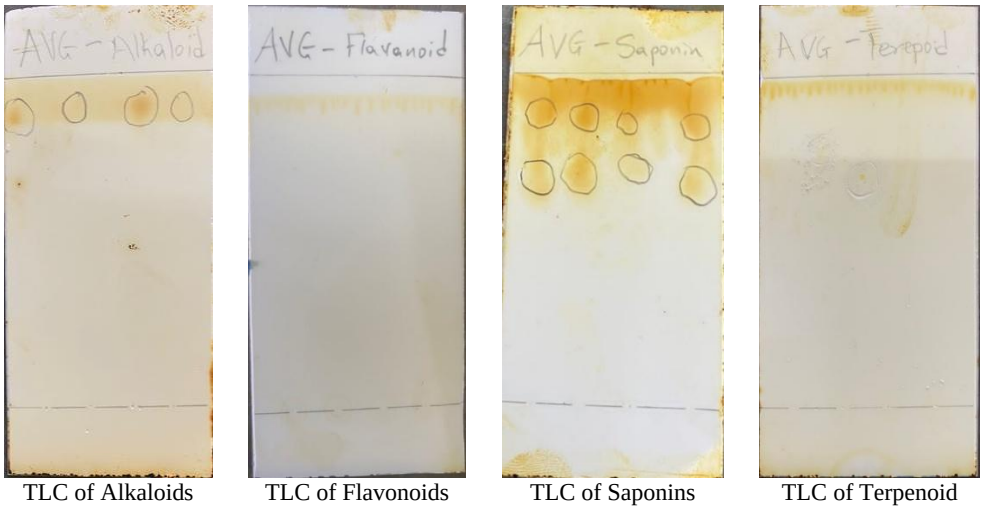
2.9 Statistical Analysis
A two-way analysis of variance (ANOVA) with replication was conducted to evaluate the effects of Extract type; Gel, Epidermis, and Whole Leaf on zone of inhibition of microbial growth, specifically for *Staphylococcus aureus* and *Escherichia coli*. The analysis was guided by three null hypotheses. First, it was hypothesized that there would be no significant difference in zone of inhibition of microbial growth across the different extract types (H_0A). Second, it was presumed that the zone of inhibition of microbial growth would not differ between the two microbes (H_0B). Third, the interaction hypothesis postulated that the effect of extract type on microbial zone of inhibition would be consistent across both microbes, indicating no interaction effect (H_0AB).
Two post hoc tests were performed, Tukey's Honestly Significant Difference (HSD) test for extract type comparison and t-Test for microbial zone of inhibition comparison. To calculate the HSD, this formula was used:

$$HSD = q \times \sqrt{\frac{MSerror}{n}}$$

Where:
- q = Studentized range value for 3 groups, 6 df error, α = 0.05 (from q-table) 4.34
- MS_{error} = Mean square error from ANOVA (0.167)
- n = number of replicates per group (4)

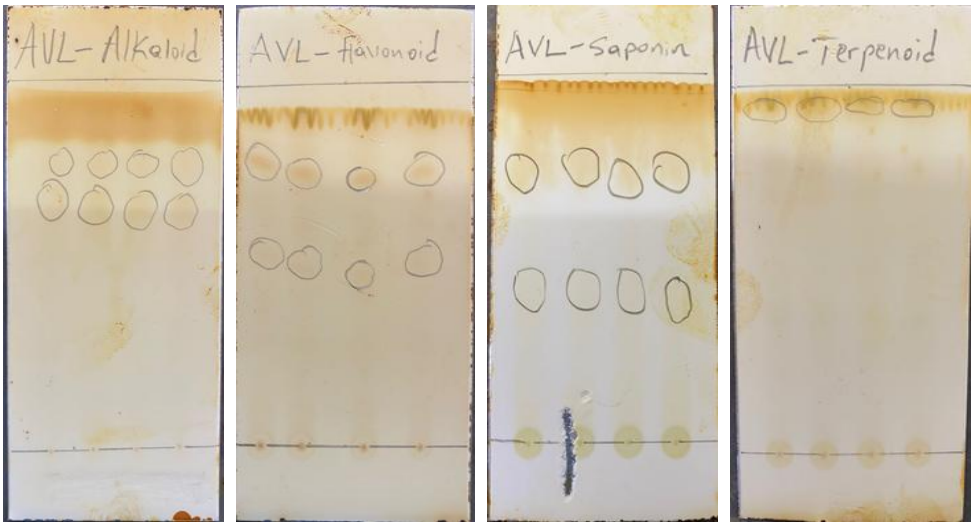
HDS = 0.887

3. Results
3.1 Determination of constituents from thin layer chromatography
Figure 1 above reveals that only two components, alkaloid, and saponin, were present in *Aloe vera* gel.



TLC of Alkaloids TLC of Flavonoids TLC of Saponins TLC of Terpenoid

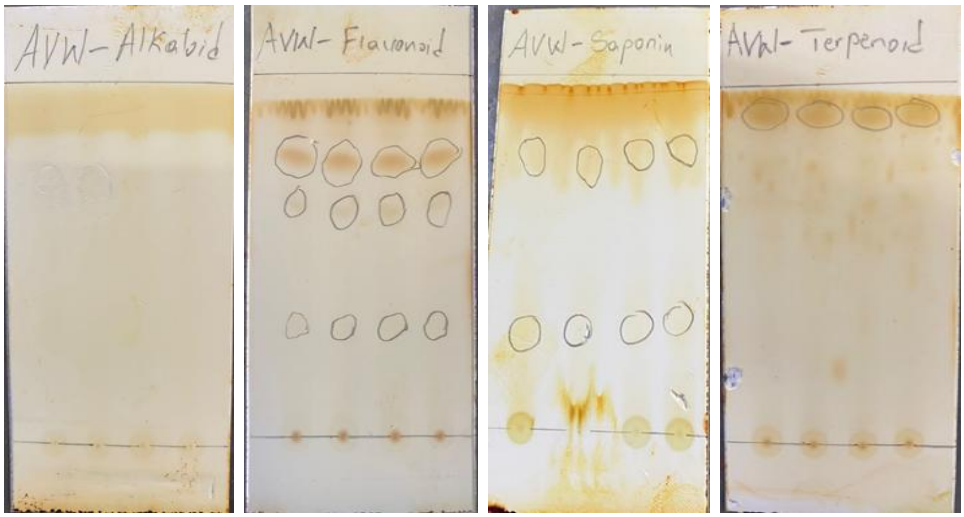
Fig 1: Separation of compound by using different solvent system for thin layer chromatography of *Aloe vera* gel extract.



TLC of Alkaloids TLC of Flavonoids TLC of Saponins TLC of Terpenoid

Fig 2: Separation of compound by using different solvent system for thin layer chromatography of *Aloe vera* epidermis extract.

Figure 2 above illustrates that the epidermis of *Aloe vera* contains all four components: alkaloids, flavonoids, saponin, and terpenoids.



TLC of Alkaloids TLC of Flavonoids TLC of Saponins TLC of Terpenoid

Fig 3: Separation of compound by using different solvent system for thin layer chromatography of *Aloe vera* whole leaf extract.

Figure 3 reveals that three components, flavonoids, saponin, and terpenoids, were present in *Aloe vera* whole leaf, but no alkaloids were detected.

Table 1: Retention factor of gel, epidermis, and whole leaf extracts of *Aloe vera* by thin layer chromatography compared to typical literature Rf range.

Phytochemical name	Plant part	Rf values	Typical Rf Range	Reference
Alkaloids	Gel	0.9	0.20 - 0.35	[23]
	Epidermis	0.66, 0.79		
	Whole leaf	----		
Flavonoid	Gel	----	0.60 - 0.80	[23]
	Epidermis	0.5, 0.77		
	Whole Leaf	0.3, 0.63, 0.77		
Saponins	Gel	0.70, 0.86	0.40 - 0.65	[24]
	Epidermis	0.40, 0.74		
	Whole leaf	0.26, 0.76		
Terpenoids	Gel	----	0.70 - 0.85	[23]
	Epidermis	0.96		
	Whole leaf	0.91		

Interpretation
TLC analysis revealed the presence of flavonoids in both epidermis and whole leaf extracts, with Rf values ranging from 0.63 to 0.77, consistent with literature standards [20]. Saponins were confirmed in the epidermis extract at Rf 0.40 [21]. Terpenoid-like compounds were observed in epidermis and whole leaf extracts, though Rf values slightly exceeded the expected range. No alkaloid-specific Rf values were detected under the solvent system used.

3.2 Antimicrobial activity of *Aloe vera* extracts against selected organisms

The antibacterial activity of ethanolic extracts of *Aloe vera* gel, epidermis, and whole leaf against the pathogens *Staphylococcus aureus* and *Escherichia coli* was tested using the well diffusion technique. The antibacterial activity of several *Aloe vera* extracts on selected microorganisms are depicted as a zone of inhibition in millimetres (mm). Using one way analysis of variance, the zone of inhibition against the test organisms on antimicrobial susceptibility tests of different *Aloe vera* extracts was studied.

C^+= Positive control (Black seed oil)
C^-= Negative control (Distil water)
G= *Aloe vera* gel extract
L= *Aloe vera* epidermis extract
S= *Aloe vera* whole leaf extract

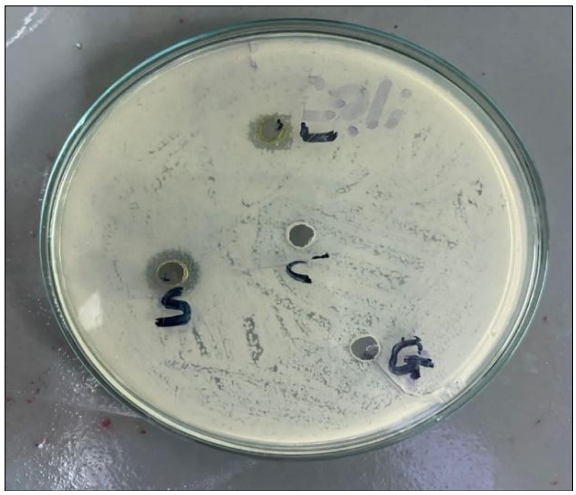


Fig 4: Antimicrobial activity of different *Aloe vera* extracts on Escherichia coli 1.

Fig 5: Antimicrobial activity of different *Aloe vera* extracts on *Escherichia coli* 2.

Figure 4 and 5 reveals that within the confines of the study, both *Aloe vera* epidermis and whole leaf extract had anti-microbial effects on *Escherichia coli*, with the epidermis having a greater impact. The gel appears to have little antimicrobial action on *E. coli*

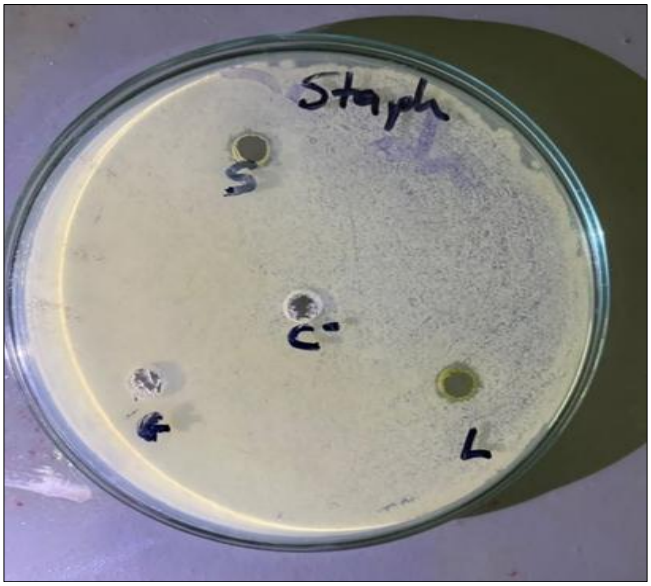


Fig 6: Antimicrobial activity of different *Aloe vera* extracts on *Staphylococcus aureus* 1.

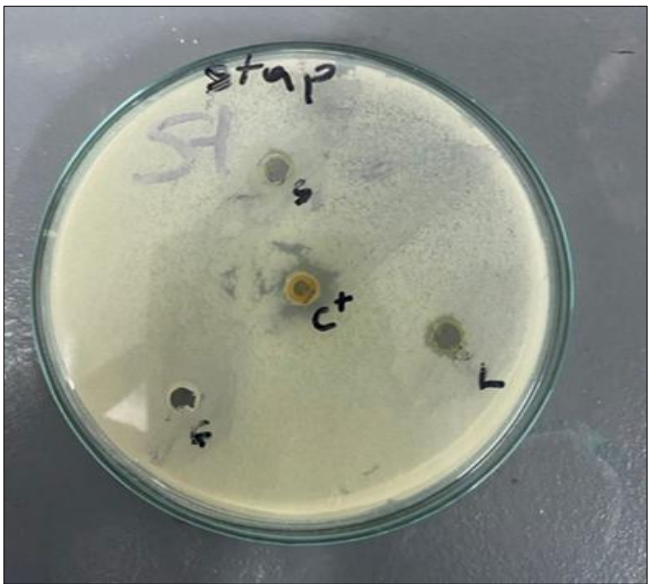


Fig 7: Antimicrobial activity of different *Aloe vera* extracts on *Staphylococcus aureus* 2.

Figure 6 and 7 above shows that both *Aloe vera* epidermis and whole leaf extract have anti-microbial effects on *Staphylococcus aureus*, with the epidermis having a greater impact again. The gel apparently shows no anti-microbial effect on *Staphylococcus aureus*.

Table 2: Shows the Diameter of zones of inhibition (mm) of *Aloe vera* gel, epidermis, and whole leaf ethanolic extracts on *Staphylococcus aureus* and *Escherichia coli*.

Microbes	Gel	Epidermis	Whole L
Staphylococcus aureus 1.	0	3	2
Staphylococcus aureus 2.	0	2	2
Escherichia coli 1.	0	5	4
Escherichia coli 2.	0	5	3

Table 3: Summary of two-way ANOVA with replication evaluating the effects of extract type and microbial species on zone of inhibition.

Source of Variation	SS	df	MS	F	P-value	F crit
Microbial type	5.333333	1	5.333333	32	0.001311	5.987378
Extract type	30.16667	2	15.08333	90.5	0.000033	5.143253
Interaction	3.166667	2	1.583333	9.5	0.013824	5.143253
Within	1	6	0.166667			
Total	39.66667	11				

Interpretation

Based on the two-way ANOVA with replication performed on microbial type, the analysis shows that, there was statistical significant for all three factors; Extract type, Microbial type and their interaction. The extract type (i.e Gel, epidermis, or whole leaf) had a highly significant impact on microbial growth with an ($F(2,6) = 90.5$, $P < 0.001$), the null hypothesis was rejected and the alternative hypothesis was accepted since the p-value < 0.05. This indicates that the choice of extract type plays an important role in controlling microbial proliferation. The Gel completely did not stop any growth across both microbes, the epidermis and the whole leaf stopped microbial growth, particularly for *E.coli.*

The microbial species themselves also showed significant difference in response, with *Escherichia coli* producing higher zone on inhibition overall. Since ($F(1,6) = 32.0$, $P < 0.01$), suggests that *Escherichia coli* may be more susceptible to the extracts. The interactions between the extract type and microbes was also statistically significant ($F(2,6) = 9.5$, $P < 0.05$), meaning that the antimicrobial potential varied not only by extract type but also by microbial species. For instance, while the gel had no zone of inhibition on both species equally, the epidermis had higher zone of inhibition on *Escherichia coli* than *Staphylococcus aureus,* and the whole leaf showed moderate zone of inhibition on both species slightly favouring *Escherichia coli.*

A post hoc test was performed to determine the location of the significant difference. The T-test (two sample assuming equal variances) was used to determine whether or not the mean differences were significant and Tukey HSD test for extract type comparison. Tukey is suited for multiple group comparisons, while t-test was used for binary comparison.

Table 4: Zone of inhibition (mm) for *Aloe vera* extract types across all microbial replicates used for Tukey's HSD post hoc analysis.

Extract type/ Microbe	*Staphylococcus aureus* 2.	*Staphylococcus aureus* 2.	*Escherichia coli* 1.	*Escherichia coli* 2.	Mean
Gel	0	0	0	0	0
Epidermis	3	2	5	5	3.75
Whole Leaf	2	2	4	3	2.75

Table 5: Pairwise comparison of mean zone of inhibition between *Aloe vera* extract types using Tukey's HSD test (critical value = 0.887).

Comparison	Mean Difference	HSD (0.887) Significant?
Epidermis vs Gel	3.75	Yes
Whole Leaf vs Gel	2.75	Yes
Epidermis vs Whole Leaf	1	Yes

Post hoc analysis using Tukey's Honestly Significant Difference (HSD) test (critical value = 0.887) confirmed that all pairwise comparisons between substances were statistically significant.

Gel showed no inhibition zone on microbial growth (mean = 0.00), while Epidermis showed the highest zone of inhibition (mean = 3.75), and Whole Leaf showed intermediate activity (mean = 2.75). These findings suggest a gradient of antimicrobial efficacy, with Gel being the least effective and Epidermis the most powerful.

Table 6: Raw zone of inhibition data (mm) for *Aloe vera* gel, epidermis, and whole leaf extracts against *Staphylococcus aureus* and *Escherichia coli* used for two-sample t-test analysis.

Extract type/ Microbes	*Staphylococcus aureus*	*Escherichia coli*
Gel	0	0
Gel	0	0
Epidermis	3	5
Epidermis	2	5
Whole L	2	4
Whole L	2	3

Table 7: Comparison of mean zone of inhibition between *Staphylococcus aureus* and *Escherichia coli* across all *Aloe vera* extracts using two-sample t-test.

	Staphylococcus aureus	*Escherichia coli*
Mean	1.5	2.833333
Variance	1.5	5.366667
Observations	6	6
Pooled Variance	3.433333	
Hypothesized Mean Difference	0	
Df	10	
t Stat	-1.24635	
P(T<=t) one-tail	0.12052	
t Critical one-tail	1.812461	
P(T<=t) two-tail	0.24104	
t Critical two-tail	2.228139	

Although *Escherichia coli* showed a higher mean zone of inhibition than *Staphylococcus* across all extract types, the difference was not statistically significant ($t(10) = -1.25$, $P = 0.241$). This suggests that species-level differences in zone of inhibition were not consistent enough to be distinguished from random variation. The significant interaction effect observed in the ANOVA further indicates that microbial zone

of inhibition response was influenced by the specific extract type used, rather than species alone.

4. Discussion

This study aimed to evaluate the antimicrobial potential of ethanolic extracts from different parts of *Aloe vera*; gel, epidermis, and whole leaf against *Staphylococcus aureus* and *Escherichia coli*. Through TLC profiling and inhibition zone assay, bioactive compounds were identified and the most therapeutically potent extract was determined.

The phytochemical profiling of *Aloe vera* extracts revealed notable variability across anatomical sources and in comparison with established literature. In the gel extract, three spots were found for alkaloid and two for saponin, but the remaining phytochemicals were not detected. Compared to existing literature such as [23] and [24], these results were far out of range, suggesting that the phytochemicals might have been other analogues. The epidermis extract showed two spots each for alkaloid, flavonoid, and saponin, and one for terpenoid. Compared to literature, the alkaloid was far from the range, while flavonoid and saponin had one spot within range and one slightly out of range; terpenoid was slightly out of range. The whole leaf extract showed no spot for alkaloid, three for flavonoid, two for saponin, and one for terpenoid. According to [23], two spots were in range for flavonoid, one for saponin, and one slightly out of range for terpenoid. These TLC results suggest varying phytochemical compositions across anatomical parts, which may influence antimicrobial performance.

The epidermis extract showed the highest zone of inhibition, with a small difference from the whole leaf extract, and no zone of inhibition for the gel extract across both microbes. Therefore, the epidermis exhibited the highest antimicrobial activity, particularly against *Escherichia coli*, which may be attributed to its high flavonoid and saponin content. To validate these observed differences in antimicrobial activity, two post hoc tests were conducted. Tukey HSD test confirmed that all pairwise comparisons between extract types were statistically significant, validating that the epidermis had the highest antimicrobial efficacy, the whole leaf was moderate, and the gel had little or no efficacy. This indicates that the antimicrobial efficacy varied meaningfully across anatomical sources, with each extract exhibiting distinct bioactivity profiles. These findings align with the phytochemical variability observed in TLC profiling. The T-test (two sample assuming equal variances) showed no statistical significance between microbes across extract types. This suggests that, despite phytochemical variability observed in TLC profiling, the therapeutic potency of the gel, epidermis, and whole leaf extracts was broadly comparable under the conditions tested.

These findings prompted comparison with existing literature on *Aloe vera* phytochemical profiles and antimicrobial efficacy. Multiple scientific studies have definitively found numerous phytochemicals in *Aloe vera* gel. [26] Demonstrated the existence of phenols, flavonoids, tannins, alkaloids, saponins, and terpenoids in *Aloe vera* gel extract. [27] Also confirmed the presence of saponins, alkaloids, glycosides, tannins, proteins, and flavonoids. The evidence across multiple independent studies is robust and consistent. The absence of detectable phytochemicals in this study may reflect possible misidentification, and these studies used aqueous extract instead of ethanolic supernatant.

Beyond gel composition, several studies have examined leaf and epidermis extracts, highlighting the role of extraction method in determining phytochemical yield. Multiple studies like [28] and [29] consistently report the presence of flavonoids, saponins, and terpenoids in *Aloe vera* leaf extracts. The specific claim about epidermis extract cannot be definitively verified with the current sources. While the general phytochemical profile is well-documented, a targeted study focusing on the epidermis extract's TLC results would be needed to conclusively support the original statement. Phytochemical screening is fundamentally dependent on extraction techniques, solvents, and specific plant material, with each factor critically influencing the quality and reliability of results. [30] Explicitly states that screening reliability depends on plant identification, pre-extraction procedures, solvent selection, and extraction methods. [31] Emphasizes extraction as a "critical step" in phytochemical analysis, noting the necessity of extracting desired components without destroying them. [32] Further elaborates that extraction involves selecting appropriate solvents (ranging from polar to non-polar) and choosing from multiple extraction procedures like maceration, ultrasound-assisted, and microwave-assisted techniques. The specific plant material's characteristics fundamentally determine the most effective extraction approach. There is need for confirmatory tests; TLC alone may not be sufficient for compound identification.

This study is supported by [33], where the leaf extract showed antimicrobial activity using the punch well method, consistent with the present study, while the gel extract failed to show any zone of inhibition across all isolates. [27] Also reported high antimicrobial activity in the epidermis (green rind), while contradicting claims that the gel has no antimicrobial activity. However, [27] used aqueous extracts, which may account for the difference. While all studies found antimicrobial activity, some noted that gel effectiveness varied by microorganism or concentration. [34] Found the least activity in *Pseudomonas aeruginosa*, with no inhibition zones at 25 mg/mL and 12.5 mg/mL, though this was due to lower concentrations rather than a complete absence of activity. It is possible that the ethanolic supernatant of the gel used in this study was at a lower concentration, which may have limited its antimicrobial efficacy.

Studies demonstrate that *Aloe vera* extracts containing phytochemicals consistently show antimicrobial activity against various pathogens. [35] Noted that *Aloe vera* extracts showed better antimicrobial activity than orthodox antibiotics. *Aloe vera*'s antibacterial properties are inconsistent and highly dependent on extraction method and plant part used. [36] Found moderate sensitivity of leaf and root extracts against both Gram-positive and Gram-negative bacteria. However, studies show significant variability: [27] noted gel extracts exhibited high activity against bacteria, while other studies found no effect. The extraction technique critically impacts results. [37] Demonstrated dose-dependent inhibitory effects, with inhibition zones increasing from 0 to 20 mm depending on concentration. This study also supports [38], which confirms *Aloe vera*'s antibacterial properties. [39] Found that freeze-dried *Aloe vera* whole leaf without juice, mesophyll, and epidermis did not exhibit antibacterial action. This contradicts findings that demonstrated antimicrobial properties of *Aloe vera* epidermis and whole leaf. These conflicting reports may be explained by methodological differences, particularly in solvent choice and gel composition.

The high water content (99%) of *Aloe vera* gel likely influences its antimicrobial efficacy, with ethanol extracts generally showing more consistent antibacterial action. These

conflicting findings underscore the need for standardized extraction protocols and comprehensive research to definitively characterize *Aloe vera*'s antimicrobial potential. In this study, the gel extract was made by immediately immersing fresh gel in ethanol, whereas in [40], the gel was exposed to air and crushed before dilution in ethanol. Given that *Aloe vera* gel contains 99% water [16], this may explain the contrasting results in this study. This also clarifies why the epidermis showed stronger antibacterial effects than the whole leaf, because the whole leaf contains both the gel and the epidermis.

In addition to extraction-related factors, the scope of microbial testing was limited by available strains, this study only used *Staphylococcus aureus* and *Escherichia coli* as microbial strains because of the limited strains available at the Sierra Leone Pharmacy Board at that moment. Follow-ups like Ultraviolet-Visible Spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), or High-Performance Liquid Chromatography (HPLC) were not done to confirm compound identities because they were not available in the research country, and ethanol was the only solvent used.

5. Conclusion

This study demonstrated that ethanolic extracts of from different parts of *Aloe vera* possess varying degrees of antimicrobial activity. Among the three extracts, the epidermis showed demonstrated the highest antimicrobial efficacy, particularly against *Escherichia coli*, which may due to higher flavonoid and saponin content. Statistical analysis confirmed significant differences in activity across extract types and microbial species. These findings support the therapeutic potential of *Aloe vera* epidermis as a natural antimicrobial agent and provide a scientific basis for its traditional use in treating infections. Further studies are recommended to isolate and characterize the specific compounds responsible for the observed antimicrobial effects and to evaluate their efficacy *in vivo*.

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