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Original Article

An In Vitro Evaluation of the Antibacterial Activity of *Aloe barbadensis* on Selected Bacterial Strains

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Abstract

Introduction: Traditional remedies, of which 95% are derived from plants, are used in Ethiopia to cure 90% of cattle and over 80% of human patients. Traditional medicine continues to be the predominant form of healthcare in underdeveloped nations. *Aloe barbadensis* is used to cure a variety of sick conditions, both infectious and not. The emergence of antimicrobial resistance (AMR) in bacteria is a significant risk and issue associated with conventional antibiotic treatment.

Methods: Disc diffusion and minimum inhibitory concentration (MIC) experiments against certain zoonotic pathogenic bacteria were used to assess the antibacterial activity of leaf extracts from chosen plants in vitro. The extraction solvents employed in this investigation were ethanol, methanol, and aqueous extraction.

Results: The ethanol extract from *A. barbadensis* was 7 ± 0.11mm to 24 ± 0.26 mm. *A. barbadensis* leaves showed 2 ± 0.11 to 10 ± 0.29 for hot conditions and 3 ± 0.11 lowest to 12 ± 0.26 highest for cold conditions. All examined microorganisms had a significant difference ($P<0.05$) in ethanol, methanol, and hot and cold aqueous extracts.

Conclusion: The findings of this study point to the potential use of *A. barbadensis* extracts as antibacterial agents for developing new pharmaceuticals to control animal pathogenic bacteria responsible for severe sickness.

Keywords: Antibacterial activity, *Aloe barbadensis*, Pathogenic bacteria

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Introduction

In conventional and contemporary medical systems, plants constitute the greatest source of pharmaceuticals, nutraceuticals, nutritional supplements, pharmaceutical intermediates, and chemical entities for synthesized medications (1). A common ethnobotanical plant provides a plentiful supply of natural medications for study and development (2). The benefits of using medications derived from medicinal plants include their ease of use, efficacy, and broad-spectrum action. Scientists are increasingly focusing on natural products to find novel approaches for creating more effective medications to treat microbial and viral infections, as well as cancer. Herbal medicine has been widely utilized worldwide, and in many nations, it is an essential component of primary healthcare. Since ancient times, plant species have been known to have pharmacological properties. This is because they are known to have various secondary metabolites used to fight pathogens that cause disease (3).

Antimicrobial resistance (AMR) is the ability of a bacterium to survive and proliferate in the presence of antibiotic doses previously thought to be effective against them, and it has emerged as a global issue. Several antimicrobial-resistant food-borne bacteria have recently surfaced in the food production chain that can infect humans and spread to other people. AMR in *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* is one of the problems that the global healthcare system is facing (4).

AMR is becoming an international issue. One way to tackle this concern is to conduct research to discover novel and inventive antimicrobials. Up to 80% of Africans receive their medical care through traditional medicine (5,6). In Ethiopia, plant remedies remain the most important and sometimes the sole source of therapeutics for around 80% of the population and over 90% of cattle (7,8).

Although people can use the plant's yellow exudate as a laxative, the WHO and IARC have published monographs indicating that it may also cause cancer (9,10). Since Aloe vera was first described as the type of the genus by Linneus, the two known therapeutic components of Aloe vera, yellow exudate and inner leaf mesophyll, have been utilized as remedies for various illnesses. According to Grace et al (11), members of the genus Aloe can be found in Madagascar, the Arabian Peninsula, East and Southern Africa, and a few smaller Indian Ocean islands.

The large intestine contains the gram-negative bacillus

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Escherichia coli, which is naturally eliminated through the urinary tract and feces. It is among the most prevalent causes of common bacterial illnesses, such as pneumonia and neonatal meningitis, as well as other clinical infections, including cholecystitis, bacteremia, cholangitis, urinary tract infections, and traveler's diarrhea (12).

Additionally, *Pseudomonas aeruginosa* is a rod-shaped, monoflagellated, gram-negative bacteria. It has a pearly appearance and manifests as a grape or tortilla. This organism can grow at 42 °C, which sets it apart from other *Pseudomonas* species. It grows well around 25–37 °C (13). It is becoming more widely acknowledged that *P. aeruginosa* is a significant nosocomial organism that can cause serious infections, particularly in hospitalized patients with burn units (14).

Healthy individuals' skin and nasal mucosa are colonized by the gram-positive bacteria *Staphylococcus aureus*. From fatal systemic difficulties to infections of the skin or soft tissues, the organism can cause a broad spectrum of illnesses (15). The formation of biofilms during an infection depends on coagulase, one of *S. aureus*'s unique virulence factors. A coagulation cascade must be started to convert soluble monomeric fibrinogen into self-polymerizing insoluble fibrin. Coagulase binds to host prothrombin and produces active staphylothrombin complexes (15).

Enterococcus faecalis is a gram-positive, cocci-shaped, non-spore-forming, fermentative, facultatively anaerobic bacteria. One common bacteria seen in prolonged, asymptomatic endodontic infections is *E. faecalis*. In smaller quantities, they can also be found in the oral cavity and female genital tracts of humans. They catabolize many energy sources, such as carbohydrates, glycerol, lactate, malate, citrate, arginine, and other keto acids (16). Extremely alkaline pH levels and high amounts of salt are among the difficult conditions that *E. faecalis* can withstand (16,17). They resist desiccation, ethanol, zeolite, detergents, heavy metals, and bile salts. They can tolerate 60 °C for 30 minutes and develop at 10-40 °C (16).

Therefore, the ongoing search must focus on finding fewer antibiotic sources. According to Doughari et al (18), plants are the safest and least expensive alternative source of antimicrobials. Despite the plant's availability in our nation, its antibacterial action has not yet been investigated.

Statement of the Problem

Public health is becoming increasingly concerned about emergencies and the emergence of bacterial resistance to traditional antibiotics. This study was conducted:

- To determine which extraction solvent would be best for extracting specific plant materials and
- To evaluate the antibacterial efficacy of *Aloe barbadensis* leaf extracts against a range of bacterial pathogens at varying concentrations.

Objectives

General Objectives

The main objectives of this study were to determine the antimicrobial activity of extracts of *Aloe barbadensis* plants for selected zoonotic antibiotic-resistant bacteria.

Specific Objectives

- To evaluate the in-vitro antibacterial activities of *A. barbadensis* plant extract against Gram-positive (*S. aureus* and *E. faecalis*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacteria.
- To assess better solvents from the extracts of *Aloe barbadensis* plant materials amongst selected extraction solvents (methanol, ethanol, and aqueous).

Materials and Methods

Study Period and Study Area

The study was conducted between February 2022 and July 2022 in the Kersa district of the Jimma zone of Oromia Regional State. The investigation was initially carried out in the southwest of Ethiopia. Jimma Town is situated 357 km from Addis Ababa. Jimma is one of Ethiopia's largest and most prominent towns. At a height of 1704 meters above sea level, Jimma was situated between latitudes 7° 13'20" and 8° 53' 16" North and longitudes 35° 51' 07" and 37° 36' 16" East. The average yearly temperature is roughly 18.5 °C, with the minimum and maximum values ranging between 6 °C and 31 °C, respectively. Unpredictable rainfall and temperature swings significantly influence the spread of disease in animals and people (19).

Plant Collection and Preparation

Preparation of the extract Leaves of Aloe (*A. barbadensis* or *Aloe vera) were* collected from Kersa woreda and Jimma town. Fresh leaves of mature and healthy Aloe vera were washed in tap water for 5 minutes and cleaned with sterile distilled water. Then, using a sterile knife, the leaves were dissected longitudinally, and the colorless aloe gel (parenchymatous tissue) was scraped out without the fibers. The drained gel was dried in the oven at 80 °C for 48 hours. Dried gels were ground to obtain powder by using mortar and pastel, and 30 g of this powder was soaked in 300 mL of solvents (ethanol, methanol, and aqueous) for 4 days for proper extraction of the active ingredients at room temperature (20).

Plant Extraction

Although a standard extraction procedure for herbal extracts has not been established, the business of herbal medicine routinely prepares plant crude extracts using 20–95 percent of the solvents (polar or non-polar). According to traditional claims and earlier investigations, all two medicinal herbs, such as *A. barbadensis,* were extracted using methanol (99.8%), aqueous, and ethanol (99.5%). A flat-bottom flask containing 200 mL of extracting solvents

was used to macerate 50 grams of air-drying powder plant materials for 24 hours while it was shaken at 121 rpm. The suspension was filtered using the Whatman No. 1 paper. After that, the filtrate was concentrated in a rotary evaporator under reduced pressure. After more drying, the solvent was extracted from the sticky residue using a water bath and an oven set to 45 °C and 42 °C, respectively. The remaining crude extracts were refrigerated in an airtight bottle until the experiment was carried out after the solvent had evaporated (21).

Determination of Extraction Yield

The percentage yield of each extract was obtained using the formula

$$Percent\ extracts = \frac{W_2 - W_1}{W_0} \times 100$$

Where W_2 is the weight of the dried extract and the container, W_1 is the weight of the container alone, and W_0 is the weight of the dried plant material (22).

Test Bacteria

To assess the antibacterial efficacy of crude plant extracts, reference bacterial strains of *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) were used. The Jimma Public Health Institute's main campus was the source of these four American-type cell cultures (ATCC), which were aseptically collected and transported through a cold chain. In the study, two gram-negative and two Gram-positive bacterial strains each comprised one of the four employed. The bacteria were grown on nutrient agar slants at 4°C and maintained on all of the test's strains, which are reference strains.

Media for Test Bacteria

The media was set at 1000 mL of distilled water and 38 g of Mueller-Hinton Agar were placed in a flask, and the mixture was heated gradually until the media was completely dissolved, as per the manufacturer's instructions. To sterilize the media, it was autoclaved for 15 minutes at 121 °C. Once the media cooled to about 50 °C, 25 mL of the sterilized medium were aseptically placed into 90 mm-diameter sterilized Petri plates. The plates were then allowed to dry until all the moisture remaining on the agar surface was removed before use. The prepared media's sterility was assessed by incubating a batch of randomly selected plates at 37 °C for a day (23).

Standardization of Inoculum

The zone of inhibition, which corresponds to the area where no bacterial growth was seen under ideal conditions for bacterial growth, is measured by culturing bacteria in the presence of the chemical or extract, and this method was used to evaluate the antimicrobial activity of a bioactive component. Bacterial susceptibility to the bioactive substances and extracts of the plant increased with increasing diameter (zone of inhibition). The technique was put into practice using the pre-outlined steps (24).

As a result, three to five colonies from pure cultures of each of the four chosen bacterial species were transferred using a sterile wire loop into a different label test tube containing 5 ml of nutrient broth and allowed to grow for two hours at 37 °C. The inoculums were made from the stock cultures, which were subcultured onto nutrient broth using a sterilized wire loop while kept on a nutrient agar slant at 4 °C. A comparison with the 0.5 McFarland standard of sodium chloride solution was used to calculate the density of suspension inoculated onto the media for the susceptibility test (25).

Preparation of Disc

Diffusion discs of 6 mm diameter were prepared from absorbent filter paper (Whatman No. 1) using a paper puncher, sterilized at 120 °C for 1 hour, and dried in an oven. The sterilized discs were then soaked aseptically by applying 30 µL of each crude plant extract at a concentration of 100 mg/mL using a sterile digital micropipette. They were then allowed to dry at room temperature for 15 minutes, placed in a sterile container, and stored at 4 °C until further use (23).

Preparation of the Test Bacterial

The test bacteria were grown on new plates using the isolate cultures acquired on agar slants. After choosing several bacterial isolate colonies, the colonies were suspended in 5 mL of nutritional broth in sterile vials with clearly labeled labels. For twenty-four hours, they were incubated at 37 °C. The broth cultures of each isolate were diluted ten times, one milliliter for each, in nine milliliters of sterile normal saline. To calculate the population density, a 0.5 mL syringe was used to choose 0.02 mL of dilutions ranging from 10-4 to 10-9 for each bacteria. After being incubated, different bacteria isolates formed variable numbers of colonies at different dilutions (26).

Antimicrobial Activity (Disc Diffusion Test)

A standard inoculum dilution of the test bacterial isolates in 1 mL was used to seed semi-solid nutrient agar plates. The inoculum on the agar surface was distributed by swirling the plates, and any extra was thrown away in a sterile jar. After setting for around 20 minutes on the bench, the plates were dried in the incubator for 30 minutes at 37 °C. Six wells were drilled around the plates using the sterile standard Cork borer. To stop the extracts from diffusing beneath the agar, the bottoms of the wells were sealed with one drop of sterile nutritional agar. Positive and negative controls were used in the fifth and sixth wells, respectively. The negative control well was filled with sterile distilled

water. Tetracyclines and ciprofloxacin for *Aloe vera* were used as the positive controls (27).

The disc diffusion technique has been widely used to assay plant extracts for antimicrobial activity (28). A sterile cotton swab was dipped into the adjusted standardized broth inoculum suspension by rotating the swab. The swab was then evenly streaked over the entire surface of the Muller-Hinton agar plate.

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) is the lowest dose that can stop detectable bacterial growth on the culture plates (29). All plant extracts were prepared as a 200 mg/mL stock solution. Ten milliliters of Muller-Hinton broth were added to each tested sterile tube. It was possible to create 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL using a sterile digital micropipette and stock solution in a double dilution procedure (24). Lastly, each tube received 20 µL of the test organism's standard suspension, adjusted to 0.5 McFarland standards. After giving the tubes a little shake to mix them, they were incubated for 24 hours at 37 °C. These are the lowest extract doses (MIC values) that stop bacterial growth during a 24-hour incubation period at 37 °C. Every test was conducted three times (23).

Data Management and Analysis

Before being transferred to statistical tools for analysis, data were entered and coded in Microsoft Excel spreadsheets and then transferred to SPSS 20. To determine whether there was any variation in the antibacterial effects of the four extracts—aqueous, ethanol, methanol, and fresh leaf, a one-way analysis of variance was conducted. The mean comparative value of the zone of inhibition obtained against each bacterium was computed for the chemical extracts of *A. vera*. A P value of less than 0.05 was considered significant for all analyses.

Results

Antibacterial Assay

The antibacterial activity of the dried leaves of *A. vera* was extracted by cold and hot water, ethanol, and methanol. The extract had more antibacterial action against gram-positive species (*S. aureus and E. faecalis*) than gram-negative pathogens (*E. coli and P. aeruginosa*). Fresh leaf gel and dried two-plant extracts in methanol and ethanol had greater antibacterial activity in this investigation than tetracycline and ciprofloxacin did. The yields of the extracts in different solvents of various forms of extract of the leaf of *A. barbadensis* against aqueous (cold), methanol, ethanol, and aqueous (hot) are shown below in Table 1 (21,30,31). The extraction solvents of methanol and ethanol have a high yield percent, and aqueous extraction is variable under different extract forms.

The different effects on the four test bacteria by Ethanol extracts of *A. barbadensis* are shown in Table 2. The four microorganisms were significantly different in response to ethanol extracts of *A. vera* compared to standard antibiotics (tetracycline and ciprofloxacin for bacteria). Table 2 displays the diameter of their inhibition zones. Aside from *P. aeruginosa,* which showed low inhibition on the extract of the dried leaves, all pathogens were sensitive to ethanol extracts of fresh and dried leaves, with their corresponding diameter zones of inhibition shown in Table 2.

Aloe barbadensis methanol extract was tested against two gram-positive and gram-negative bacteria. The extract was inhibitory to some pathogens tested, with the corresponding diameter zone of inhibition shown in Table 3. For *P. aeruginosa and E. coli,* the fresh leaf of methanol extract had low inhibitory effects. The Aloe vera gel of the methanol extract was highly inhibitory for *E. faecalis and P. aeruginosa*. Among the four studied pathogens, *S. aureus* was likewise found to be highly sensitive ($P<0.05$) to distilled water, and tetracycline was typically used as negative and positive controls in this extract form.

Table 4 shows significant differences in activity among the hot and cold aqueous extracts of *A. barbadensis* parts used compared to standard antibiotics (tetracycline). For *S. aureus and E. faecalis,* the diameter of the inhibitory zones was 12 ± 0.34 and 10 ± 0.37mm, respectively, when leaf extracts were cold aqueous extracted, and also *E. coli* 6 ± 0.26 mm *and P. aeruginosa* 3 ± 0.22 mm could grow by these extracts. Additionally, warm aqueous extracts of dried leaves and Aloe vera had a comparable antibacterial effect on *S. aureus and E. faecalis* but moderately inhibited *E. coli* and *P. aeruginosa*.

Based on the above result data analysis, plant parts used and test bacteria were highly significant at $P<0.00$, while the extraction solvent (methanol, ethanol, and water) was significant at $P<0.05$ in determining the production of inhibition zones (response) (Table 5).

Minimum Inhibitory Concentration

The following table displays the minimal inhibitory concentrations of the various extracts and the significance tests against the tested bacterial pathogens. Antibacterial activity of *Aloe barbadensis* (*Aloe vera)* extract against bacteria of *S. aureus, E. faecalis, P. aeruginosa,* and *E. coli* at different concentrations (mg/mL). The *A. barbadensis*

Table 1. Yield of the Extracts in Different Solvents

Scientific Name	Solvents	Weight of Dry Powder	Weight of Extract Powder	% Yield
Aloe barbadensis	Aqueous (cold)	25 g	7.5 g	30%
	Methanol	30 g	14.8 g	49.3%
	Ethanol	30 g	12.5 g	41.7%
	Aqueous (hot)	25 g	5 g	20%

extracts by methanol (mean inhibition zone 25 ± 0.19 mm) appear more effective *in E. faecalis* than the gel extracts (mean inhibition zone (MIZ) of 15 ± 0.26) against *S. aureus*, with significant differences in activity from each other. There was no significant difference in *Aloe vera* extracts (MIZ of 23 ± 0.29) against *E. coli*, whereas significantly higher activity (MIZ of 28 ± 0.11) against *P. aeruginosa*.

Table 6 below shows the different effects of ethanol extracts of Aloe barbadensis on the four test bacteria. The four microorganisms responded significantly differently to ethanol extracts of *Aloe vera* compared to standard antibiotics (tetracycline and ciprofloxacin for bacteria).

Discussion

Antibacterial Assay

The objective of the current investigation was to gather preliminary data on the antibacterial activity of water,

Table 2. Antibacterial Activity of Medicinal Plant Extracts by Ethanol Against Selected Bacteria

	Name of Bacteria	Zones of Inhibition (mm) Ethanol Extract (Mean ± SE)		*P* Value
		Aloe barbadensis	**Tetracycline (Standard)**	
Gram positives	*S. aureus*	24 ± 0.26	18 ± 0.34	0.003
	E. faecalis	21 ± 0.19	20 ± 0.37	0.001
Gram negatives	*E. coli*	13 ± 0.29	12 ± 0.26	0.000
	P. aeruginosa	7 ± 0.11	19 ± 0.22	0.000

Table 3. Antibacterial Activity of Plants Extracted by Methanol Against Selected Bacteria

Name of Bacteria		Zones of inhibition (mm) Methanol extract (Means ± SE)			*P* Value
		Aloe barbadensis	**Distilled Water**	**Tetracycline (Standard)**	
Gram Positives	*S. aureus*	15 ± 0.26	N	18 ± 0.34	0.003
	E. faecalis	25 ± 0.19	N	20 ± 0.37	0.001
Gram Negatives	*E. coli*	23 ± 0.29	N	12 ± 0.26	0.000
	P. aeruginosa	28 ± 0.11	N	19 ± 0.22	0.000

Data are presented as means ± SE, the Value of the triplicate experiment.

Table 4. Antibacterial Activity of Plants Extracted by Aqueous Against Selected Bacteria

	Name of Bacteria	*Aloe vera*				*P* Value
		Hot Gel	**Cold Gel**	**Distilled Water**	**Tetracycline**	
Gram Positives	*S. aureus*	5 ± 0.26	12 ± 0.26	N	18 ± 0.34	0.003
	E. faecalis	5 ± 0.19	10 ± 0.19	N	20 ± 0.37	0.001
Gram Negatives	*E. coli*	10 ± 0.29	6 ± 0.29	N	12 ± 0.26	0.000
	P. aeruginosa	2 ± 0.11	3 ± 0.11	N	19 ± 0.22	0.000

Diameter of the zone of inhibition, including a diameter of disc 6 mm; Data are presented as means ± SE, the Value of a triplicate experiment.
N = No inhibition.

Table 5. ANOVA table for the significance tests at different concentrations (mg/mL)

Source of variation	*df*	Sum of Squares	Mean Square	F value	*P* value
a. ANOVA table type of *Aloe barbadensis* extract against bacteria of *S. aureus*.					
Treatment	21	2970	141.4286	2088.21	0.000
Residual	44	2.98	0.06773		
Total	65	2972.98			
b. ANOVA table type of Aloe barbadensis extract against bacteria of *E. faecalis*					
Treatment	21	3514.364	167.3507	4720.15	0.000
Residual	44	1.56	0.03545		
Total	65	3515.924			
c. ANOVA table type of Aloe barbadensis extract against bacteria of *E.coli*					
Treatment	21	2374.36	113.065	1383.83	0.002
Residual	44	3.595	0.0817		
Total	65	2377.96			
d. ANOVA table type of *Aloe barbadensis* extract against bacteria of *P. aeruginosa*					
Treatment	21	3399.64655	161.8879	12757.74	<0.005
Residual	44	0.55833	0.01269		
Total	65	3400.20489			

df, degrees of freedom.
Note: Based on this analysis, extraction solvent, plant (Treatment) used, and test-organism factors are significant at *P* value e < 0.05.

ethanol, methanol, and fresh leaf juice extracts from *A. barbadensis*. The ethanol extracts of fresh gel *A. barbadensis* exhibit high antibacterial activity in the development of the organisms examined, except for *E. coli* and *P. aeruginosa*, while they showed a minimal inhibitory impact when compared to the standardized control tetracycline and ciprofloxacin ($P > 0.05$). The antibacterial properties of different *A. vera* extracts vary. Compared to ethanol and aqueous solutions, *A. vera*'s methanol extract exhibited the highest antibacterial activity. This research was comparable to one conducted in 2011 by Ibrahim et al. to examine the antibacterial assessment of *A. vera* gel against a variety of human and plant pathogens by the disc diffusion method, which was done with three different forms (ethanol, methanol, and aqueous extracts) (32).

In this study, methanol and ethanol extracts from dried leaves exhibited higher antibacterial activity in the growth of gram-positive bacteria tested than tetracycline. This is because methanol and ethanol can extract more of the plant's active ingredient than aqueous extracts. Many studies have shown that plants inhibit the growth of gram-positive bacteria more than gram-negative bacteria (33,34). This may be attributed to the high permeability barrier of gram-negative bacteria to numerous antibiotic molecules similar to aqueous and ethanol. This corroborates the work of Nair et al (35), who had similar findings. In the other direction, comparing the extracts, the methanol extract of the *A. barbadensis* gel exhibited broad antibacterial activity against all the tested bacteria (*S. aureus, E. faecalis, E. coli,* and *P. aeruginosa*). The activity of the plants against both gram-positive and gram-negative bacteria may indicate the presence of broad-spectrum antibiotic compounds in the leaves of the plants (36,37).

The hot and cold aqueous extract of the fresh gel *A. barbadensis* showed appreciable antibacterial activity on *S. aureus and E. faecalis*, while it did not affect *E.coli or P. aeruginosa*. The cold water extract of leaves displayed a relatively better antibacterial effect against *S. aureus* and *E. faecalis*, with their diameter zones of inhibition recorded at the fresh gel *A. vera* on two-gram positives being 12 ± 0.26 mm and 10 ± 0.19 mm. In earlier research done by other researchers, hot water extracts could not inhibit any organisms (38).

However, in this study, a hot water extract of fresh gel *A. barbadensis* inhibited two organisms: *Staphylococcus aureus* and *E. faecalis*. This result is interesting because, in the traditional method of treating a bacterial infection, a decoction of the plant part or boiling the plant in water is employed (Tables 7-9). Another reason for the ineffectiveness of aqueous extracts could be that the active compounds were not soluble in water, or the aqueous extracts in this study were not prepared according to traditional methods, which in many cases involve boiling and soaking with water for several hours (39). Success in traditional medicines may be due to administering the extracts in large quantities over a long period (40). Also, Pandey and Mishra reported that there was no inhibitory effect of the aqueous extract on the Gram-negative bacteria and a weak inhibitory effect on gram-positive bacteria (41). Differences in the antimicrobial effects of various extracts of *A. vera* may be attributed to the different solubility of various compounds found in *A. vera*, particularly some solvents with a specific antimicrobial activity.

Evidence (42,43) indicates that the global rise of AMR

Table 6. Antibacterial Activity of *Aloe vera* in Different Concentrations of Extracts by Ethanol

Concentrations (g/mL)	Zone of Inhibition			
	Ethanol Extract			
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	24 ± 0.26^{a}	21 ± 0.19^{b}	13 ± 0.29^{c}	7 ± 0.11^{f}
0.5	12 ± 0.26^{d}	14 ± 0.19^{c}	8 ± 0.29^{c}	5 ± 0.11^{k}
0.25	10 ± 0.26^{e}	12 ± 0.19^{c}	6 ± 0.29^{g}	2 ± 0.11^{j}
0.125	6 ± 0.26^{h}	5 ± 0.19^{b}	4 ± 0.29^{i}	0.87 ± 0.11^{k}
Distilled water (2 mL)	0 ± 0.00^{i}	0 ± 0.00^{j}	0 ± 0.00^{k}	0 ± 0.00^{l}
Positive control	18 ± 0.26^{a}	20 ± 0.19^{b}	12 ± 0.29^{b}	19 ± 0.11^{b}

Mean values with different superscripts in the same column are significantly different at
$P < 0.05$. *Means inhibition zones (mm) within a column followed by the same letter are not significantly different from each other at $p < 0.05$.

Table 7. Antibacterial Activity of *Aloe vera* in different Concentrations of Extracts by Methanol

Concentrations (g/mL)	Zone of Inhibition Methanol Extract			
	Gram-Positive Bacteria		Gram-Negative Bacteria	
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	15 ± 0.26^{b}	25 ± 0.19^{a}	23 ± 0.29^{a}	28 ± 0.11^{a}
0.5	14 ± 0.26^{c}	13 ± 0.19^{d}	13 ± 0.29^{c}	15 ± 0.11^{c}
0.25	12 ± 0.26^{d}	10 ± 0.19^{f}	10 ± 0.29^{d}	10 ± 0.11^{d}
0.125	8 ± 0.26^{f}	8 ± 0.19^{g}	7 ± 0.29^{f}	8 ± 0.11^{e}
Distilled water (2 mL)	0 ± 0.00^{g}	0 ± 0.00^{h}	0 ± 0.00^{h}	0 ± 0.00^{g}
Positive control	18 ± 0.26^{a}	20 ± 0.19^{b}	12 ± 0.29^{b}	19 ± 0.11^{b}

Mean values with different superscripts in the same column are significantly different at $P < 0.05$.

Table 8. *Aloe barbadensis* in Different Concentrations of Extracts by Aqueous (Cold)

Concentrations (g/mL)	Zone Of Inhibition			
	Aqueous (Cold) Extract			
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	12 ± 0.26^{d}	10 ± 0.19^{f}	6 ± 0.29^{h}	3 ± 0.11^{i}
0.5	6 ± 0.26^{h}	5 ± 0.19^{i}	2 ± 0.29^{k}	2 ± 0.11^{j}
0.25	5 ± 0.26^{i}	3 ± 0.19^{k}	0 ± 0.00^{l}	0 ± 0.00^{m}
0.125	3 ± 0.26^{k}	2 ± 0.19^{l}	0 ± 0.00^{l}	0 ± 0.00^{m}
Distilled water (2 mL)	0 ± 0.00^{l}	0 ± 0.00^{m}	0 ± 0.00^{a}	0 ± 0.00^{a}
Positive control	18 ± 0.26^{a}	20 ± 0.19^{b}	12 ± 0.29^{b}	19 ± 0.11^{b}

Mean values with different superscripts in the same column are significantly different at $P < 0.05$.

Table 9. *Aloe barbadensis* in Different Concentrations of Extracts by Aqueous (Hot)

Concentrations (g/mL)	Zone of Inhibition			
	Aqueous(Hot) Extract			
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	5 ± 0.26^{i}	5 ± 0.19^{i}	10 ± 0.29^{d}	2 ± 0.11^{j}
0.5	4 ± 0.26^{j}	2 ± 0.19^{l}	8 ± 0.29^{e}	0 ± 0.00^{m}
0.25	2 ± 0.26^{l}	0 ± 0.00^{m}	6 ± 0.29^{g}	0 ± 0.00^{m}
0.125	0 ± 0.00^{m}	0 ± 0.00^{m}	3 ± 0.29^{j}	0 ± 0.00^{m}
Distilled water (2 mL)	0 ± 0.00^{m}	0 ± 0.00^{m}	0 ± 0.00^{l}	0 ± 0.00^{m}
Positive control	18 ± 0.26^{a}	20 ± 0.19^{b}	12 ± 0.29^{b}	19 ± 0.11^{b}

Mean values with different superscripts in the same column are significantly different at $P < 0.05$.

is mainly due to the discriminatory use of drugs for the treatment of both human and animal diseases. Although different antibiotic classes are used in animal health management and human medicine, selecting resistance to one drug class may lead to cross-resistance to another (44).

In general, the antibacterial activity of the crude plant extracts on the test organisms supports the active ingredient found in herbal remedies, and the bioactive substance's antibacterial potential was quite equivalent to the antibiotic utilized in the sensitivity test. Additionally, the involvement of plants in the development of the chosen bacteria may indicate the presence of broad-spectrum antibiotic chemicals in the plant's leaf. To inhibit pathogenic bacteria, *A. barbadensis* may thus develop into a promising natural antibacterial agent with prospective applications in the pharmaceutical sector. However, if plant extracts are to be used for medicinal purposes, safety and toxicity should be considered.

The potency of the extract depends on the method used to obtain it. The failure of some of the extracts to have an antibacterial effect on the test organism is insufficient to conclude that the extract does not contain substances that can have an antibacterial effect on the test organism (45-47). The age of the plant utilized, the freshness of the plant material, physical factors (temperature, light, or water), contamination by field bacteria, improper processing of the plant, etc., may all have a role in the antimicrobial activities of different plant extracts (36).

Minimum Inhibitory Concentration

The sensitivity of the test bacteria to the plant extracts varied depending on the bacteria. Most of the extracts were antibacterial, especially against *S. aureus*, the most susceptible bacterium to the extracts, compared to less affected *E. coli*. The differential sensitivity of bacteria to plant extracts may be explained by the cell wall composition of Gram-positive and Gram-negative bacteria. The cell wall of a gram-negative bacterium (*E. coli*) contains an outer membrane and lipid bilayer embedded with proteins and porins (carrier proteins). These proteins allow the passage of certain small molecules or ions into or out of the cell periplasm. The active compounds may not be able to pass into the cells, making them inactive. Many molecules are prevented from passing through the outer membrane, which keeps out many dangerous compounds and reduces its sensitivity to numerous extracts (48,49). The size of the molecule that can pass through a porin channel is directly determined by its size. The peptidoglycan membrane of the gram-positive bacterium S. aureus is quite thick. Yet, most extracts can still pass through it since it is permeable to various compounds, such as sugars, ions, and amino acids (50).

Conclusions and Recommendations

In recent years, there has been an increase in interest in using plants as a source of new chemicals to fight microbial diseases. The need to find plant-based antimicrobials is growing due to the high cost, decreased efficacy, and rising resistance to conventional medications. Several discoveries suggest a new method for uncovering an effective antibacterial agent from Aloe barbadensis. The results, including the current result, generally showed that the active principle of the plants, which had effective antibacterial results towards the tested pathogens, could become a promising natural antimicrobial agent with potential applications in pharmaceutical industries for controlling pathogenic bacteria. However, the value of the extract for bacterial cultivation depends on the anti-biogram output of the experiment. The findings of this research demonstrated that all of the extracts have antibacterial activity against the examined pathogens to varying degrees. Thus, based on the above-concluding remarks, the following recommendable points are forwarded:

- People ought to be advised on possible ways of cultivating plants.
- Additional research should be done to evaluate the antibacterial properties of *A. barbadensis* extracts against pathogens other than those chosen in the initial investigations.
- To determine the potential variables influencing the antibacterial effect of the plant extract, research should be conducted.
- It is important to raise awareness of the plant's historical use in the region as a remedy for various illnesses.

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Competing Interests

The authors declare no conflict of interest.

Ethical Approval

This study was approved by Toke Kutaye Woreda Agricultural Office, Ambo, Oromia, Ethiopia under ethics number 3457.

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