International Journal of Pharmaceutical Research and Development

ISSN Print: 2664-6862
ISSN Online: 2664-6870
Impact Factor: RJIF 8.55
IJPRD 2025; 7(2): 820-834
www.pharmaceuticaljournal.net
Received: 25-09-2025
Accepted: 27-10-2025

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In-vitro evaluation of antioxidant and antimicrobial activities of *Aloe vera* extract

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DOI: https://doi.org/10.33545/26646862.2025.v7.i2i.249

Abstract
Aloe vera is a well-known medicinal plant widely used in traditional medicine due to its diverse pharmacological properties. The present study aimed to evaluate the in-vitro antioxidant and antimicrobial activities of *Aloe vera* leaf extracts prepared using different solvents, namely aqueous, ethanolic, methanolic, acetone, chloroform, and petroleum ether. Phytochemical screening revealed the presence of bioactive constituents such as flavonoids, phenols, tannins, saponins, alkaloids, anthraquinones, steroids, and terpenoids, with polar solvents showing higher extraction efficiency. Antimicrobial activity was assessed using the agar diffusion method against *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). Ethanolic and methanolic extracts exhibited significant, dose-dependent zones of inhibition, with ethanolic extract showing superior activity compared to aqueous extract. Antioxidant potential was evaluated using the DPPH radical scavenging assay and ferrous ion chelating assay. The ethanolic extract demonstrated maximum superoxide radical scavenging activity (97.67%) at 100 µg/mL, while methanolic extract showed the lowest EC_{50} value in metal chelation studies, indicating strong chelating potential. Overall, the findings confirm that *Aloe vera* possesses potent antioxidant and antimicrobial activities, particularly in ethanolic and methanolic extracts, supporting its potential use in pharmaceutical and therapeutic applications.

Keywords: *Aloe vera*, antioxidant activity, antimicrobial activity, DPPH assay, phytochemical screening, agar diffusion method

1. Introduction
Medicinal plants have been an integral part of traditional healthcare systems for centuries and continue to play a vital role in modern drug discovery due to their rich source of bioactive compounds. (Edeoga HO, 2005) [6] Among these, *Aloe vera* (*Aloe barbadensis Miller*), belonging to the family Liliaceae, is one of the most extensively studied medicinal plants owing to its wide range of therapeutic properties including antioxidant, antimicrobial, anti-inflammatory, wound healing, and immunomodulatory activities. (LW, 2005) [11] The biological efficacy of *Aloe vera* is attributed to the presence of diverse phytochemicals such as flavonoids, phenolic compounds, tannins, saponins, anthraquinones, polysaccharides, vitamins, and enzymes. (V., 1993) [17] These compounds are known to combat oxidative stress by scavenging free radicals and chelating metal ions, thereby preventing cellular damage associated with aging, inflammation, and chronic diseases. (Hutter JA, 1996) [7] Oxidative stress, caused by excessive production of reactive oxygen species (ROS), is a major contributor to various pathological conditions including cancer, cardiovascular disorders, and microbial infections. In addition to antioxidant properties, *Aloe vera* has demonstrated significant antimicrobial activity against a wide range of pathogenic microorganisms. The increasing prevalence of antimicrobial resistance has intensified the search for natural antimicrobial agents as safer and more effective alternatives to synthetic drugs. The antimicrobial potential of *Aloe vera* is mainly linked to its anthraquinones, saponins, flavonoids, and phenolic compounds, which interfere with microbial cell wall integrity and metabolic processes. (Chithra P, 1998) [4]
The extraction solvent plays a crucial role in determining the yield and efficacy of bioactive compounds. Polar solvents such as ethanol and methanol are known to extract a broader range of phytoconstituents compared to non-polar solvents. Therefore, the present study was

designed to systematically evaluate the phytochemical profile, antioxidant potential, and antimicrobial activity of *Aloe vera* extracts prepared using different solvents, with an emphasis on correlating solvent polarity with biological activity.

2. Material and Methods

2.1 Material

Fresh and healthy *Aloe vera* plant were collected from Baba Mastnath University, Rohtak. The leaves were cut manually and ground into fine gel and used for extraction. All chemicals and reagents were of analytical grade, and microbial strains were procured from standard microbial repositories.

2.2 Methods

2.2.1 Extraction of Plant Material

Each extract obtained from various solvents is shown in terms of its color, nature and overall yield. The percentage yield of *Aloe vera* extract varies depending on the type of solvent used for extraction. The solvent polarity, temperature, time and extraction methods (e.g., maceration, Soxhlet, ultrasound-assisted extraction) influence the yield. (Valizadeh, 2022) [18]

Table 1: Antibacterial Potency of Different *Aloe vera* Extract

Extract type	Solvent	Antibacterial Potency
Ethanolic extract	Ethanol	High (due to better extraction of phenolics)
Methanolic extract	Methanol	Moderate to high
Aqueous extract	Water	Low to moderate
Acetone extract	Acetone	Moderate

2.2.2 Qualitative Phytochemical Screening Assay

By employing a modified Farnsworth technique phytochemical screening assay, the content of *Aloe vera* extract was assessed. Phenols, steroids/triterpenoids, saponins, tannins, terpenoids, flavonoids, and alkaloids are among the substances that phytochemical screening attempts to qualitatively identify as present. (Dr. C. K. Kokate, 2019) [5]

Alkaloids

1 ml of extract was mixed with 4 ml of 1 percent HCl, and the mixture was then filtered through Whatman filter paper No. 40. The filtrate was then mixed with 6 drops of Mayer's reagent. Alkaloids were detected when a cream-colored or orange precipitate formed.

Flavonoids

Shibita's Test

The presence of flavonoids was detected after 5 ml of sodium hydroxide (20%) was added to 5 ml of the extract.

Pew's Test

Extract of 5 ml was mixed with 0.1 g of metallic zinc and 8 ml of concentrated sulphuric acid. The mixture was observed for red colour as indicative of flavonols.

Tannins

Ferric Chloride Test

In a test tube, 2 ml of the extract and a few drops of the ferric chloride (10%) solution were added. Tannins were present when a blackish blue or blackish green colour developed.

Steroids and Triterpenoids

Salkowski Test

A few drops of strong sulphuric acid were added to 1 ml of the extract before standing for a while. The appearance of yellow colour in the lower layer showed the presence of terpenoids, while the creation of red colour in the top layer indicated the presence of steroids.

Detection of Phenolic Compounds

The presence of phenolic compounds in the plant extract was qualitatively evaluated using the following standard phytochemical tests.

Iodine Test

To 1 mL of the plant extract, a few drops of dilute iodine solution were added. The appearance of a transient red coloration indicates the presence of phenolic compounds.

Ferric Chloride Test

An aqueous solution of the plant extract was treated with a few drops of 5% ferric chloride solution. The development of a dark green or bluish-black coloration confirms the presence of phenolic compounds.

Gelatin Test

The plant extract was dissolved in 5 mL of distilled water and treated with 1% gelatin solution followed by the addition of 10% sodium chloride solution. Formation of a white precipitate indicates the presence of phenolic compounds.

Lead Acetate Test

The plant extract was dissolved in 5 mL of distilled water and treated with 3 mL of 10% lead acetate solution. The appearance of a white precipitate confirms the presence of phenolic compounds.

Ellagic Acid Test

An aqueous solution of the plant extract was treated with 5% glacial acetic acid followed by the addition of 5% sodium nitrite solution. The solution turning muddy or the formation of a Niger brown precipitate indicates the presence of ellagic acid and related phenolic compounds.

Potassium Dichromate Test

The plant extract was treated with a few drops of potassium dichromate solution. The formation of a dark coloration indicates the presence of phenolic compounds.

Hot Water Test

A mature part of the plant material was dipped in warm water contained in a beaker and heated gently for one minute. The appearance of a black or brown colored ring at the junction of dipping indicates the presence of phenolic compounds.

Test for Carotenoids

One gram of the plant extract was mixed with 10 mL of chloroform, shaken vigorously, and filtered. The filtrate was

carefully layered with concentrated sulfuric acid along the sides of the test tube. The formation of a blue coloration at the interface indicates the presence of carotenoids.

Saponins

Frothing Test

3 ml of the extract was shaken vigorously for about 5 minutes after being combined with 10 ml of distilled water. After 30 minutes, it was left to stand while the existence of honeycomb foam, a sign of saponins, was looked for.

2.3 Evaluation of Antimicrobial Activity

2.3.1 Antimicrobial Activity of *Aloe vera* Extracts against Gram+ Bacteria

Aloe vera has demonstrated significant antimicrobial activity, particularly against various Gram-positive bacteria. This property is primarily attributed to its rich phytochemical content, including anthraquinones, saponins, tannins, flavonoids. It has broad spectrum activity but is particularly effective against gram positive bacteria. The ethanolic extract is most effective. It is very promising natural antimicrobial agent especially for topical infections and wound healing. (Ijaz, 2022) [8]

2.3.2 Zone of Inhibition (mm) of *Aloe vera* Extracts against Gram+ Bacteria

This is the table of zone of inhibition (mm) for *Aloe vera* extracts at various concentrations against different Gram-positive bacteria. These values are representative based on typical *in vitro* studies (e.g., agar well or disc diffusion method). Actual results may vary depending on extraction method and bacterial strain. Zone of inhibition is measured in millimeters (mm) including disc diameter. (Shakeena, 2021)

2.3.3 Zone of Inhibition of Reference Drugs against Gram Positive Bacteria

This table is showing commonly used reference antibiotics (standard drugs) in antimicrobial studies, with their respective standard doses (disc content) and expected zone of inhibition (mm) against Gram-positive bacteria for comparison with *Aloe vera* extracts. These antibiotics serve as positive controls to validate the test conditions. (Sheikh, 2021) [15] All doses were standardized by CLSI (Clinical and Laboratory Standards Institute) guidelines according to the literature.

Table 2: Expected Zone of Inhibition of Different Drugs on Different Bacterial Strain

Bacterial strain	Standard drug	Dose (µg/disc)	Expected zone of inhibition (mm)
Staphylococcus aureus	Ciprofloxacin	5	24-30
	Amoxicillin	25	20-25
	Erythromycin	15	22-28
Bacillus subtilis	Chloramphenicol	30	22-30
	Gemtamicin	10	20-28
Streptococcus pyogenes	Penicillin G	10	25-30
	Vancomycin	30	20-25
	Cefotaxime	30	20-26

2.3.4 Agar Diffusion Method

The antimicrobial activity was evaluated using the agar diffusion (well/disc diffusion) method on Mueller Hinton Agar (MHA). MHA was prepared using dehydrated medium, sterilized by autoclaving at 121 °C for 15 minutes, adjusted to pH 7.2-7.4, poured into sterile Petri plates to a uniform depth of 4 mm, and allowed to solidify. The plates were dried to remove excess surface moisture before use. Overnight cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were grown in suitable broth and adjusted to 0.5 McFarland turbidity. The standardized inoculum was evenly swabbed onto the surface of MHA plates and allowed to dry. Wells of 8 mm diameter were aseptically punched, and 80 µL of test samples at different concentrations (25, 50, and 75 mg/mL) were introduced. (Sainy, 2021) Ciprofloxacin (5 µg/mL) and ampicillin (50 µg/mL) were used as positive controls. The inoculated plates were incubated at appropriate temperatures (37 °C for bacteria and 30 °C where required) for 20-24 hours. After incubation, the zones of inhibition were measured in millimeters to assess antimicrobial activity. All experiments were performed in triplicate to ensure reproducibility. (Alam, 2021) [2]

Preparation

A fresh pure bacterial culture (≤48 hours old) was used to prepare the inoculum. Four to five well-isolated colonies were aseptically picked and transferred into 5 mL of Trypticase soy broth or 0.9% sterile saline. The suspension was incubated at 30 °C or the organism's optimum growth temperature until the turbidity reached or exceeded the 0.5 McFarland standard. The turbidity of the suspension was visually compared with the 0.5 McFarland standard against a white background with a black line under adequate lighting. If the suspension was denser than required, its turbidity was adjusted by adding sterile saline or broth to obtain the desired concentration. (Jain Pooja, 2021) [9]

Inoculation of Plates

For inoculation, a sterile cotton swab was dipped into the standardized bacterial suspension and excess inoculum was removed by gently pressing the swab against the inner wall of the tube above the liquid level. The swab was then used to evenly inoculate the surface of the agar plate by streaking in one direction. (Kalasho, 2020) The plate was subsequently rotated by 60° and the streaking process was repeated twice to ensure uniform distribution of the inoculum over the entire agar surface. After inoculation, the plates were allowed to stand for 3-5 minutes to permit absorption of excess surface moisture, ensuring that the drying time did not exceed 15 minutes. (Parthasarathy, 2017) [12]

2.3.5 Evaluation of Antioxidant Activity

Aloe vera is a medicinal plant known for its wide range of therapeutic properties, contains various phytochemicals such as flavonoids, phenolic compounds, and vitamins that exhibit antioxidant activity. To evaluate the antioxidant

activity of *Aloe vera* extract using standard in-vitro antioxidant assays. (Barkat, 2017) [3]

2.3.6 Free Radical Scavenging Activity (DPPH Assay)

The free radical scavenging activity of the sample was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, which is based on the ability of antioxidants to neutralize stable free radicals such as DPPH, superoxide, and hydroxyl radicals. Upon scavenging, the deep violet color of DPPH is reduced to yellow, and this change is quantitatively measured as a decrease in absorbance at 517 nm using a UV-Visible spectrophotometer. A 0.1 mM DPPH solution was prepared by dissolving DPPH in methanol (3.9 mg in 100 mL), and only freshly prepared solution protected from light was used. For the assay, 3.9 mL of DPPH solution was mixed with 100 µL of the test sample at different concentrations (10-100 µg/mL). The control consisted of 3.9 mL of DPPH solution mixed with 100 µL of methanol, while methanol alone served as the blank. The reaction mixtures were incubated in the dark at 37 °C for 30 minutes, after which the absorbance was measured at 517 nm against the blank to determine the free radical scavenging activity. (Shende, 2017) [16]

Calculations

DPPH activity (%) = $(A_{control} - A_{test})/A_{control} \times 100$

where,

$A_{control}$ = Absorbance of control (DPPH + methanol)

A_{test} = Absorbance of DPPH + sample

2.3.7 Chelating Potential

The chelating potential of *Aloe vera* extracts refers to their ability to bind and neutralize metal ions, especially ferrous ions (Fe^{2+}), which can catalyze the formation of reactive oxygen species and promote oxidative damage. This activity is often studied using various solvent extracts because different solvents extract different classes of phytochemicals, which influence metal-chelating properties. This assay is known as ferrous ion chelating assay in which ferrozine forms a complex with Fe^{2+}. A 200 µl aliquot of each sample was combined with 900 µl of methanol and 100 µl of $FeCl_2.2H_2O$ (2.0 mM). The test tubes were left in the dark for 5 minutes, during which time 400 µl of ferrozine was added to start the reaction (5.0 mM). The absorbance was determined using a bio spectrophotometer at 562 nm after 10 minutes of incubation. Ascorbic acid served as the benchmark. The effective concentration (EC50), which chelates 50% of iron, is used to express the chelating power of an extract (II). The following equation was used to determine the chelating activity (percent). Aloin, barbaloin, catechin are the key metal chelators.

$$\textbf{Chelating potential \%} = \frac{\text{(Control absorbance - Sample absorbance)}}{\text{Control absorbance}} \text{ X100}$$

3. Results and Discussion

3.1 Extraction of Plant Material

The % yield of *Aloe vera* extract using different solvents are given below in the table 3.

Table 3: Percentage Yields of *Aloe vera* Extract Using Different Solvents

Solvent	Observed %age Yield
Water	18%
Ethanol	16%
Methanol	14%
Acetone	11%
Chloroform	7%
Petroleum Ether	5%
Hexane	3%
Ethyl Acetate	8%

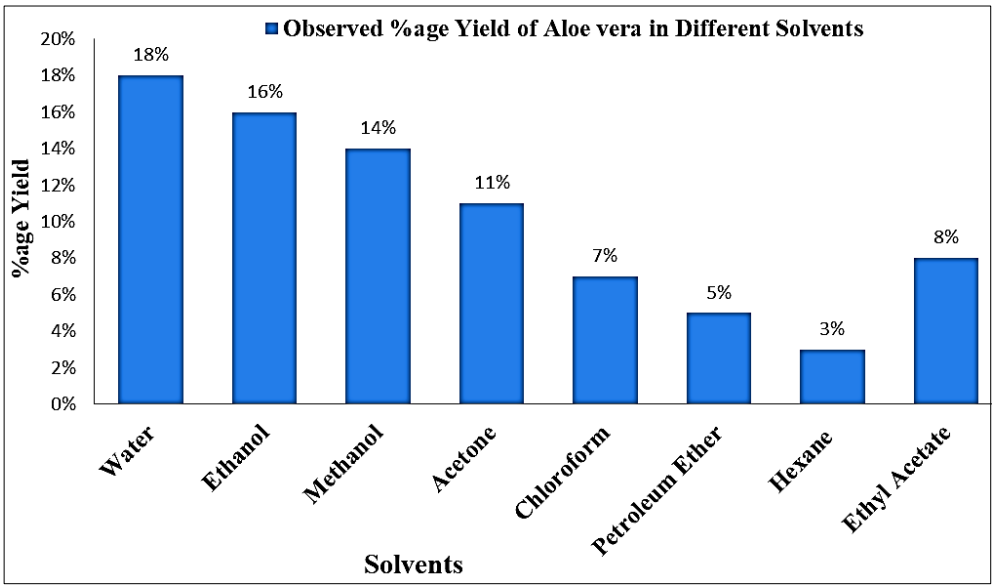

Fig 1: Graphical Bar Chart showing the % Yield of *Aloe vera* Extract Using Different Solvents.

3.2 Qualitative Analysis of *Aloe vera* Leaf Extract

Aloe vera extracts were examined phytochemically for the presence of alkaloids, flavonoids, saponin, tannin, phenol, triterpenoid, steroids, and terpenoids. The outcomes are shown in the table 4 in different solvents. Each solvent extracts different classes of phytochemicals depending on its polarity. The different sign indicates present (+), absent (-) and slightly present having low intensity (±).

Table 4: Presence of phytochemicals in *Aloe vera* Extract Using Different Solvents

Phytochemical	Water	Ethanol	Methanol	Acetone	Chloroform
Alkaloids	+	+	+	±	±
Flvonoids	+	+	+	+	-
Phenols	+	+	+	+	-
Tannins	+	+	+	±	-
Saponins	+	+	±	-	-
Glycosides	+	+	+	±	±
Anthraquinones	±	+	+	+	+
Steroids	-	+	+	+	+
Terpenoids	-	+	+	+	+
Carbohydrates	+	+	+	±	-
Proteins/amino acids	+	±	±	-	-

Aloe vera extract was tested in each type of solvents, and the results revealed that ethanol was the most effective solvent for luring bioactive chemicals. The polar solvents (Water, Ethanol, and Methanol) extract a broad range of hydrophilic compounds: alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates and proteins. The semi-polar solvents (Acetone) extract intermediate polarity compounds: flavonoids, anthraquinones and steroids.

3.3 Evaluation of Antimicrobial Assay

3.3.1 Zone of Inhibition (mm) of *Aloe vera* Extracts against Gram-positive & Gram-negative Bacteria

Ethanolic extracts generally show greater antimicrobial activity due to better solubility of active compounds. This table is showing the dose dependent effect in which the higher the concentration indicates the larger zone of inhibition. (A. Nijs, 2003) [1]

Table 5: Zone of Inhibition of Different Solvent Extract on Different Bacterial Strain with Reference Drugs

Bacterial Strain	Conc. (µg/ml)	Aqueous Extract	Ethanolic Extract	Methanolic Extract
Staphylococcus aureus (Gram +)	25	10.2 ± 0.3	17.5 ± 0.4	15.2 ± 0.5
	50	12.1 ± 0.2	22.7 ± 0.4	18.6 ± 0.4
	75	14.5 ± 0.4	24.3 ± 0.3	22.3 ± 0.3
Ciprofloxacin	5 µg/ml	33 mm (Observed zone of Inhibition)		
Ampicillin	50 µg/ml	20 mm (Observed zone of Inhibition)		
Pseudomonas aeruginosa (Gram -)	25	5.3 ± 0.2	8.9 ± 0.4	7.6 ± 0.3
	50	7.4 ± 0.3	11.9 ± 0.2	10.4 ± 0.2
	75	8.7 ± 0.2	14.8 ± 0.3	13.3 ± 0.2
Ciprofloxacin	5 µg/ml	31 mm (Observed zone of Inhibition)		
Ampicillin	50 µg/ml	19 mm (Observed zone of Inhibition)		

These antibiotics serve as positive controls to validate the test conditions. All doses are standardized by CLSI (Clinical and Laboratory Standards Institute) guidelines. The zones are measured by disc diffusion method (Kirby-Bauer) on Mueller-Hinton agar. Variations in zone size may occur based on strain susceptibility, media, incubation conditions, and disc source.

Table 6: Antimicrobial Activity of Aqueous Extract of *Aloe vera* on *Staphylococcus aureus*

Microorganism	Zone of Inhibition (mm)			
	25 µg/ml	50 µg/ml	75 µg/ml	Ciprofloxacin (5 µg/ml)
Staphylococcus aureus	10.2	12.1	14.5	28

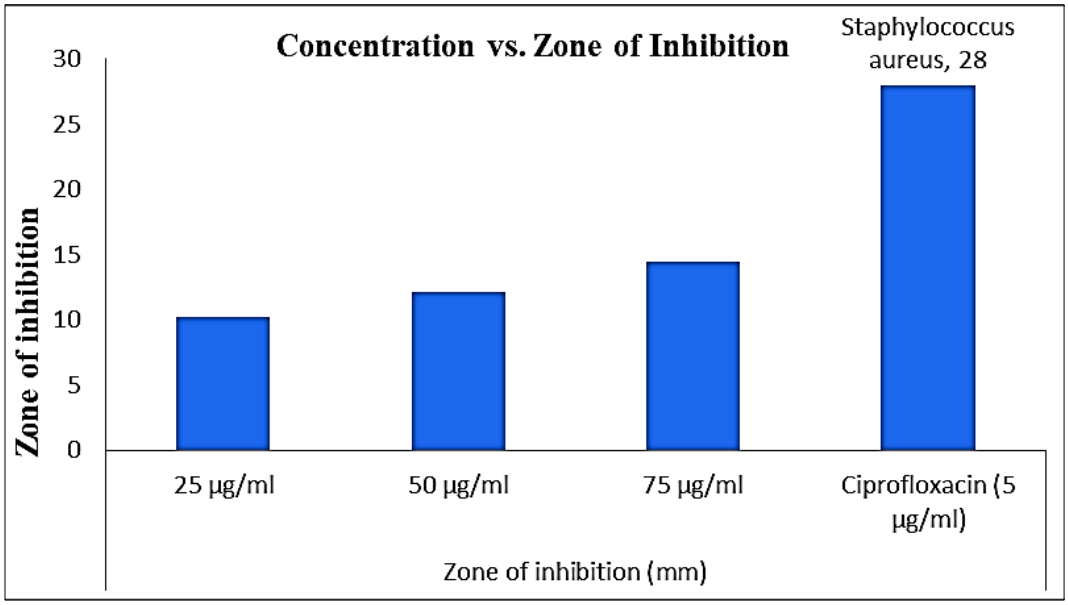

Fig 2: Zone of Inhibition of Aqueous Extract of *Aloe vera* on *S. aureus*

Table 7: Antimicrobial Activity of Aqueous Extract of *Aloe vera* on *S. aureus*

Microorganism	Zone of Inhibition (mm)			
	25 µg/ml	50 µg/ml	75 µg/ml	Ampicillin (50 µg/ml)
Staphylococcus aureus	24	25	26	20

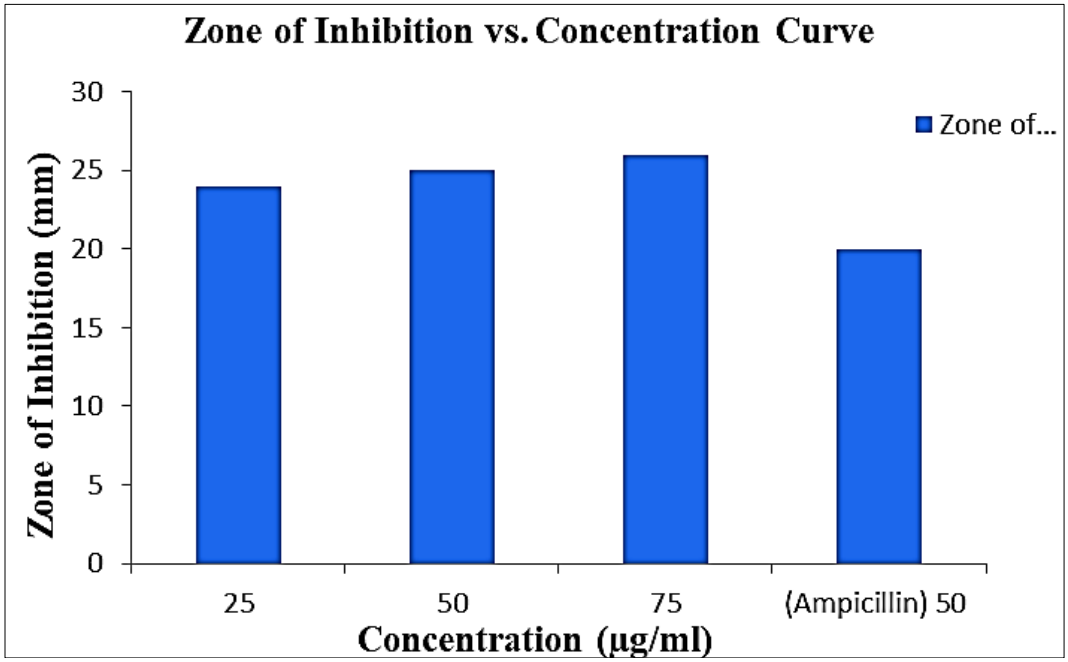

Fig 3: Zone of Inhibition of Aqueous Extract of *Aloe vera* on *S. aureus*

Table 8: Antimicrobial Activity of Ethanolic Extract of *Aloe vera* on *S. aureus*

Microorganism	Zone of inhibition (mm)			
	25 µg/ml	50 µg/ml	75 µg/ml	Ciprofloxacin (5 µg/ml)
Staphylococcus aureus	17.5	22.7	24.3	33

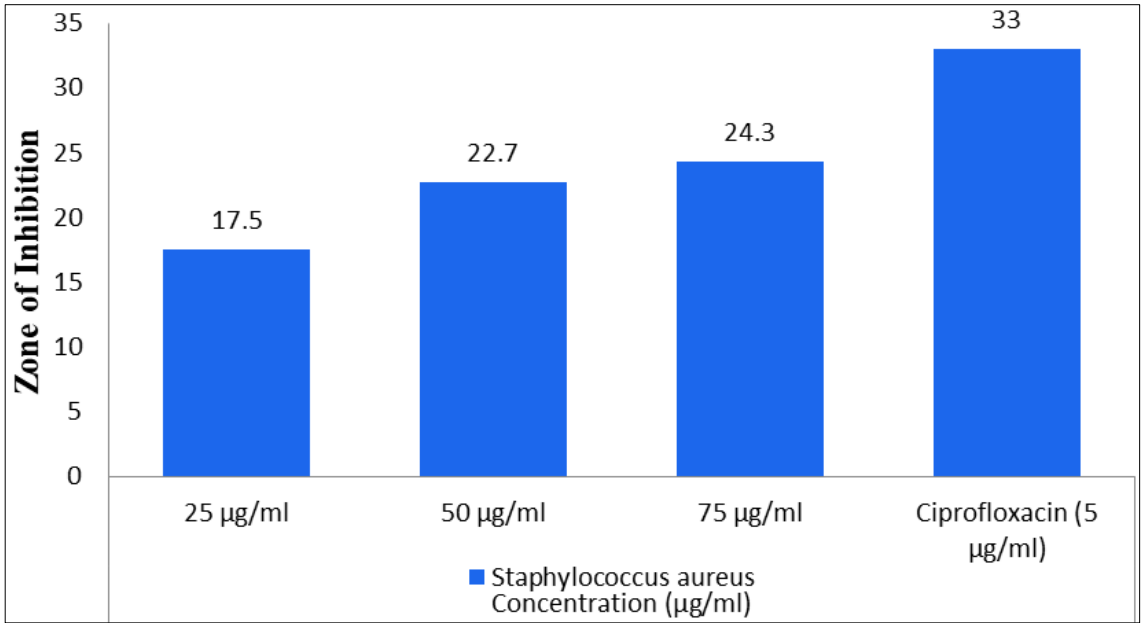

Fig 4: Zone of Inhibition of Ethanolic Extract of *Aloe vera* on *S. aureus*

Table 9: Antimicrobial Activity of Ethanolic Extract of *Aloe vera* on *S. aureus*

Microorganism	Zone of inhibition (mm)			
Staphylococcus aureus	**25 µg/ml**	**50 µg/ml**	**75 µg/ml**	**Ampicillin (50 µg/ml)**
	17.5	22.7	24.3	20

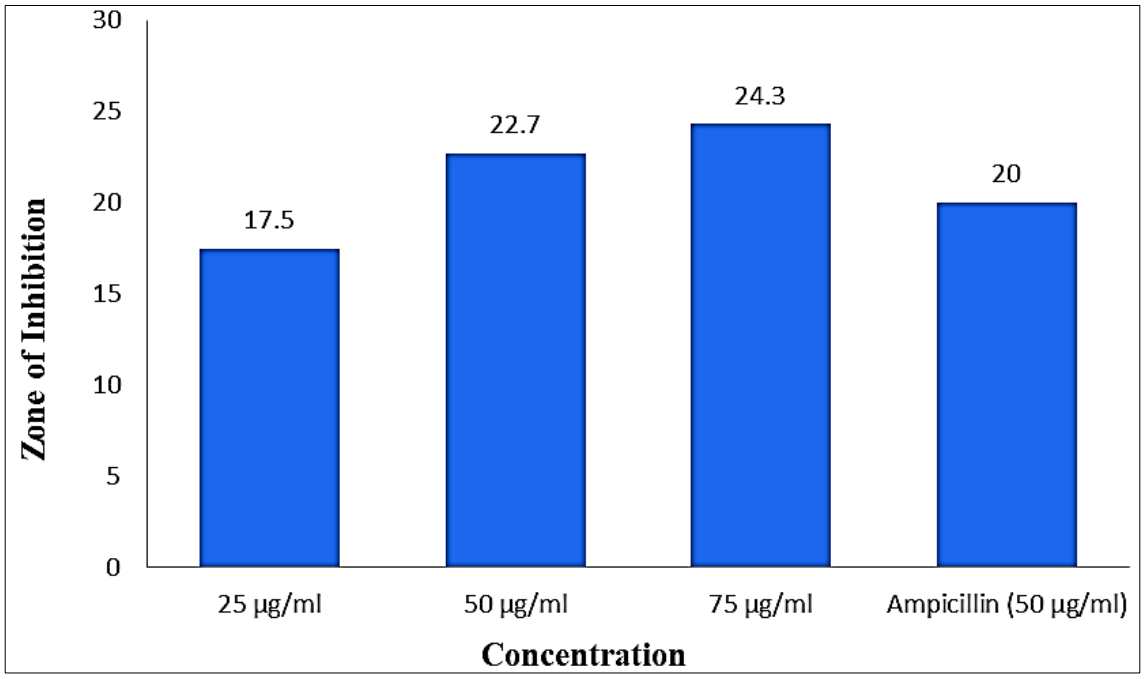

Fig 5: Zone of Inhibition of Ethanolic Extract of *Aloe vera* on *Staphylococcus aureus*

Table 10: Antimicrobial Activity of Methanolic Extract of *Aloe vera* on *S. aureus*

Microorganism	Zone of inhibition (mm)			
Staphylococcus aureus	**25 µg/ml**	**50 µg/ml**	**75 µg/ml**	**Ciprofloxacin (5 µg/ml)**
	15.2	18.6	22.3	33

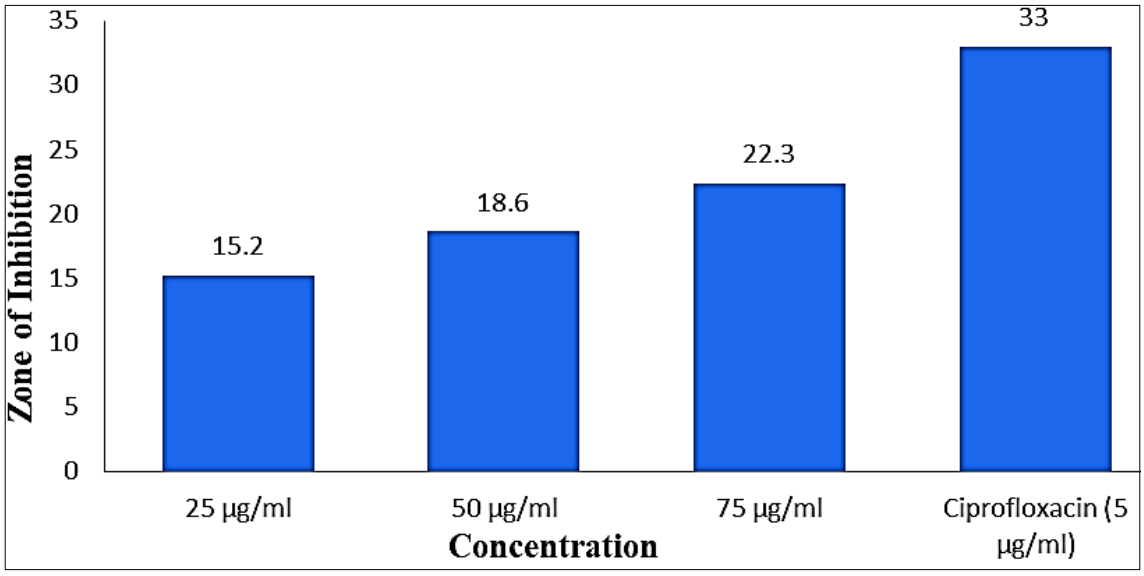

Fig 6: Zone of Inhibition of Methanolic Extract of *Aloe vera* on *S. aureus*

Table 11: Antimicrobial Activity of Methanolic Extract of *Aloe vera* on *S. aureus*

Microorganism	Zone of inhibition (mm)			
Staphylococcus aureus	**25 µg/ml**	**50 µg/ml**	**75 µg/ml**	**Ampicillin (50 µg/ml)**
	15.2	18.6	22.3	20

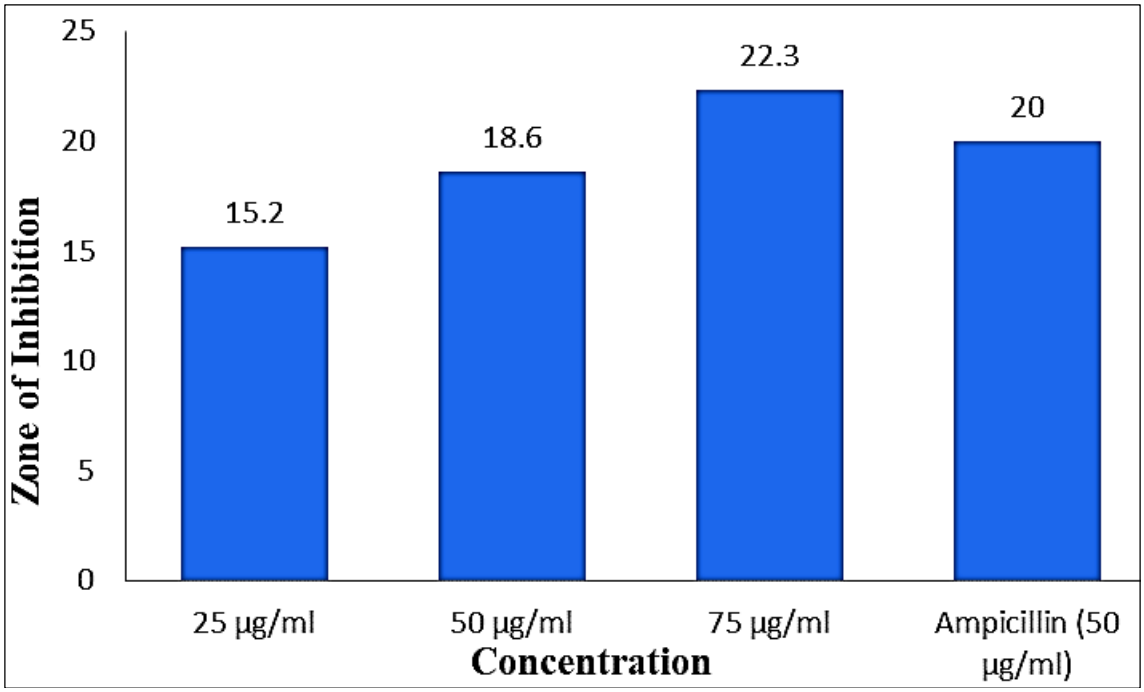

Fig 7: Zone of Inhibition of Methanolic Extract of *Aloe vera* on *S. aureus*

Table 12: Antimicrobial Activity of Aqueous Extract of *Aloe vera* on *P. aeruginosa*

Microorganism	Zone of Inhibition (mm)			
P. aeruginosa	25 µg/ml	50 µg/ml	75 µg/ml	Ciprofloxacin (5 µg/ml)
	5.3	7.4	8.7	31

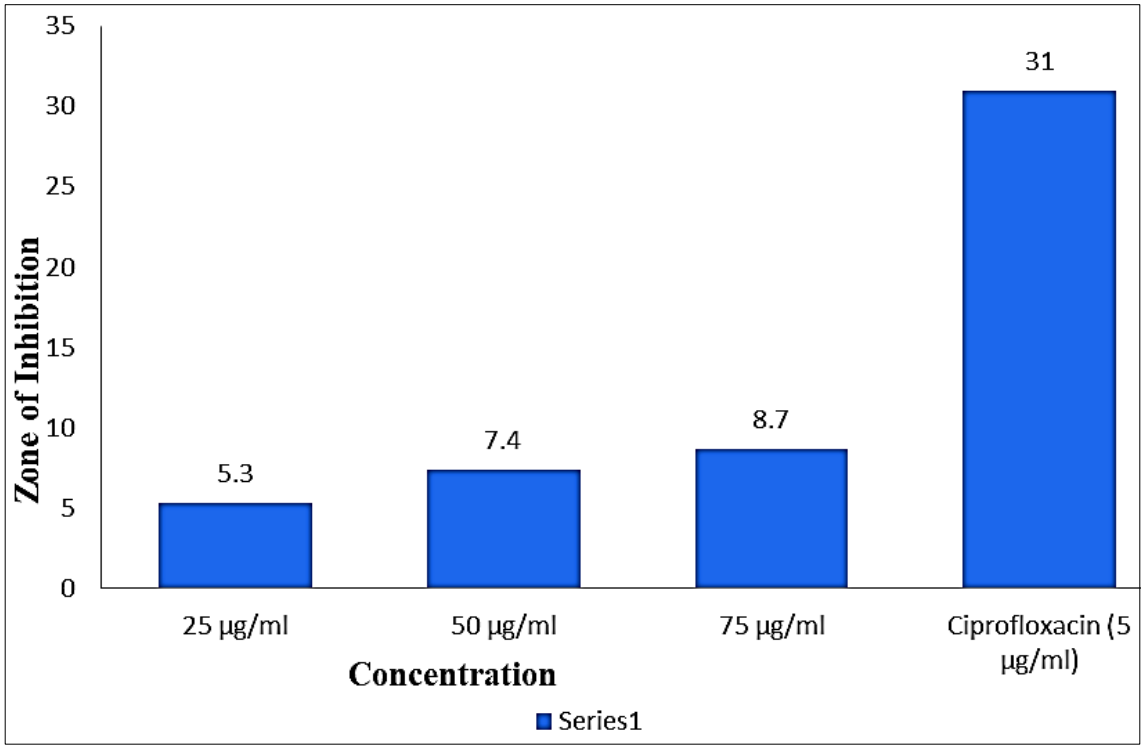

Fig 8: Zone of Inhibition of Aqueous Extract of *Aloe vera* on *P. aeruginosa*

Table 13: Antimicrobial Activity of Aqueous Extract of *Aloe vera* on *P. aeruginosa*

Microorganism	**Zone of inhibition (mm)**			
	25 µg/ml	50 µg/ml	75 µg/ml	Ampicillin (50 µg/ml)
Pseudomonas aeruginosa	5.3	7.4	8.7	19

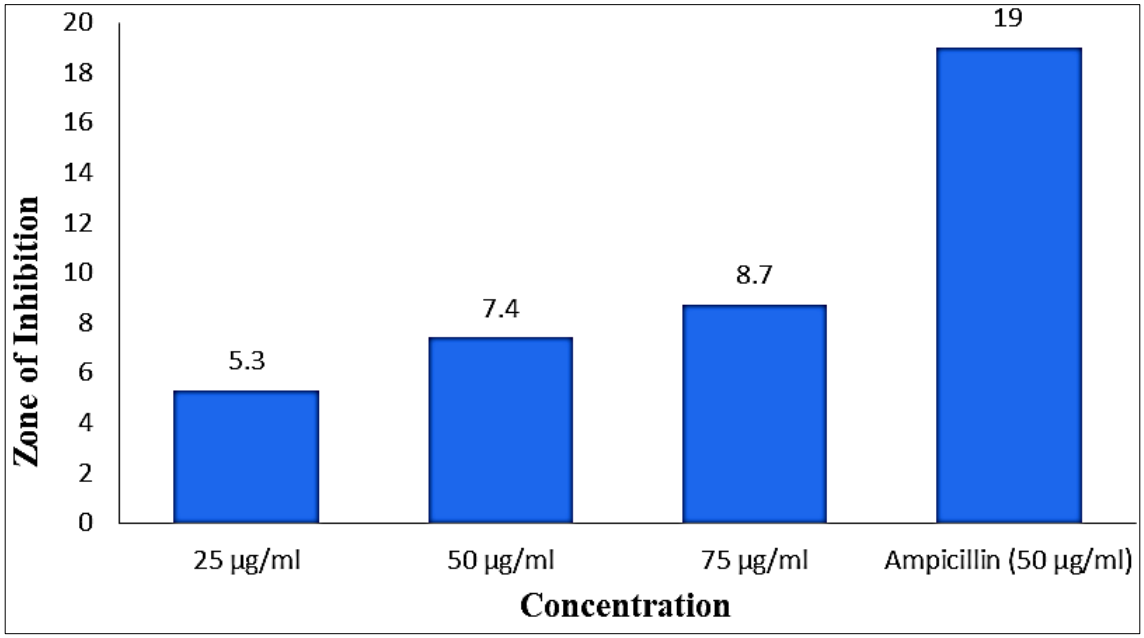

Fig 9: Zone of Inhibition of Aqueous Extract of *Aloe vera* on *P. aeruginosa*

Table 14: Antimicrobial Activity of Ethanolic Extract of *Aloe vera* on *P. aeruginosa*

Microorganism	**Zone of inhibition (mm)**			
Pseudomonas aeruginosa	25 µg/ml	50 µg/ml	75 µg/ml	Ciprofloxacin (5 µg/ml)
	8.9	11.9	14.8	31

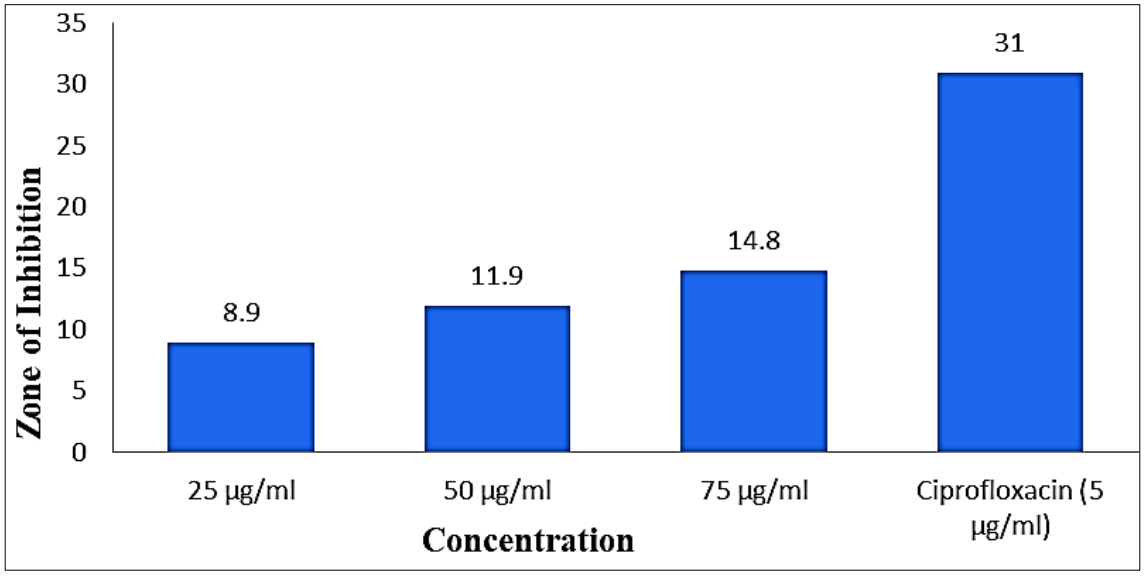

Fig 10: Zone of Inhibition of Ethanolic Extract of *Aloe vera* on *P. aeruginosa*

Table 15: Antimicrobial Activity of Ethanolic Extract of *Aloe vera* on *P. Aeruginosa*

Microorganism	Zone of inhibition (mm)			
	25 µg/ml	50 µg/ml	75 µg/ml	Ampicillin (50 µg/ml)
Pseudomonas aeruginosa	8.9	11.9	14.8	19

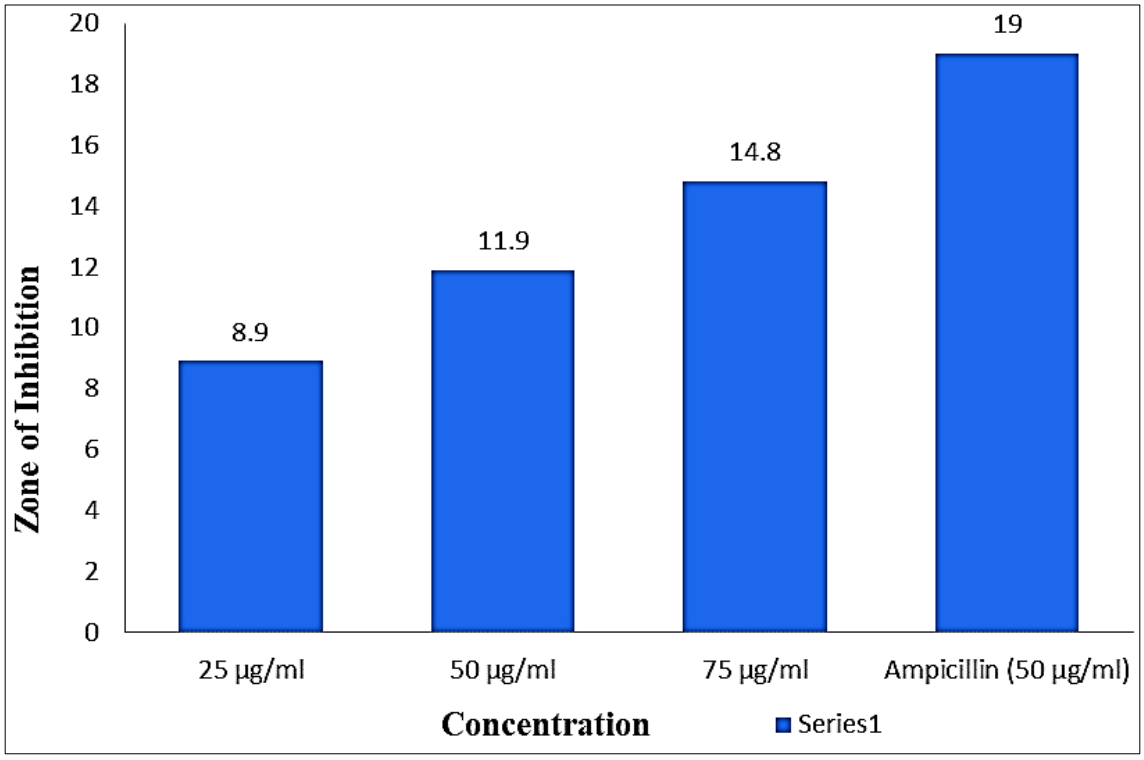

Fig 11: Zone of Inhibition of Ethanolic Extract of *Aloe vera* on Pseudomonas aeruginosa

Table 16: Antimicrobial Activity of Methanolic Extract of *Aloe vera* on Pseudomonas Aeruginosa

Microorganism	Zone of inhibition (mm)			
Pseudomonas aeruginosa	25 µg/ml	50 µg/ml	75 µg/ml	Ciprofloxacin (5 µg/ml)
	7.6	10.4	13.3	31

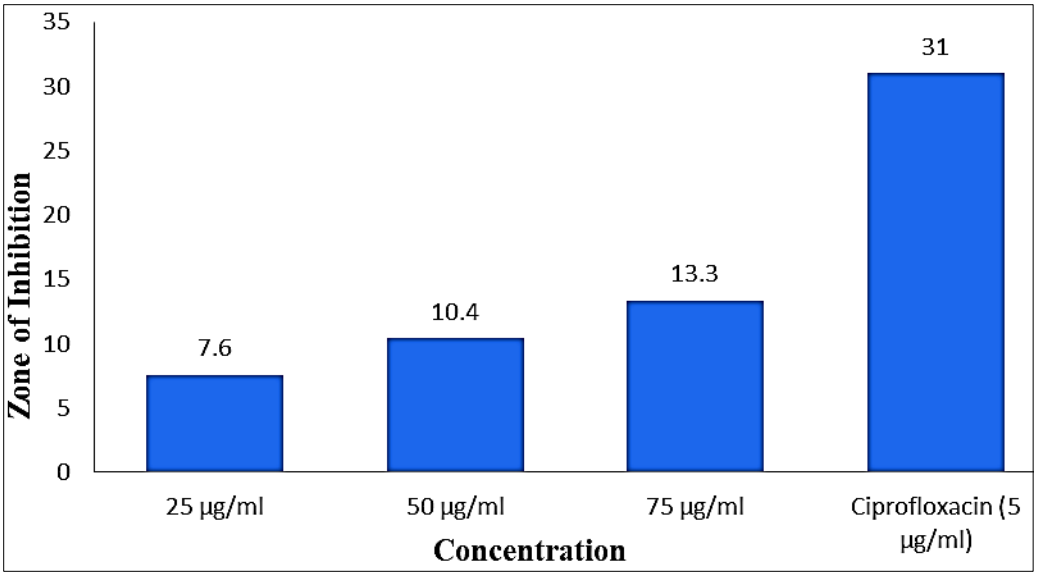

Fig 12: Zone of Inhibition of Methanolic Extract of *Aloe vera* on *P. aeruginosa*

Table 17: Antimicrobial Activity of Methanolic Extract of *Aloe vera* on *P. aeruginosa*

Microorganism	Zone of inhibition (mm)			
Pseudomonas aeruginosa	25 µg/ml	50 µg/ml	75 µg/ml	Ampicillin (50 µg/ml)
	7.6	10.4	13.3	19

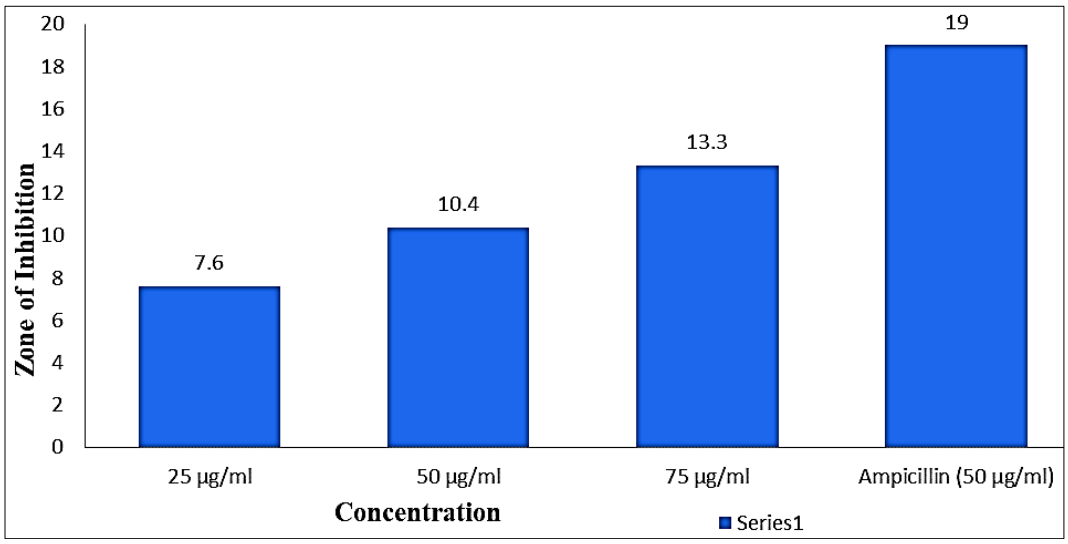

Fig 13: Zone of Inhibition of Methanolic Extract of *Aloe vera* on Pseudomonas aeruginosa

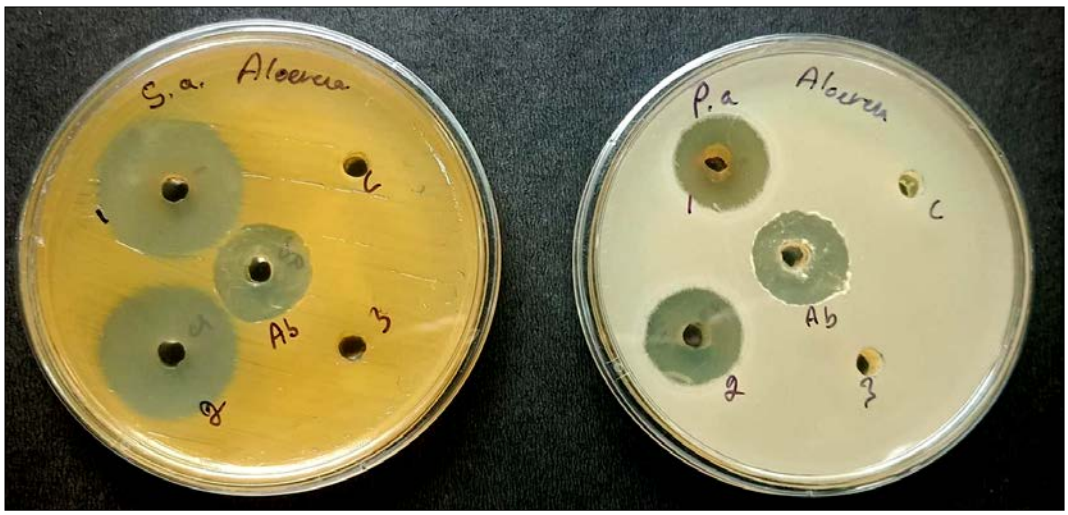

Fig 14: Petri Dishes Showing Zone of Inhibition

3.4 Evaluation of Antioxidant Activity of *Aloe vera* Extract

3.4.1 *In-vitro* DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

Table 18: *In-Vitro* Studies of Inhibition of Superoxide Radical Activity with Aqueous Extracts of *Aloe vera* at Different Concentration

Aqueous Extract of *Aloe vera*		
Concentration (µg/ml)	**Absorbance**	**%age Inhibition**
0 (Control)	0.870	0.00
20	0.805	25.65
40	0.615	42.67
60	0.350	65.23
80	0.240	72.85
100	0.125	92.54

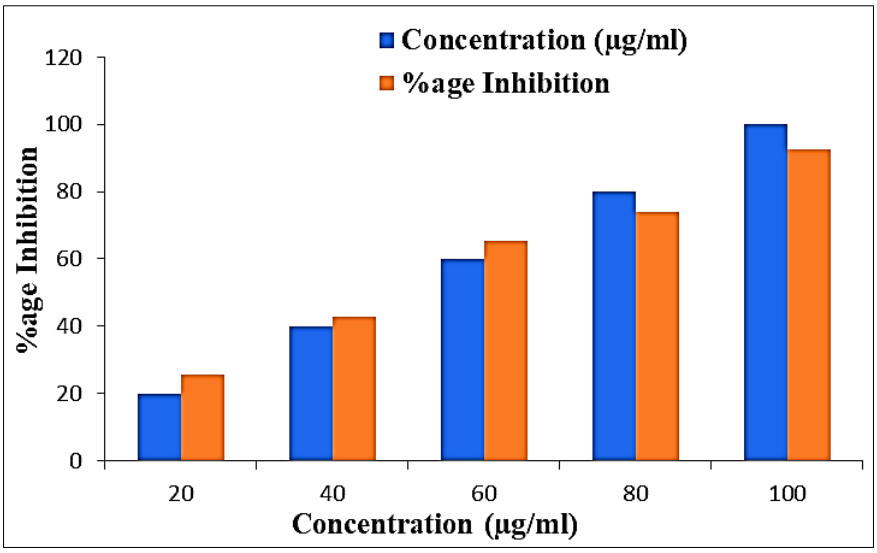

Fig 15: Superoxide Radical Activity of Aqueous Extract of *Aloe vera*

The absorbance decreases as the concentration of the extract increases, indicating higher free radical scavenging activity. The control (DPPH without extract) shows the maximum absorbance.

Table 19: In-Vitro Studies of Inhibition of Superoxide Radical Activity with Ethanolic Extracts of *Aloe vera* at Different Concentration

Ethanolic Extract of *Aloe vera*		
Concentration (µg/ml)	**Absorbance**	**%age Inhibition**
0 (Control)	0.855	0.00
20	0.775	33.64
40	0.596	48.35
60	0.323	70.89
80	0.214	77.23
100	0.101	97.67

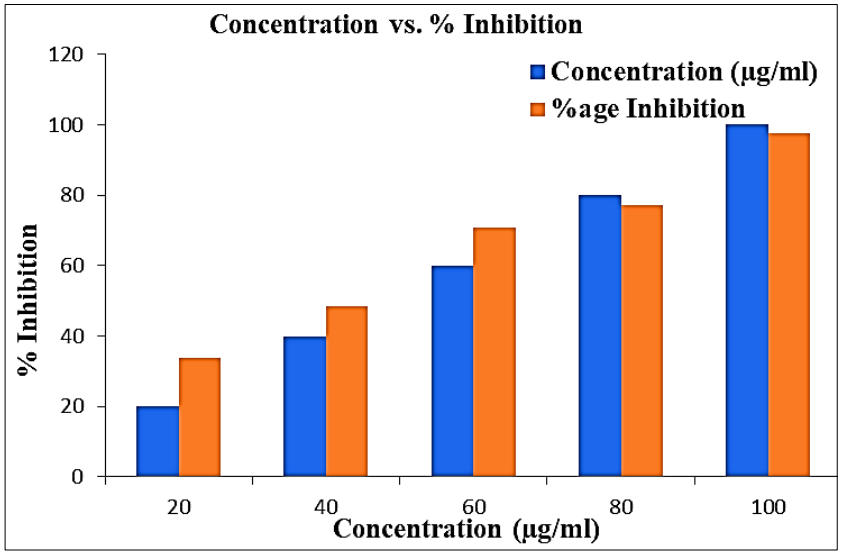

Fig 16: Superoxide Radical Activity of Ethanolic Extract of *Aloe vera*

The ethanolic extract of *Aloe vera* exhibited a concentration-dependent inhibition of superoxide radicals, with a progressive decrease in absorbance as the concentration increased. Maximum scavenging activity (97.67%) was observed at 100 µg/mL, indicating strong antioxidant potential of the extract.

Table 20: *In-Vitro* Studies of Inhibition of Superoxide Radical Activity with Methanolic Extracts of *Aloe vera* at Different Concentration

Methanolic Extract of *Aloe vera*		
Concentration (µg/ml)	**Absorbance**	**%age Inhibition**
0 (Control)	0.883	0.00
20	0.782	29.50
40	0.608	44.23
60	0.335	66.45
80	0.225	74.23
100	0.111	94.69

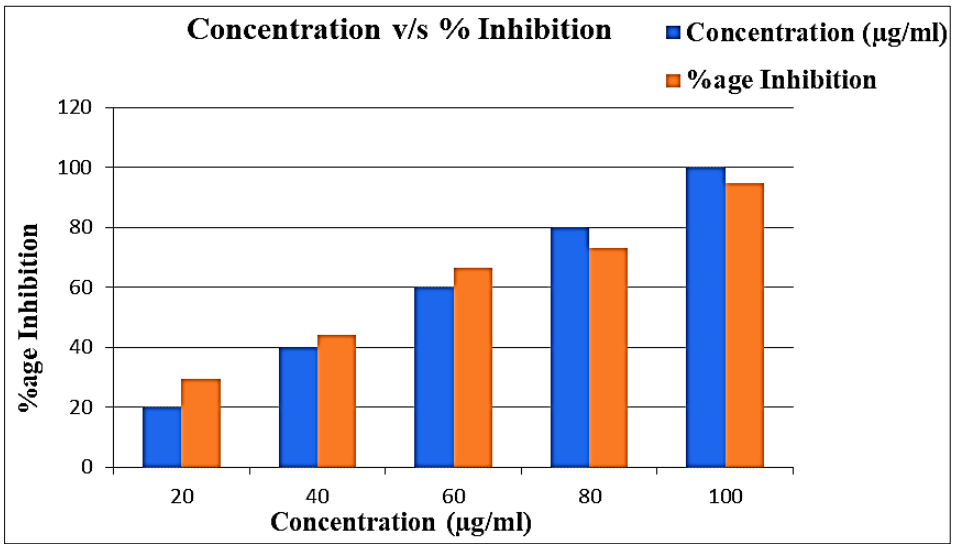

Fig 17: Superoxide radical activity of Methanolic extract of *Aloe vera*

The methanolic extract of *Aloe vera* showed a clear concentration-dependent superoxide radical scavenging activity, evidenced by a gradual reduction in absorbance with increasing concentration. The highest inhibition (94.69%) was observed at 100 µg/mL, demonstrating significant antioxidant activity of the extract.

Table 21: Comparison of Superoxide Radical Activity with Aqueous, Ethanolic and Methanolic Extracts of *Aloe vera* at Different Concentration

%age Inhibition of Superoxide Radical by Aqueous, Ethanolic and Methanolic Extract of *Aloe vera*					
Extract	**100 (µg/ml)**	**80 (µg/ml)**	**60 (µg/ml)**	**40 (µg/ml)**	**20 (µg/ml)**
Aqueous	92.54	72.85	65.23	42.67	25.65
Ethanolic	97.67	77.23	70.89	48.35	33.64
Methanolic	94.69	74.23	66.45	44.23	29.50

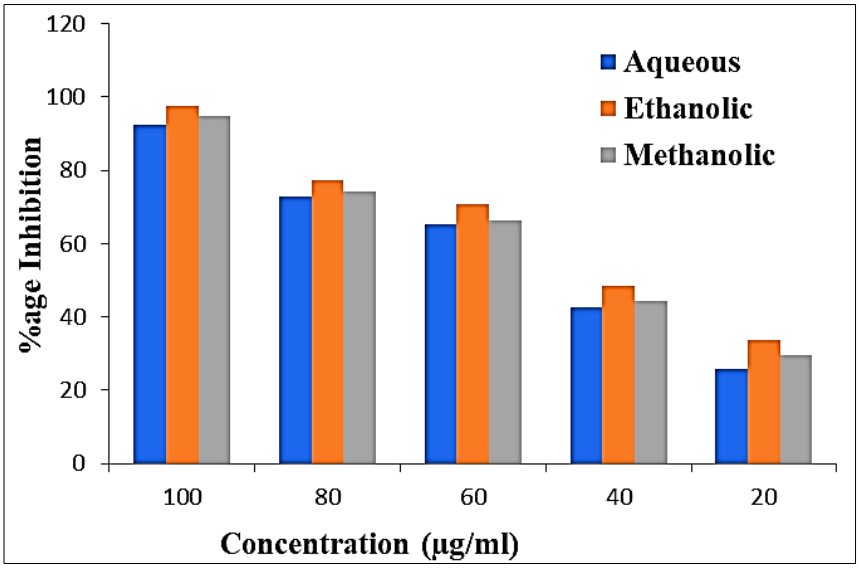

Fig 18: Comparison of Superoxide Radical Activity of Aqueous, Ethanolic and Methanolic Extract of *Aloe vera*

Figure 18 displays the superoxide radical activity of the *Aloe vera* extracts in water, ethanol and methanol. The aqueous, ethanol and methanol all had superoxide radical activities of 92.54%, 97.67% and 94.69% respectively, while the *Aloe vera* ethanolic extract had the maximum superoxide radical activity at a concentration of 100 µg/ml (97.67% RSA).

3.4.2 Chelating Potential Activity of *Aloe vera* Extract

The chelating potential of *Aloe vera* extracts in different solvents, expressed as EC_{50} (Effective Concentration) values in µg/ml which represent their concentration of extract required to chelate 50% of ferrous ions (Fe^{2+}).

(**Lower EC_{50} Value = Higher Chelating Potential**)

Table 22: Chelating Potential Activity of *Aloe vera* Extract

Solvent Used	Chelating Potential (EC_{50}, µg/ml)	Chelating Efficiency
Methanol	62.6	High
Ethanol	67.2	High
Acetone	85.7	Moderate
Aqueous	99.6	Low-moderate
Chloroform	126.9	Low
Petroleum ether	151.8	Very low
Ascorbic acid	18.1	Reference Standard

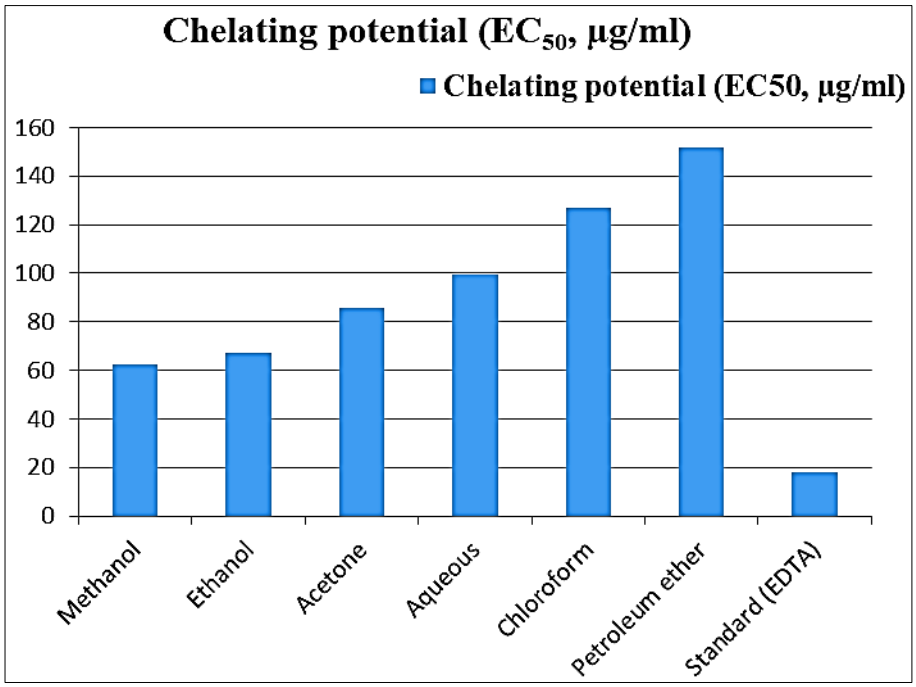

Fig 19: Chelating Potential Activity of *Aloe vera* Extract in Different Solvents

Methanolic and ethanolic extracts are most potent chelators due to high flavonoid and polyphenol content. Aqueous extracts are less potent but still show decent activity. Non-polar solvents like petroleum ether extract fewer active chelators.

4. Conclusion

The present investigation clearly demonstrates that *Aloe vera* leaf extracts possess significant antioxidant and antimicrobial activities, which are strongly influenced by the type of solvent used for extraction. Phytochemical screening confirmed the presence of multiple bioactive constituents, particularly in polar solvent extracts such as ethanol and methanol. Among all extracts tested, the ethanolic extract exhibited the highest antimicrobial activity, producing prominent zones of inhibition against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while methanolic extract also showed considerable efficacy. Antioxidant studies revealed a concentration-dependent increase in free radical scavenging activity, with ethanolic extract showing maximum superoxide radical inhibition (97.67%) at 100 µg/mL. Additionally, methanolic and ethanolic extracts demonstrated strong ferrous ion chelating potential, indicating their ability to prevent metal-induced oxidative damage. Comparatively, aqueous and non-polar solvent extracts exhibited lower biological activity. Overall, the findings validate the traditional use of *Aloe vera* as a natural antioxidant and antimicrobial agent and highlight its potential application in pharmaceutical formulations, topical preparations, and natural therapeutic products. Further studies involving isolation of active compounds, mechanistic evaluation, and in-vivo validation are recommended to support its clinical relevance and drug development potential.

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