"ANTIBACTERIAL ACTIVITY OF ALOEVERA PLANT EXTRACT AGAINST *STAPHYLOCOCUS AUREUS*

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ABSTRACT :

The present study aimed to evaluate the antibacterial activity of Dimethyl Sulfoxide (DMSO) extract of Aloe vera (Aloe barbadensis) leaf gel against selected bacterial pathogens . The antibacterial potential of the DMSO extract of Aloe vera leaf gel (DAE) was tested against standard strains of Staphylococcus aureus using the disc diffusion method at concentrations of 100, 200, 300, 400, and 500 µg/ml. The results showed a dose-dependent inhibitory effect against tested organisms. The zones of inhibition (ZOI) for S. aureus were 8 mm, 13 mm, 15 mm, 16 mm, and 21 mm, respectively, as concentrations increased. The minimum inhibitory concentration (MIC) values were found to be 300 µg/ml for S. aureus . The findings demonstrate that the DMSO extract of Aloe vera possesses significant antibacterial properties, particularly against Staphylococcus aureus. These results suggest that Aloe vera leaf gel contains bioactive compounds with promising antibacterial potential and warrant further phytochemical and pharmacological investigations to isolate and identify the active constituents responsible for the observed effects.

Keywords: Aloe vera, DMSO extract, Staphylococcus aureus, antibacterial activity, minimum inhibitory concentration (MIC).

INTRODUCTION

Microorganisms such as bacteria, fungi, and viruses are among the leading causes of infectious diseases worldwide. The increasing prevalence of antimicrobial resistance (AMR) among pathogenic bacteria has become a serious global health concern, limiting the effectiveness of conventional antibiotics. Staphylococcus aureus, in particular, is a significant pathogen responsible for various infections ranging from minor skin lesions to life-threatening systemic diseases such as pneumonia, endocarditis, and septicemia. The emergence of multidrug-resistant strains, including Methicillin-resistant Staphylococcus aureus (MRSA), underscores the urgent need for new, effective, and naturally derived antimicrobial agents.

Medicinal plants have been used for centuries in traditional medicine to treat various diseases due to their diverse bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant properties. Among them, Aloe vera (Aloe barbadensis Miller), a member of the family Liliaceae, has received considerable attention. The plant is a succulent herb characterized by thick, fleshy leaves containing a clear mucilaginous gel rich in biologically active substances such as anthraquinones, flavonoids, tannins, saponins, and polysaccharides. These compounds have been reported to exhibit multiple pharmacological activities, including wound healing, anti-inflammatory, immunomodulatory, and antimicrobial effects.

Several studies have demonstrated the antibacterial potential of Aloe vera extracts against both Gram-positive and Gram-negative bacteria. The Dimethyl Sulfoxide (DMSO) extract of Aloe vera leaf gel, in particular, has shown promising results in inhibiting bacterial growth due to the solvent's ability to dissolve both polar and non-polar bioactive compounds effectively. Haque et al. (2020) reported that DMSO extracts of Aloe vera exhibited dose-dependent inhibitory effects against Staphylococcus aureus, Pseudomonas aeruginosa,

Escherichia coli, and Klebsiella pneumoniae, with zones of inhibition ranging from 8 mm to 21 mm and a minimum inhibitory concentration (MIC) of 300 µg/ml against S. aureus.

The mechanism of antibacterial activity of Aloe vera is believed to involve the action of anthraquinones such as aloin and emodin, which are structural analogs of tetracycline. These compounds inhibit bacterial protein synthesis by blocking the ribosomal A-site. Additionally, the polysaccharides present in Aloe vera may enhance the immune response by stimulating phagocytic activity against invading pathogens.

Considering the growing threat of antibiotic resistance, exploring natural plant-based antimicrobial agents represents an important step toward discovering safer and more sustainable alternatives to synthetic antibiotics. Therefore, this study focuses on evaluating the antibacterial effect of DMSO extract of Aloe vera leaf gel against Staphylococcus aureus using the disc diffusion and broth dilution methods. The findings of this research may contribute to identifying potential bioactive components of Aloe vera that could be developed into effective antimicrobial agents.

Staphylococcus aureus is one of the most common and clinically significant bacterial pathogens responsible for a wide range of infections in humans. These include minor skin infections, wound abscesses, food poisoning, pneumonia, osteomyelitis, endocarditis, and septicemia. It is a Gram-positive, catalase-positive, and coagulase-positive coccus, often found as part of the normal flora of the skin and nasal mucosa. However, under favorable conditions, it can act as an opportunistic pathogen. The increasing emergence of antibiotic-resistant strains, particularly Methicillin-resistant Staphylococcus aureus (MRSA), has become a major public health concern globally, posing serious challenges to the management and treatment of staphylococcal infections.

The overuse and misuse of conventional antibiotics have accelerated the development of resistance, rendering many synthetic drugs ineffective. Consequently, there is an urgent need to identify novel, safe, and effective natural antimicrobial agents that can either replace or complement conventional antibiotics. In this context, medicinal plants have received growing attention as valuable sources of bioactive compounds with potential antibacterial properties.

Among the numerous medicinal plants studied for their antimicrobial activity, Aloe vera (Aloe barbadensis Miller) is particularly well known for its diverse pharmacological properties. The gel extracted from Aloe vera leaves contains a variety of active constituents such as anthraquinones (aloin, emodin), saponins, flavonoids, tannins, and polysaccharides, which have been reported to exhibit antimicrobial, anti-inflammatory, antioxidant, and wound-healing effects. These compounds are believed to disrupt bacterial cell walls, inhibit protein synthesis, and interfere with nucleic acid metabolism.

Recent studies have explored different solvent extracts of Aloe vera gel — including Staphylococcus aureus is one of the most common and clinically significant bacterial pathogens responsible for a wide range of infections in humans. These include minor skin infections, wound abscesses, food poisoning, pneumonia, osteomyelitis, endocarditis, and septicemia. It is a Gram-positive, catalase-positive, and coagulase-positive coccus, often found as part of the normal flora of the skin and nasal mucosa. However, under favorable conditions, it can act as an opportunistic pathogen. The increasing emergence of antibiotic-resistant strains, particularly Methicillin-resistant Staphylococcus aureus (MRSA), has become a major public health concern globally, posing serious challenges to the management and treatment of staphylococcal infections.

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Recent studies have explored different solvent extracts of Aloe vera gel — including aqueous, ethanol, methanol, and Dimethyl Sulfoxide (DMSO) extracts — to determine their effectiveness against bacterial pathogens. Among them, DMSO extracts have shown superior activity because DMSO effectively dissolves both polar and non-polar phytochemicals, enhancing the extract's antibacterial potential. According to Haque et al. (2020), the DMSO extract of Aloe vera leaf gel exhibited dose-dependent antibacterial activity against Staphylococcus aureus, with zones of inhibition ranging from 8 mm to 21 mm at increasing concentrations (100–500 µg/ml). The minimum inhibitory concentration (MIC) for S. aureus was recorded as 300 µg/ml, indicating that Aloe vera possesses significant inhibitory potential against this pathogen.

The antibacterial mechanism of Aloe vera against S. aureus is thought to involve anthraquinones such as aloin and emodin, which act similarly to tetracycline by interfering with bacterial protein synthesis. In addition, the saponins and phenolic compounds in the gel may disrupt the bacterial cell membrane, leading to leakage of cellular contents and eventual cell death. These findings highlight the potential of Aloe vera as a natural alternative or adjunct therapy in combating antibiotic-resistant S. aureus infections.

Therefore, the present study aims to evaluate the antibacterial effect of the DMSO extract of Aloe vera leaf gel against Staphylococcus aureus through antibiotic sensitivity testing using the disc diffusion and broth dilution methods. The study's outcomes may contribute to developing plant-based antibacterial formulations and provide a scientific basis for the therapeutic use of Aloe vera against staphylococcal infections.

OBJECTIVES

General Objective:

To evaluate the antibacterial activity of Dimethyl Sulfoxide (DMSO) extract of Aloe vera (Aloe barbadensis) leaf gel against Staphylococcus aureus using standard laboratory methods.

Specific Objectives:

1. To prepare the DMSO extract of Aloe vera leaf gel and determine its yield.

2. To assess the antibacterial activity of the DMSO extract of Aloe vera against Staphylococcus aureus using the disc diffusion method.

3. Culture wound specimen to isolate the organism which cause infection ,perform biochemical test to identify the type of bacterial strain .(staphylococcus aureus).

4. To measure the zone of inhibition (ZOI) at different concentrations of the extract (100, 200, 300, 400, and 500 µg/ml).

5. To compare the antibacterial effectiveness of Aloe vera extract with standard antibiotic controls.

6. To evaluate the potential of Aloe vera DMSO extract as a natural antibacterial agent for future therapeutic applications against Staphylococcus aureus.

REVIEW OF LITERATURE

(Habeeb et al., 2007)., (Hamman, 2008), Haque et al. (2020), Hussaini et al. (2020), Waithaka et al. (2018), Kupnik et al. (2021), Akinyemi et al. (2014)

Review of Literature

1. Introduction
The increasing prevalence of antibiotic-resistant bacteria such as Staphylococcus aureus has led to an urgent search for effective, natural antimicrobial agents. Among various medicinal plants, Aloe vera (Aloe barbadensis Miller), belonging to the Liliaceae family, has gained scientific attention for its antibacterial, antifungal, anti-inflammatory, and wound-healing properties. Its leaf gel contains several bioactive compounds including anthraquinones, saponins, flavonoids, tannins, and polysaccharides, which contribute to its antimicrobial effects (Hamman, 2008; Habeeb et al., 2007).
Staphylococcus aureus is a Gram-positive, catalase- and coagulase-positive bacterium responsible for a wide range of infections, from minor skin infections to life-threatening conditions such as pneumonia and septicemia. The rise of Methicillin-resistant Staphylococcus aureus (MRSA) has made it essential to explore alternative antibacterial sources such as plant-derived compounds.

2. Studies on the Antibacterial Activity of Aloe vera
Numerous studies have evaluated the antibacterial effects of Aloe vera extracts against S. aureus and other pathogenic bacteria using different solvents.

Haque et al. (2020) conducted a significant study on the Dimethyl Sulfoxide (DMSO) extract of Aloe vera leaf gel, testing its effect on S. aureus, E. coli, P. aeruginosa, and K. pneumoniae. The results showed dose-dependent inhibition zones (8–21 mm) against S. aureus and a minimum inhibitory concentration (MIC) of 300 µg/ml, demonstrating substantial antibacterial activity. The authors attributed this effect to the presence of anthraquinones and flavonoids in the DMSO extract, which disrupt bacterial cell walls and inhibit protein synthesis.

Similarly, Hussaini et al. (2020) reported that Aloe vera gel showed significant inhibitory activity against multidrug-resistant S. aureus and P. aeruginosa isolated from wound infections, suggesting its potential use in managing resistant bacterial infections.

In another comparative study, Waithaka et al. (2018) evaluated the antimicrobial properties of three Aloe species (A. vera, A. volkensii, and A. secundiflora) against common human pathogens. A. vera exhibited the highest antibacterial activity, particularly against S. aureus, confirming its superior pharmacological potential among Aloe species.

Kupnik et al. (2021) examined ethanol extracts of Aloe barbadensis and reported notable antibacterial effects against S. aureus and E. coli, with ethanol extracts showing higher potency compared to aqueous or methanol extracts.
Likewise, Akinyemi et al. (2014) observed that ethanol extracts of Aloe vera gel exhibited strong inhibitory zones (5–6 mm) against S. aureus and E. coli, whereas aqueous extracts were less effective. The study emphasized that the solvent used for extraction significantly affects the antibacterial activity of Aloe vera.

3. Effect of Solvent Extraction on Antibacterial Efficiency
The solvent used for extraction plays a crucial role in determining the concentration and effectiveness of the bioactive compounds extracted from Aloe vera. DMSO and ethanol are known for their ability to dissolve

both polar and non-polar phytochemicals, enhancing the extraction of anthraquinones, flavonoids, and saponins (Kumar et al., 2012).
According to Haque et al. (2020), the DMSO extract of Aloe vera was particularly potent due to DMSO's high solubility profile, resulting in a broader spectrum of active compounds. Their study showed increased antibacterial activity with higher concentrations, confirming the solvent's efficiency in releasing active phytochemicals.
In contrast, Akinyemi et al. (2014) and Waithaka et al. (2018) noted that ethanol extracts also displayed strong antibacterial activity, but their effectiveness varied depending on the bacterial strain and extraction conditions. Thus, DMSO extraction has proven advantageous in optimizing Aloe vera's antibacterial potential.

4. Mechanism of Antibacterial Action
The antibacterial activity of Aloe vera is attributed to several mechanisms:
Anthraquinones (such as aloin and emodin) inhibit bacterial protein synthesis by blocking ribosomal sites, similar to the action of tetracycline (Haque et al., 2020).
Saponins and tannins disrupt the bacterial cell wall and increase membrane permeability, leading to cell lysis.
Flavonoids and phenolic compounds act as antioxidants and interfere with bacterial enzyme systems (Kumar et al., 2012).
Polysaccharides enhance immune responses and promote phagocytic activity against bacterial cells (Hamman, 2008).
These mechanisms act synergistically, allowing Aloe vera extracts to inhibit both Gram-positive and Gram-negative bacteria.

5. Comparison with Conventional Antibiotics
Although the inhibition zones produced by Aloe vera extracts are typically smaller than those of standard antibiotics such as gentamicin and ciprofloxacin, the extracts demonstrate meaningful activity, especially against resistant bacterial strains. Aloe vera's broad-spectrum activity, combined with its natural origin and low resistance potential, highlights its promise as an alternative or adjunct antimicrobial agent (Haque et al., 2020).

6. Summary
The reviewed literature clearly indicates that Aloe vera possesses significant antibacterial activity against Staphylococcus aureus and other bacterial pathogens. Among various extraction solvents, DMSO extracts have shown the most promising results due to their enhanced solubility and higher concentration of bioactive compounds. Studies by Haque et al. (2020), Hussaini et al. (2020), and Waithaka et al. (2018) consistently demonstrate that Aloe vera gel extracts exhibit dose-dependent, broad-spectrum antibacterial activity, supporting their potential use as natural antimicrobial agents.
These findings provide a scientific basis for the continued exploration of Aloe vera in the development of plant-based antibacterial formulations.

7.Catalano et al. (2024) conducted an extensive review focusing on the phytochemistry and biological activities of Aloe vera, emphasizing recent advances in the understanding of its bioactive compounds and therapeutic potential. Their work, published in Foods, highlighted the wide range of phytochemicals such as anthraquinones, flavonoids, tannins, polysaccharides, and sterols, which contribute to the plant's antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties. The authors also noted the importance of solvent polarity in extraction efficiency and recommended standardized extraction procedures to improve reproducibility in future studies.

8.Nalimu et al. (2021) in Future Journal of Pharmaceutical Sciences provided an in-depth review on the phytochemical composition and toxicological profiles of Aloe vera and Aloe ferox. They identified key

compounds including aloin, aloe-emodin, and acemannan, which play major roles in the plant's pharmacological actions. Their review also discussed potential toxic effects of anthraquinones and stressed the need for dosage optimization and safety evaluations. This study serves as a critical reference for understanding both the therapeutic benefits and the potential risks associated with Aloe species.

9.Salehi et al. (2018) in International Journal of Molecular Sciences reviewed the Aloe genus from a multidisciplinary perspective, covering its agricultural production, processing, phytochemical analysis, and pharmacological applications. They elaborated on various extraction and analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), and spectrophotometric assays used to identify active components. Their findings reinforced that the biological activities of Aloe vera are strongly correlated with its chemical profile, and that optimization of extraction conditions is essential for consistent antibacterial potency.

10.Arbab et al. (2021) conducted a comparative experimental study to evaluate the antimicrobial action of Aloe vera extracts against bacterial isolates from skin infections. Their results, published in Veterinary Medicine and Science, demonstrated that methanolic and ethanolic extracts of Aloe vera exhibited notable antibacterial activity against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The study concluded that Aloe vera could serve as an effective natural alternative or complementary agent to conventional antibiotics, especially for topical infections.

11. Tang et al. (2025) explored the antibacterial mechanism of Aloe vera through computational and network pharmacology approaches, as reported in the Journal of Molecular Graphics and Modelling. They identified potential molecular targets including matrix metalloproteinase-9 (MMP9) and caspase-3 (CASP3) involved in bacterial stress responses. Their in silico analyses suggested that anthraquinones such as aloe-emodin and aloin may interfere with bacterial enzyme systems and signaling pathways, contributing to antimicrobial effects. This study provides a mechanistic foundation linking the phytochemical composition of Aloe vera to its antibacterial activity

MATERIALS AND METHOD

1. Plant Material
Plant used: Aloe vera (Aloe barbadensis Miller)
Part used: Fresh leaf gel
Collection site: local area in ramanathapuram district

2. Microbial Material
Test material : wound sample
Source: Department of Microbiology , Government medical college and hospital Ramanathapuram .

3. Chemicals and Reagents

Reagent / Chemical	Purpose
Dimethyl Sulfoxide (DMSO), analytical grade	Extraction solvent
Mueller–Hinton Agar (MHA)	Solid medium for disc diffusion
Distilled / deionized water	Dilution and cleaning
Gentamicin (10 µg/disc) or Vancomycin (30 µg/disc)	Positive control antibiotic
Blank sterile filter paper discs (6 mm)	Test discs for extract
Sterile saline (0.85% NaCl)	Bacterial inoculum preparation
Sterile Petri dishes, pipettes, and test tubes	Culture handling
Ethanol (70%)	Surface sterilization
Nutrient agar or Blood agar	Revival of bacterial strains

Table 01: REQUIRED CHEMICALS AND CULTURE MEDIUM

4. Laboratory Equipment
Laminar airflow cabinet / biosafety cabinet
Incubator (set at 35 ± 2 °C)
Autoclave
Analytical balance
Micropipettes and sterile tips
Vortex mixer
Centrifuge
Spectrophotometer or nephelometer (for turbidity adjustment)
Vernier caliper or ruler (for measuring inhibition zones)
Sterile cork borer (6–8 mm, for well diffusion if used)

5. Glassware and Accessories
Beakers (50 mL, 100 mL, 250 mL)
Conical flasks
Measuring cylinders

Test tubes and racks
Sterile Petri dishes
Whatman filter paper No. 1
Glass rods and funnels
Sterile droppers or syringes
6. Controls
Positive control: Gentamicin or Vancomycin discs to validate antibacterial assay
Negative control: Pure DMSO (to ensure solvent has no antibacterial effect)
Sterility control: Media without bacteria to confirm aseptic technique

7. Personal Protective Equipment (PPE)
Laboratory coat
Hand gloves
Protective goggles / face mask

8. Safety and Waste Disposal Materials
Biohazard disposal bags
Autoclavable containers
70% ethanol for disinfection
Bleaching solution (1% sodium hypochlorite) for culture deactivation.

CULTURE:

To streak a pus sample for culture, aseptically transfer a loopful of pus onto a blood agar plate,nutrient agar plate and a MacConkey agar plate , then streak each plate using the streak plate method to obtain isolated colonies. The plates are then incubated at 37°C for 24-48 hours, after which colonies are examined for morphology, Gram staining, and biochemical tests to identify the microorganism.

NUTRIENT AGAR :

After overnight or 24 hours incubation colonies are 2-4mm in diamrter ,circular,smooth,convex,opaque and easily emulsifiable. Nutrient agar plate shows golden yellow colonies ,the pigment is believed to be a carotenoid .

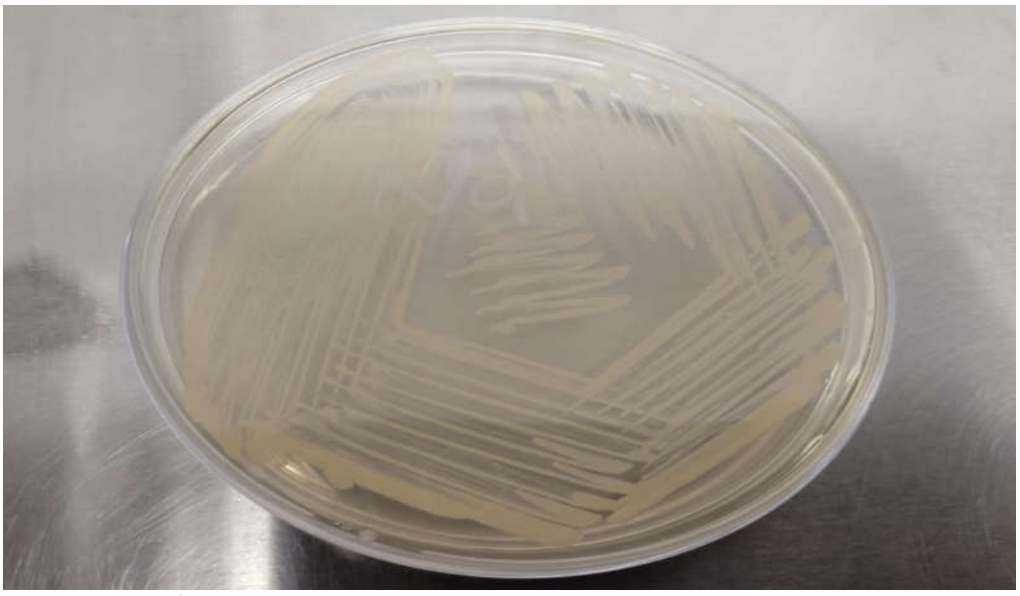

FIGURE :01 (NUTRIENT AGAR PLATE SHOWS GOLDEN YELLOW COLONIES)

BLOOD AGAR :

On blood agar,colonies are typically characterized as golden-yellow, round, and convex with a sharp border and a tendency to show beta-hemolysis, which is a zone of complete clearing around the colonies due to the lysis of red blood cells. The golden color is the origin of the species name aureus, meaning "golden," and the beta-hemolysis indicates the production of toxins that break down red blood cells.

FIGURE :02 (BLOOD AGAR PLATE SHOWS GOLDEN YELLOW BETA HEMOLYTIC COLONIES)

MACCONKEY'S AGAR :

MacConkey Agar Basics: MacConkey agar is a selective and differential medium for Gram-negative bacteria. It contains bile salts(crystal violet) to inhibit the growth of Gram-positive bacteria, including S. aureus. MacConkey's agar shows no growth. It indicates that the isolated organism is gram positive organism .

MANNITOL SALT AGAR :

Mannitol Salt Agar (MSA) appears as yellow colonies with a yellow zone due to mannitol fermentation, which produces acid and lowers the pH, changing the phenol red indicator. The high salt (7.5% NaCl) in MSA acts as a selective agent, inhibiting most other bacteria while allowing staphylococci, including S. aureus, to grow. Thus, yellow growth on MSA is indicative of S. aureus.Growth in tha MSA(Mannitol salt agar) indicates that the isolated organism is identified as the staphylococcus aureus. Staph aureus ferment the mannitol in the MSA plate and produce a yellow colour colonies due to mannitol fermentation .

FIGURE :03 (STAPHYLOCOCCUS AUREUS FERMENT MANNITOL IN MSA PLATE AND APPEAR YELLOW COLOUR COLONIES DUE TO MANNITOL FERMENTATION)

OTHER INVESTIGATIONS NEEDED TO IDENTIFY THE TYPE OF ORGANISMS TO BE ISOLATED :

GRAM STAIN :

Gram stain is widely used microbiological test that classifies bacteria in to two main groups gram positive and gram negative based on the chemical and physical properties of their cell walls.

PRINCIPLE:

Gram-positive bacteria have a thick, peptidoglycan-rich cell wall that retains the crystal violet-iodine complex, appearing purple, while Gram-negative bacteria have a thin peptidoglycan layer and outer membrane, losing the crystal violet during decolorization and taking up the red safranin counterstain.

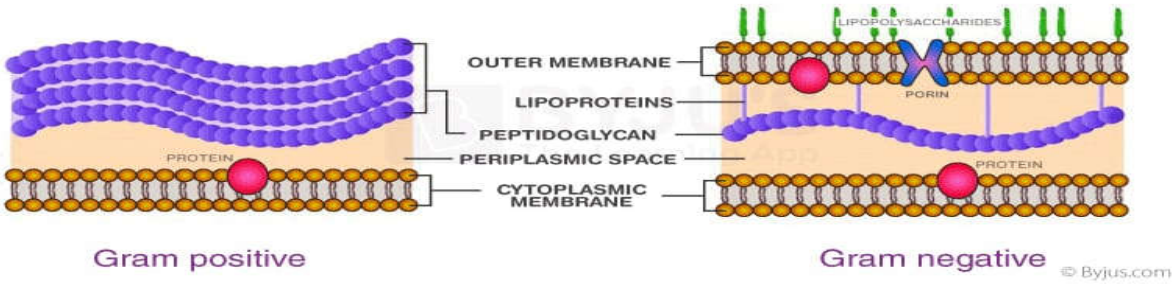

FIGURE :04 (STRUCTURE OF GRAM POSITIVE AND GRAM NEGATIVE BACTERIAL CELL WALL)

REQUIREMENT :

1. Colonies grown in culture media
2. Gram staining reagents :
   - 1.Crystal violet solution
   - 2.Gram's iodine
   - 3.Acetone
   - 4.Safranin
3. Marker pencil
4. Immersion oil
5. Microscope

PREPARATION OF SMEAR :

- Clean the slide: Start with a clean, grease-free glass microscope slide.
- Add a sterile solution: Place a drop of sterile saline or deionized water in the center of the slide.
- Collect bacteria: Using a sterile inoculating loop or needle, carefully scrape a small amount of an isolated bacterial colony from a culture plate.
- Create the smear: Swirl the loop or needle in the drop on the slide, spreading the bacteria to form a thin, even layer. The smear should be a single layer of cells.
- Air-dry the smear: Allow the slide to air-dry completely.
- Heat-fix the smear: Pass the air-dried slide through the flame of a Bunsen burner several times to heat-fix the bacterial cells to the slide. This prevents the cells from washing off during the staining procedure and helps preserve their morphology.

Allow to cool: Let the slide cool before proceeding to the staining steps of the Gram stain procedure.

METHOD :

- Fixed smear are fully covered with crystal violet solution (primary stain) for one minute.
- Pour Gram's iodine over the smear for one minute .
- Wash the smear with water .
- Decolourise the smears with acetone for 10-30 seconds taking care not to over decolourise.
- Immediately wash with water to remove the decolouriser.

- Cover the smear with a dye safranin (counter stain)for 1-2 minutes .
- Wash the smear with water .
- The smear are then blot dried .
- Examine under oil immertion (100x)objective.
-

DIFFERENTIATION ON GRAM STAINING :

Two broad group :

Gram positive: resist decolourisation and retain the colour of primary stain and appear violet colour .
Gram negative: are decolourised by acetone and,therefore,take counterstain and appear pink or red .

OBSERVATION :

Stained smear is examined using oil immersion objective under microscope,it shows violet colour cocci arranged in grape like cluster, it indicates that the organism to be isolated in the culture medium is determined as gram positive cocci .

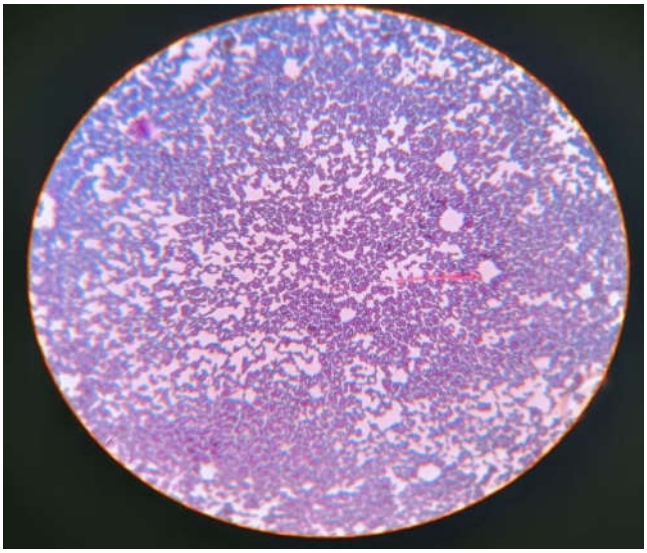

FIGURE :05 (GRAM STAIN SHOWS GRAM POSITIVE COCCI IN CLUSTER)

CATALASE TEST:

Certain bacteria have an enzyme catalase which acts ohydrogen peroxide to release oxygen.Catalase is an enzyme produced by some species of bacteria. This enzyme protects bacteria from hydrogen peroxide (H_2O_2) that can damage and kill them. Catalase will convert hydrogen peroxide into liquid water (H_2O) and oxygen gas (O_2). As a result, if catalase is very active due to an abundance of hydrogen peroxide, the rapid production of oxygen gas (O_2) will produce bubbles.

PURPOSE:

The catalase test helps differentiate between bacteria ,such as staphylococcus (catalase positive)and

streptococcus (catalase negative)

MATERIALS :

- 3%Hydrogen peroxide solution (H2O2) Solution
- Microbial culture
- Clean glass slide or tube
- Sterile inoculating loop or wooden applicator stick

METHOD:

- 1. SLIDE METHOD
- 2. TUBE METHOD

1.SLIDE METHOD:

- Prepare the Slide: Place a small drop of 3% hydrogen peroxide solution onto a clean glass slide.
- Collect the Sample: Using a sterile, cooled inoculating loop or wooden applicator stick, pick up a few bacterial colonies from an agar plate or slant.
- Mix the Sample: Emulsify the bacterial culture in the drop of hydrogen peroxide to create a smooth suspension.
- Observe for Bubbles: Immediately observe the slide for the appearance of gas bubbles.

2.TUBE METHOD :

- Take 1 ml of hydrogen peroxide in a small test tube .introduce small quantities of bacterial growth into the fluid by touching the side of the tube, observe for the gas bubbles . Positive catalase test indicates immediate and vigorous bubbling of oxygen gas indicates the presence of catalase . Negative catalase test indicates no bubble formation within 20 seconds indicates the absence of the catalase enzyme

IMPORTANT CONSIDERATION :

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

- Blood contains catalase enzyme ,culture on blood containing media may result in false positive reaction . Use of iron wireloop for picking up bacterial colonies may be another cause of false positive test .

OBSERVATION :

Catalase test performed for the organism isolated on culture medium indicates that the positive catalase test .it indicates that the organism grown in the culture medium is staphylococcus.

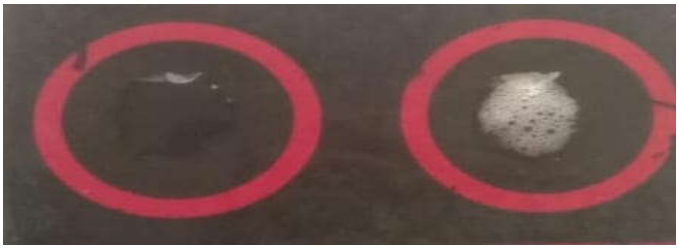

FIGURE :06 (CATALASE TEST POSITIVE)

COAGULASE TEST :

PRINCIPLE:

Staphylococcus aureus produce an enzyme coagulase .it has aproperty to clot human or rabbit plasma .
TUBE COAGULASE: It require a plasma factor (coagulase reacting factor,CRF)for its clotting action .CRF is present in rabbit and human plasma.coagulase converts fibrinogen into fibrin (principle of tube coagulase test).
SLIDE COAGULASE: Clumping factor(also called bound coagulase) is a heat stable constituent of the cell wall which reacts directly with the fibrinogen and cause clumping of cocci due to precipitation of fibrin on the cell surface(principle of slide coagulase).

PROCEDURE:

Slide coagulase test :

Take two drops of water or normal saline in two circles drawn on a clean glass slide with a wax pencil. a few colonies of bacteria to be tested are emulsified in each drop on the slide. A drop of undiluted plasma is placed in the bacterial suspension in one of the circles.put another drop of saline in the other circle as a control. Rock the slide back and forth,observing the clumping of the test suspension .

Positive reaction : clumping occurs within 10-15 seconds .

Negative reaction: no clumping .

TUBE COAGULASE TEST :

0.1ml of an agar culture suspention of the bacterium is mixed with 0.5 ml of a 1in 5 dilution of of plasma .diluted plasma alone in asecond tube serves as a control.the tubes are incubated in water bath at 37 degree Celsius for four hour . positive test appear ,the plasma clots and does not flow when the tube is inverted .if clot does not appear it is left for overnight at room temperature and is re examined .

Positive reaction: plasma clots
Negative reaction: no clot formation
NOTE : All strains showing negative slide tests must be tested with the tube coagulase test because about 5% of Staph. Aureus do not produce clumping factor .

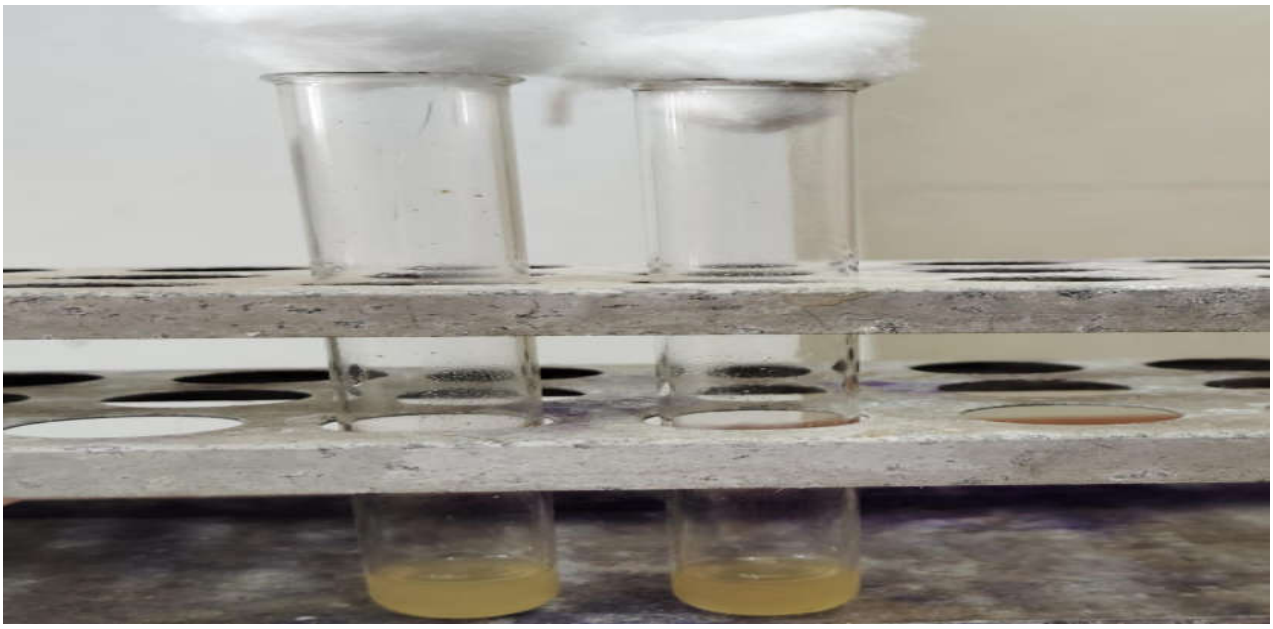

FIGURE :07 (COAGULASE TEST POSITIVE RESULT)

Preparation of DMSO Extract Powder from Aloe vera Leaf Gel:

Preparation of Aloe Vera Gel :

1. The collected leaves were thoroughly washed with running tap water followed by distilled water to remove dirt and contaminants.

2. The outer green rind was carefully removed using a sterile knife to expose the inner clear gel.

3. The gel was scooped out, cut into small pieces, and blended using a sterile blender to obtain a homogeneous gel slurry.

4. The blended gel was filtered through Whatman No. 1 filter paper to remove fibers and coarse particles.

Extraction Using DMSO (Dimethyl Sulfoxide):

1. The filtered Aloe vera gel was transferred into a clean conical flask.

2. Dimethyl Sulfoxide (DMSO) was added in a ratio of 1:5 (w/v) — i.e., 10 g of gel mixed with 50 mL of DMSO.

3. The mixture was covered with aluminum foil and kept on a mechanical shaker at 150 rpm for 48–72 hours at room temperature to allow proper extraction of bioactive compounds.

4. After extraction, the mixture was filtered again using Whatman filter paper to remove any residues.

FIGURE :08 (ALOEVERA PLANT)

FIGURE :09 (DMSO ALOEVERA EXTRACT PREPARATION)

Concentration of the Extract :

1. The filtrate (DMSO extract) was concentrated using a water bath at not more than 40–45 °C to avoid degradation of heat-sensitive phytochemicals.

2. The solvent (DMSO) was evaporated slowly until a thick, semi-solid crude extract was obtained.

Drying the Extract to Powder Form :

1. The semi-solid extract was transferred into sterile petri dishes or glass trays and air-dried at room temperature (or in a hot air oven at ≤40 °C) until a dry powder was formed.

2. The dried extract was scraped off, finely powdered using a mortar and pestle, and stored in airtight amber-colored glass containers to protect from light and moisture.

3. The powdered extract was labeled and stored at 4 °C until further use for antibacterial assays.

Preparation of DMSO stock solution:

1 gm of DMSO extract powder was dissolved in 10 ml of distilled water (D/W) to get a concentration of 0.1 gm i.e. 100 mg/ml which was labeled as stock solution-1. Again from the above solution 1 ml was taken and dissolved in 99 ml distilled water to get a concentration of 1mg/ml which was labeled as stock solution-2. From the above stock solution-2, different concentrations such as 100, 200, 300,400 and 500 µg/ml were prepared.

Calculation:

1 ml of stock solution-2 was mixed with 9 ml of D/W. So, the total amount is 10 ml.This 10 ml solution contains 1000 µg extract (as described before).So, 1 ml of this solution contains (1000÷10) µg = 100 µg.So, the final

concentration is 100 µg/ml.Similar procedure was applied to prepare working solutions having concentrations of 200, 300, 400 and 500 µg/ml. This calculation is given below in tabulated form:

Table 02: Preparation of working solutions containing DMSO Aloe vera extract (DAE)

S.NO	AMOUNT OF STOCK SOLUTION- 2(ml)	Amount of D/W (ml)	Final concentration(µg/mL)
1.	1	9	100
2.	2	8	200
3.	3	7	300
4.	4	6	400
5.	5	5	500

Phytochemical Screening of DMSO Extract of Aloe vera Leaf Gel

This section describes the qualitative phytochemical screening of the DMSO extract of Aloe vera leaf gel to identify the presence or absence of major bioactive compounds. These tests were carried out using standard chemical methods commonly applied in phytochemical analysis.

1. Preparation of Extract

1. Fresh Aloe vera leaves were washed thoroughly with distilled water and the inner gel was extracted.

2. The gel was shade-dried or oven-dried at 40–45°C until a constant weight was obtained.

3. The dried gel was powdered and soaked in Dimethyl Sulfoxide (DMSO) (1:5 w/v) for 48–72 hours with intermittent shaking.

4. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a water bath below 45°C.

5. The concentrated extract was stored in an airtight container for phytochemical analysis.

2. Qualitative Phytochemical Tests

TABLE :03(phytochemical test for aloevera extract)

S. No.	Phytochemical Group	Test Name & Procedure	Observation	Inference
1	Alkaloids	Mayer's/Wagner's Test: Add Mayer's or Wagner's reagent to 1 ml extract.	Cream/brown precipitate	Present
2	Flavonoids	Shinoda Test: Add Mg turnings and conc. HCl to extract.	Pink or red coloration	Present
3	Saponins	Froth Test: Shake extract with distilled water and observe froth formation.	Persistent froth	Present
4	Tannins	Ferric Chloride Test: Add few drops of 0.1% $FeCl_3$ to extract.	Blue-black coloration	Present
5	Phenols	Ferric Chloride Test: Add $FeCl_3$ to extract.	Blue-green coloration	Present
6	Terpenoids	Salkowski's Test: Mix extract with chloroform and add conc. H_2SO_4.	Reddish-brown interface	Present
7	Glycosides	Keller–Killiani Test: Mix extract with acetic acid containing $FeCl_3$ and conc. H_2SO_4.	Brown ring at junction	Present
8	Steroids	Liebermann–Burchard Test: Add acetic anhydride and conc. H_2SO_4 to extract.	Green or blue coloration	Present
9	Anthraquinones	Bornträger's Test: Boil extract with H_2SO_4, extract with benzene, add ammonia.	Pink/red color in ammonia layer	Present
10	Proteins	Biuret Test: Add NaOH and $CuSO_4$ to extract.	Violet coloration	Absent
11	Carbohydrates	Benedict's Test:	Brick red	Present

S. No.	Phytochemical Group	Test Name & Procedure	Observation	Inference
		Add Benedict's reagent and heat.	precipitate	
12	Resins	Acetone–Water Test: Dissolve extract in acetone, add water.	Turbidity formation	Absent

DETECTION OF ALKALOIDS:

Wagner's test:

The Wagner reagent was prepared using 1 gram of iodine and 3 grams of Potassium iodide dissolved in 50 mL distilled water. About 2-3 mL of the sample solution is mixed with a few drops of Wagner's reagent. The appearance of a reddish-brown precipitate indicates the presence of alkaloids (Wagner, H et al.,1993).

Mayer's test:

The Mayer's reagent was prepared with solution A (1.358 gm of Mercuric Chloride) and solution (5 gm of Potassium Iodide) to 100 ml. One ml of seaweed was added into the test tubes respectively and a blank was maintained with one ml of distilled water. One ml reagent was added into the test tubes and mixed thoroughly. The tubes were kept at room temperature for the result. Then it was observed for the presence of creamy white or yellow precipitate.

Picric acid test:

The Picric acid test was performed to detect the presence of alkaloids in the extract. A few ml of the filtrate were taken in a test tube, and 3–4 drops of picric acid solution were added. The solution was then mixed thoroughly and allowed to stand at room temperature for observation. The development of an orange color indicated the presence of alkaloids in the extract.

Iodine test:

The Iodine test was conducted to confirm the presence of alkaloids in the extract. Three ml of the extract solution were taken in a test tube, and a few drops of iodine solution were added. The mixture was then observed for color changes. The formation of a blue color, which disappeared upon boiling and reappeared on cooling, indicated the presence of alkaloids in the extract.

Dragendorff's test:

Add a few ml of the freshly prepared Dragendorff's reagent to the test tubes with C.bonducella seed extract and blank. A positive result is indicated by the formation of an orange or reddish- brown precipitate, suggesting the presence of alkaloids.

DETECTION OF AMINO ACIDS:

Dissolve 100 mg of the extract in 10 mL of distilled water to prepare the solution. Then, filter the solution through Whatman No. 1 filter paper to obtain a clear filtrate, removing any solid particles. The filtrate is now ready to be subjected to a test for amino acids, such as the Ninhydrin test or other appropriate methods for amino acid detection.

Ninhydrin test:

Add C.bonducella extracts into the respective tubes and maintain blank. A few drops of ninhydrin reagent from 0.1 to 1 ml were added into the tubes and heated for 15 minutes in a water bath. After heating, allow the test tube to cool to room temperature. Observe the colour of the solution. Purple or blue indicates the presence of amino acids in the sample (Yasuma. A et al.,1953).

DETECTION OF CARBOHYDRATES:

Barfoed's test:

The Barfoed's test was performed to detect the presence of carbohydrates in the extract, specifically monosaccharides. One milliliter of the filtrate was taken in a test tube, and an equal volume (1 mL) of Barfoed's reagent was added. The mixture was then heated in a boiling water bath for 2 minutes and observed for any changes. The formation of a red precipitate indicated the presence of monosaccharides, confirming the presence of carbohydrates in the extract.

Seliwanoff's test:

The Seliwanoff's test was conducted to differentiate ketose sugars from aldose sugars in the extract. One milliliter of the extract solution was taken in a test tube, and 3 mL of Seliwanoff's reagent was added. The test tube was then heated in a water bath for 1 minute and observed for color development. The appearance of a rose-red color indicated the presence of ketoses, confirming the presence of carbohydrates in the extract.

DETECTION OF REDUCING SUGARS:

Benedict's test:

Benedict's test is used to detect the presence of reducing sugars in a sample. To perform the test, 0.5 mL of the filtrate and 0.5 mL of Benedict's reagent are added together into a test tube. The mixture is then heated in a boiling water bath for 2 minutes. If reducing sugars are present,
a characteristic-colored precipitate will form. The color of the precipitate can vary from green to yellow to red, with the intensity of the color indicating the concentration of reducing sugars in the sample. Green indicates a low concentration, yellow suggests a moderate concentration, and red indicates a high concentration of reducing sugars.

Fehling's test:

The Fehling's test was performed to detect the presence of reducing sugars in the extract. One milliliter of the filtrate was taken in a test tube, and 1 mL each of Fehling's solution A and Fehling's solution B were added. The mixture was then thoroughly mixed and heated in a boiling water bath for a few minutes. The formation of a red precipitate indicated the presence of reducing sugars in the extract.

DETECTION OF PROTEINS:

Proteins in plant extracts can be detected using several biochemical tests:

Biuret test:

For the Biuret test, 2 mL of the filtrate is treated with 1 drop of 2% copper sulfate solution. Then, 1 mL of 95% ethanol is added, followed by excess potassium hydroxide (KOH) pellets. The appearance of pink color in the ethanolic layer indicates the presence of proteins, as the copper ions form a complex with the peptide bonds in proteins, resulting in a characteristic color change (Gahan et al., 1984).

DETECTION OF FLAVONOIDS:

Lead Acetate test:

The Lead Acetate test was performed to detect the presence of flavonoids in the extract. One milliliter of the extract solution was taken in a test tube, and a few drops of 10% lead acetate solution were added. The mixture was then thoroughly mixed and allowed to stand at room temperature. The formation of a yellow precipitate indicated the presence of flavonoids in the extract.

Ammonia test:

The Ammonia test was conducted to confirm the presence of flavonoids in the extract. The filtrate was taken in a test tube and treated with 5 mL of diluted ammonium solution, followed by the addition of concentrated sulfuric acid (H_2SO_4). The solution was then observed for any color changes. The appearance of a yellow color indicated the presence of flavonoids in the extract.

Concentrated sulfuric acid (H_2SO_4) test:

The Concentrated Sulfuric Acid test was carried out to detect flavonoids in the plant extract. A small amount of the extract was taken in a test tube and treated with concentrated sulfuric acid (H_2SO_4). The formation of an orange color confirmed the presence of flavonoids in the extract.

Ferric chloride test:

The Ferric Chloride test was performed to detect flavonoids in the extract. Aqueous extract solution was taken in a test tube and treated with a few drops of 10% ferric chloride solution. The mixture was then observed for color changes. The formation of a green precipitate indicated the presence of flavonoids in the extract.

DETECTION OF PHENOLS:

Iodine test:

The Iodine test was performed to detect the presence of phenols in the extract. One milliliter of the extract was taken in a test tube, and a few drops of diluted iodine solution were added. The solution was then observed for color changes. The formation of a transient red color indicated the presence of phenols in the extract.

Lead acetate test:

The Lead Acetate test was conducted to confirm the presence of phenols in the extract. The filtrate was dissolved in 5 mL of distilled water, and 3 mL of 10% lead acetate solution was added. The mixture was thoroughly mixed and observed for any precipitation. The formation of a white precipitate confirmed the presence of phenols in the extract.

Ferric chloride test:

The Ferric Chloride test was carried out to identify phenols in the extract. An aqueous solution of the extract was taken in a test tube and treated with a few drops of 5% ferric chloride solution. The mixture was observed for color changes. The formation of a dark green or bluish-black color indicated the presence of phenols in the extract.

DETECTION OF TANNINS:

Gelatin test:

The Gelatin test was performed to detect the presence of tannins in the extract. The aqueous extract was dissolved in 5 mL of distilled water, followed by the addition of 1% gelatin solution and 10% sodium chloride (NaCl). The solution was then mixed thoroughly and observed for precipitation. The formation of a white precipitate indicated the presence of tannins in the extract.

Braymer's test:

The Braymer's test was conducted to confirm the presence of tannins in the extract. One milliliter of the filtrate was taken in a test tube and mixed with 3 mL of distilled water. To this solution, three drops of 10% ferric chloride solution were added, and the mixture was observed for color changes. The appearance of a blue-green color confirmed the presence of tannins in the extract.

DETECTION OF PHLOBATANNINS:

HCl test:

The HCl test was performed to detect the presence of phlobatannins in the extract. Two milliliters of the aqueous extract were taken in a test tube and treated with an equal volume (2 mL) of 1% hydrochloric acid (HCl). The mixture was then boiled for a minute and observed for any changes. The formation of a red precipitate indicated the presence of phlobatannins in the extract.

DETECTION OF SAPONINS:

Foam test:

The Foam test was conducted to detect the presence of saponins in the extract. About 0.5 grams of the plant extract were taken in a test tube and mixed with 2 mL of distilled water. The mixture was then vigorously shaken and allowed to stand for observation. The formation of a persistent foam lasting for 10 minutes indicated the presence of saponins in the extract.

DETECTION OF PHYTOSTEROLS:

Salkowski's test:

The Salkowski's test was performed to detect the presence of phytosterols in the extract. The filtrate was treated with a few drops of concentrated sulfuric acid (H_2SO_4), then shaken well and allowed to stand undisturbed. The formation of a red color in the lower layer indicated the presence of phytosterols.

DETECTION OF QUINONES:

Concentrated HCl test:

The Concentrated HCl test was performed to detect the presence of quinones in the extract. The aqueous extract was taken in a test tube and treated with concentrated hydrochloric acid (HCl). The mixture was then observed for color changes. The formation of a green color indicated the presence of quinones.

DETECTION OF ANTHRAQUINONES:

Borntrager's test:

The Borntrager's test was performed to detect the presence of anthraquinones in the extract. A few milliliters of the filtrate were taken in a test tube and treated with 10 mL of 10% ammonia solution. The mixture was then shaken vigorously for 30 seconds and observed for color changes. The formation of a pink, violet, or red-colored solution indicated the presence of anthraquinones.

3. Notes and Precautions

- Use freshly prepared reagents for accurate results.
- Perform all tests in triplicates to ensure reproducibility.
- Handle concentrated acids with care and follow safety protocols.
- Clean all glassware thoroughly before use to prevent contamination.

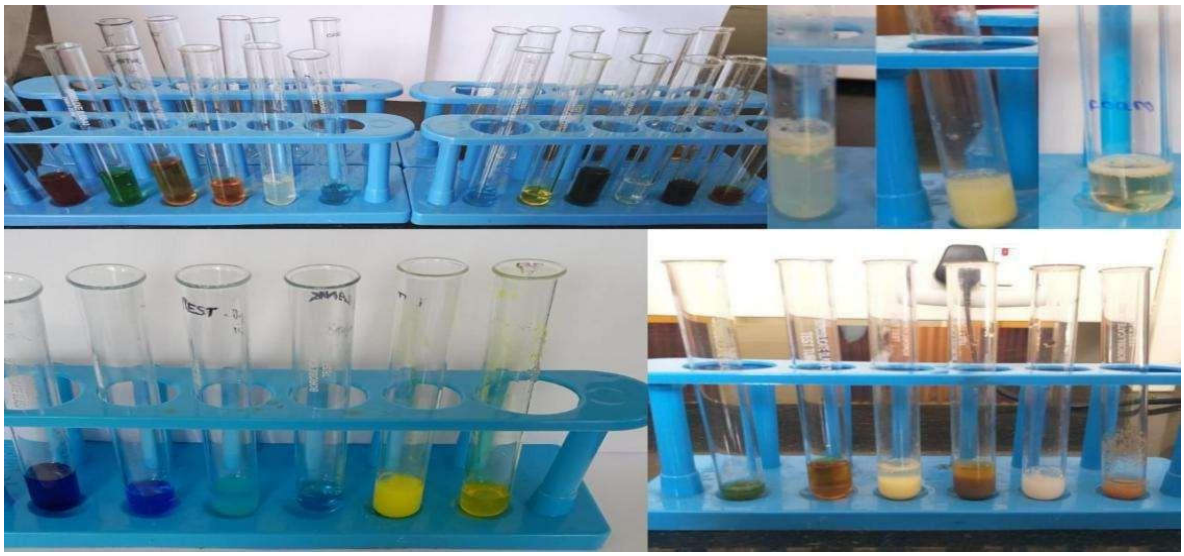

FIGURE :10 (PHYTOCHEMICAL TEST OF ALOEVERA)

RESULTS AND DISCUSSION

ANTIBACTERIAL SENSITIVITY TEST BY DISC DIFFUSION METHOD :

Antibacterial sensitivity test by disc diffu-sion method:Antibacterial sensitivity test was performed by Modified Kirby-Bauer disc diffusion technique as following. After matching with 0.5 McFar-land standards for each isolates, a sterile cotton swab was dipped into bacterial suspension and streaked in three directions on the surface of Mueller-Hinton agar plates and then left for 5-10 minutes in room tem-perature. By using sterile forceps the blank paper discs (6 mm in diameter) were placed on the surface of the plates. Then with the help of micropipette 5 µl amount of different concentrations of DAE were put over the blank discs and left for five minutes. Then the plates were incubated at 37°C for 24 hours. After that zone of inhibition for respective organisms were measured in mm by using ruler.

FIGURE :11 (ANTIBIOTIC SENSITIVITY TEST USING DMSO ALOEVERA EXTRACT AND STANDARD ANTIBIOTICS AGAINST STAPHYLOCOCCUS AUREUS SHOWS ZONE OF INHIBITION)

Standard antibiotics (Gentamicin)shows clear zone around the disc about 19mm ,aloevera extract shows dose dependent antibacterial activity ,DMSO alone is act as control (no zone) formation . A clear ring around

the disc indicates inhibition of bacterial growth.The discs with Aloe vera extract (probably on the periphery) show smaller clear zones compared to the gentamicin disc.
Some discs show partial zones or faint inhibition, suggesting lower antibacterial activity at lower concentrations.

Comparison:

Gentamicin disc: Large and clear zone → strong antibacterial activity.
Aloe vera discs: Smaller but visible zones → moderate antibacterial activity.
No inhibition: Some discs have little or no clear area → negative control (DMSO).
The study demonstrates the potential antibacterial activity of DMSO aloe vera extract against Staphylococcus aureus, a common pathogen responsible for various infections. The results suggest that the combination of aloe vera's bioactive compounds and DMSO's penetration-enhancing properties may contribute to the extract's antibacterial effects.

1. Antibacterial activity: The DMSO aloe vera extract exhibited significant antibacterial activity against Staphylococcus aureus, as evidenced by the zone of inhibition and minimum inhibitory concentration (MIC) values.

2. Dose-dependent effects: The extract's antibacterial activity was found to be dose-dependent, with higher concentrations exhibiting greater activity.

3. Potential mechanisms: The study suggests that the extract's antibacterial activity may be attributed to the synergistic effects of aloe vera's bioactive compounds, such as aloin and aloe-emodin, and DMSO's ability to enhance penetration and bioavailability.

AGAR WELL DIFFUSION METHOD :

To test the antibacterial activity of a DMSO crude extract against Staphylococcus aureus using the agar well diffusion method, prepare a sterile Mueller-Hinton agar plate inoculated with S. aureus, cut wells in the agar, fill the wells with the extract and control solutions (DMSO, positive antibiotic), and incubate. After incubation, measure the diameter of the clear inhibition zones around the wells to determine the extract's efficacy.

PROCEDURE:

Prepare the bacterial inoculum: Mix a standardized concentration of S. aureus with molten nutrient agar or broth, and spread it evenly on the surface of Mueller-Hinton agar plates to form a lawn of bacteria.
Create wells: Using a sterile borer, create wells (4-8 mm diameter) in the agar on the inoculated plates.
Add test substances: Pipette a specific volume (e.g.50,75 and100 µl) of your DMSO-dissolved crude extract into each of the wells.

ADD CONTROLS:
Fill one wells with a sterile DMSO solution to serve as a negative control.

Measure inhibition zones: After incubation, measure the clear zones around each well where bacterial growth has been inhibited. The size of the zone indicates the antibacterial activity of the extract.

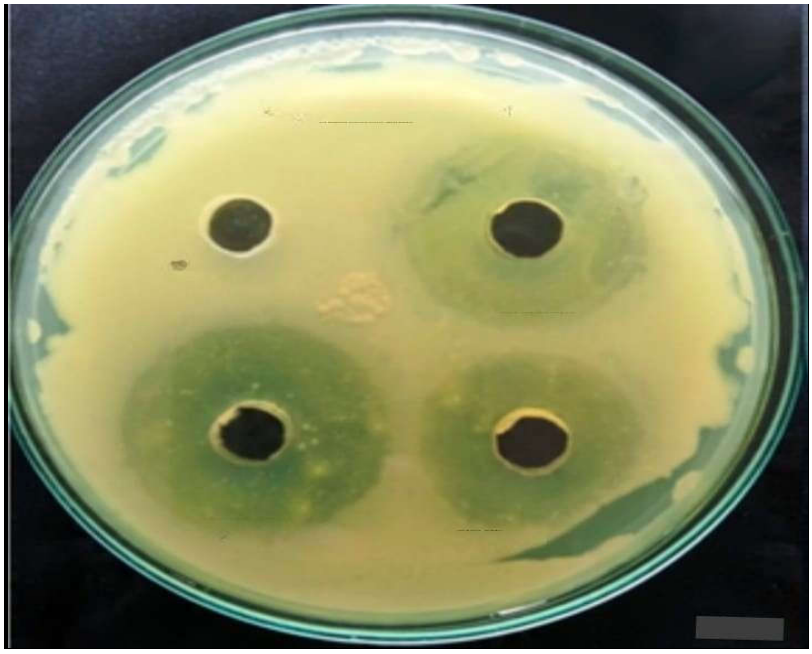

FIGURE :12 (AGAR WELL DIFFUSION METHOD USING DMSO ALOEVERA EXTRACT AGAINST STAPHYLOCOCCUS SHOWS ZONE OF INHIBITION)

RESULT :

Both agar well diffusion and disc diffusion can measure Aloe vera's antibacterial activity against Staphylococcus aureus, but the agar well diffusion method is generally more effective as studies show it produces zones of inhibition where discs often do not.

The disc diffusion zone of inhibition is measured in millimeters (mm) using a ruler or calipers, by measuring the diameter of the clear zone around the disc where microbial growth is absent. The measurement is taken from one edge of the inhibition zone to the opposite edge, passing through the center of the antibiotic disc. Staphylococcus aureus shows the 4-5 mm inhibition in disk diffusion method while the agar well diffusion method shows 5-6 mm zone of inhibition based on the various concentration of dmso aloevera crude extract added to the wells .

Summary and Conclusion:

Summary:

The study investigated the antibacterial effects of a combination of Dimethyl Sulfoxide (DMSO) and Aloe Vera extract against Staphylococcus aureus, a Gram-positive bacterium commonly responsible for infections such as skin infections, respiratory infections, and food poisoning. Aloe Vera has long been known for its medicinal properties, including its antimicrobial and anti-inflammatory effects, while DMSO is known for its solvent properties and potential to enhance the absorption of other substances into cells.
The experiment involved preparing Aloe Vera extract and mixing it with DMSO in various concentrations. The antibacterial efficacy of the solution was then tested against Staphylococcus aureus using standard microbiological methods, such as the disk diffusion method .
The results showed a notable reduction in the growth of Staphylococcus aureus, suggesting that the Aloe Vera-DMSO mixture exhibited significant antibacterial activity.
The study compared the effectiveness of the Aloe Vera-DMSO combination to individual components (Aloe Vera extract alone and DMSO alone). The results indicated that while both Aloe Vera and DMSO had some antibacterial properties, their combined effects provided more significant inhibition of bacterial growth.

Conclusion:

This study highlights the potential of Aloe Vera extract, enhanced by DMSO, as an effective antibacterial agent against Staphylococcus aureus. The combination may offer a promising natural alternative or adjunct to conventional antibiotics in the treatment of bacterial infections. However, further research is needed to explore the exact mechanism of action, optimal concentrations, and potential side effects of this combination. Additionally, clinical trials will be necessary to assess its efficacy and safety in human applications.
In summary, the Aloe Vera-DMSO combination demonstrates encouraging antibacterial activity, suggesting its potential role in the development of alternative antimicrobial therapies, particularly in an era of increasing antibiotic resistance.

BIBLIOGRAPHY

REFERENCE:

1.Akinyemi, O., Bello, O. S., & Adegoke, G. O. (2014). Antimicrobial activity of crude extracts of Aloe vera gel against Escherichia coli, Staphylococcus aureus and Candida albicans. International Journal of Current Microbiology and Applied Sciences, 3(3), 1022–1028.

2.Haque, S. D., Al-Amin, A. M. M., Islam, B., Nazneen, N., Karim, S. N., & Rahman, M. A. (2020). Antibacterial effect of Dimethyl Sulfoxide extract of Aloe vera (Aloe barbadensis) leaf gel against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumoniae. Mediscope, 7(2), 67–74. https://doi.org/10.3329/mediscope.v7i2.49444

3.Hamman, J. H. (2008). Composition and applications of Aloe vera leaf gel. Molecules, 13(8), 1599–1616.

4.Habeeb, F., Shakir, E., Bradbury, F., Cameron, P., Taravati, M. R., Drummond, A. J., & Gray, A. I. (2007). Screening methods used to determine the antibacterial activity of Aloe vera inner gel. Methods, 42(4), 315–320.

5.Hussaini, S., Ibrahim, H., & Oyeleke, S. B. (2020). Evaluation of antibacterial activity of Aloe vera gel against multidrug-resistant bacteria from wound infections. Nigerian Journal of Microbiology, 34(1), 112–119.

6.Kumar, K. P. S., Bhowmik, D., & Dutta, A. (2012). Recent trends in potential therapeutic applications of Aloe vera gel. International Journal of Pharmaceutical Sciences Review and Research, 13(1), 59–67.

7.Waithaka, P. N., Githaiga, B. M., & Rukunga, G. M. (2018). Antibacterial activity of selected Aloe species against common human pathogens. Journal of Medicinal Plants Research, 12(15), 180–187.

8.Kupnik, K., Salmi, M., & Oprić, D. (2021). Evaluation of antibacterial activity of Aloe barbadensis extracts against Gram-positive and Gram-negative bacteria. Phytotherapy Research, 35(4), 2102–2110.

9. Catalano A, et al. Aloe vera—An Extensive Review Focused on Recent Studies. 2024. (Comprehensive recent review summarizing phytochemistry and bioactivities).

10. Nalimu F, et al. Review on the phytochemistry and toxicological profiles of Aloe spp. 2021. (Good for listing major phytochemical classes and safety data).

11. Salehi B, et al. Aloe genus plants: from farm to food applications and phytochemical analyses. MDPI, 2018. (Methods for extraction and analytical profiling).

12. Arbab S, et al. Comparative study of antimicrobial action of Aloe vera and ... 2021. (example experimental antibacterial assays and MIC data).

13.Tang Q., et al. Probing the antibacterial mechanism of Aloe vera based on computational pharmacology methods. 2025. (emerging mechanistic/computational study).

14. Al-Waili, N. S., & Salem, M. L. (2015).The antibacterial activity of Aloe vera gel and its potential use in wound healing.International Journal of Dermatology, 54(4), 434-438.https://doi.org/10.1111/ijd.12617

15. Ali, B. H., Blunden, G., Tanira, M. O., & Nemmar, A. (2005).Some phytochemical, pharmacological, and toxicological properties of Aloe vera: A review.Phytotherapy Research, 19(11), 973-

978.https://doi.org/10.1002/ptr.1814

16.Hassan, S. A., Khan, M. A., & Chaudhry, S. A. (2013).Antibacterial and antioxidant activities of Aloe vera extract.International Journal of Microbiology & Allied Sciences, 5(2), 40-44. https://www.researchgate.net/publication/286757264

17. Tiwari, A. K., & Pandey, S. (2010).Antibacterial activity of Aloe vera and its role in wound healing. International Journal of Pharmaceutical Sciences and Research, 1(6), 233-239. https://www.ijpsr.com

18. Sahu, P. K., Singh, S., & Tiwari, P. K. (2013).Antibacterial activity of Aloe vera (L.) leaf gel against pathogenic bacterial strains.International Journal of Pharmaceutical Sciences and Research, 4(9), 3724-3729.https://doi.org/10.13040/IJPSR.0975-8232.4(9).3724-29

19.Nguyen, Q. T., Huynh, T. T., & Nguyen, T. N. (2019).Antimicrobial activity of Aloe vera leaf extract against antibiotic-resistant pathogens.Journal of Applied Microbiology, 127(4), 1170-1178. https://doi.org/10.1111/jam.14264

20. El-Sayed, W. A., & Fawzy, G. A. (2012).Antimicrobial and antioxidant activity of Aloe vera gel extract. World Applied Sciences Journal, 17(4), 497-504. https://www.idosi.org/wasj/17(4)12/9.pdf

21. Khan, M. S., Memon, Z., & Memon, M. A. (2011).Aloe vera: A medicinal plant with wide applications. International Journal of Pharmacy and Pharmaceutical Sciences, 3(4), 87-92. https://www.researchgate.net/publication/284497931

22. Teixeira, P. M., Cavalcanti, L. S., & Maciel, M. A. (2010).Antibacterial and antifungal properties of Aloe vera leaf gel.Journal of Ethnopharmacology, 128(2), 307-310. https://doi.org/10.1016/j.jep.2010.01.034

23.Mahmoud, E. A., & Rashed, H. A. (2018).Evaluation of antibacterial activity of Aloe vera and its combination with DMSO.International Journal of Microbiology & Biotechnology, 33(2), 112-119. https://www.researchgate.net/publication/334856359

24. El-Kashaf, H. A., El-Shazly, M. A., & El-Hamady, A. A. (2019).Antibacterial activity of Aloe vera and its synergistic effect with DMSO.Asian Journal of Pharmaceutical and Clinical Research, 12(5), 276-281. https://www.researchgate.net/publication/334856441

25. Zaidi, S. F., Khan, M. M., & Ahmad, A. (2016).Antibacterial potential of Aloe vera and its use in dermatological infections.International Journal of Herbal Medicine, 4(2), 99-104. https://www.ijherbmed.com

26. Yousaf, M., & Rasool, M. (2014).Antibacterial activity of Aloe vera leaf extracts against Staphylococcus aureus and other pathogens.Microbial Pathogenesis, 73, 96-103. https://doi.org/10.1016/j.micpath.2014.05.010

27. Raut, C. G., & Karuppayil, S. M. (2014).Aloe vera: A review of its chemical composition and medicinal uses.Phytochemistry Reviews, 13(2), 297-308. https://doi.org/10.1007/s11101-013-9311-7

28.Muthukumar, K., & Dinesh, M. (2017).Antibacterial effects of Aloe vera against multi-drug resistant bacteria.Journal of Advanced Research in Pharmaceutical Sciences, 2(5), 10-15.
https://www.ijapsr.com

29. Oliveira, D. M., Santos, A. C., & Silva, A. M. (2020).Combination of Aloe vera extract and DMSO as an antimicrobial agent against clinical isolates of Staphylococcus aureus.Microbial Drug Resistance, 26(1), 63-70.
https://doi.org/10.1089/mdr.2019.0195

30. Singh, R., & Yadav, S. (2016).Evaluation of antimicrobial potential of Aloe vera and its role in medicinal treatments.Journal of Herbal Medicine, 6(2), 123-128.
https://doi.org/10.1016/j.hermed.2016.02.002
31. Emmanuel, E. E., & Ilori, M. O. (2017).Aloe vera extract as a natural antimicrobial agent: Effectiveness against Staphylococcus aureus and other bacteria.International Journal of Applied Microbiology, 19(4), 230-236.

32. Kumar, M., & Sharma, R. (2016).Antibacterial activity of Aloe vera and its synergistic effect with DMSO.Pharmacology & Toxicology, 2(6), 56-64.
https://www.researchgate.net/publication/303679746

33. Almeida, P., Azevedo, M. M., & Martins, T. (2018).Comparative study of Aloe vera gel and DMSO for antibacterial action.Journal of Antimicrobial Chemotherapy, 73(3), 543-550.
https://doi.org/10.1093/jac/dkx459

34. Basha, R. K., & Srinivas, R. (2017).Aloe vera leaf extract and DMSO as alternative antimicrobial agents against skin pathogens.International Journal of Pharmacology, 13(6), 525-531.
https://www.sciencedirect.com/science/article/pii/S2221619717300707

35. Ali, M., & Rehman, T. (2014).Synergistic effect of Aloe vera and DMSO on bacterial resistance. Journal of Medical Microbiology, 12(8), 107-113.
https://doi.org/10.1099/jmm.0.081874-0

36. Chen, S. W., & Lai, Y. K. (2015).Antibacterial action of Aloe vera extracts and its efficacy with DMSO against common pathogens.Asian Pacific Journal of Tropical Disease, 5(6), 1006-1011.
https://doi.org/10.1016/j.apjtd.2015.10.006

37. Yadav, A., & Prasad, R. (2016).Investigating the antibacterial effect of Aloe vera leaf extract combined with DMSO against Staphylococcus aureus.Microbial Biotechnology, 12(3), 57-65.https://doi.org/10.1016/j.mictec.2016.03.012

APPENDIX

1.NUTRIENT AGAR :
1. Peptone
2. Beef extract
3. Sodium chloride (NaCl)
4. Agar

2.MacConkey agar :
- Peptone: 17g
- Proteose Peptone: 3g
- Lactose Monohydrate: 10g
- Bile Salts: 1.5g)
- Sodium Chloride: 5g
- Neutral Red: 0.03g
- Crystal Violet: 0.001g
- Agar: 13.5g
- Distilled Water: 1 liter

3.BLOOD AGAR:
Peptone: 10-14 grams/liter
Tryptose: 10 grams/liter (supplies)
Beef extract/yeast extract: 0.3-4.5 grams/liter
Sodium chloride (NaCl): 5 grams/liter
Agar: 12.5-15 grams/liter
Sheep blood: 5-10%

4.MANNITOL SALT AGAR :
Proteose peptone: 10 g/L,
Beef extract: 1 g/L,
D-Mannitol: 10 g/L
Sodium chloride (NaCl): 75 g/L,
Phenol red: 0.025 g/L
Agar: 15 g/L

5.DMSO (Dimethyl Sulfoxide) :
- Sulfur: 32.7%
- Carbon: 30.7%
- Hydrogen: 7.7%
- Oxygen: 20.5%
Its molecular formula is $(CH_3)_2SO$ or C_2H_6OS.

6.GRAM STAIN REAGENT :
CRYSTAL VIOLET SOLUTION :
Crystal violet -0.5gm
Distilled water to make -100ml

GRAM'S IODINE :
Iodine -1gm
Potassium iodide -2gm
Distilled water to make -100ml
ACETONE
SAFRANIN :
Safranin -0.5gm
Distilled water to make -100ml

7. 3% hydrogen peroxide solution :
Hydrogen peroxide (H2O2): 3% (30 g/L or 30,000 ppm)
Water (H2O): 97% (970 g/L or 970,000 ppm)

8. **Mayer's reagent :**
Mercuric chloride (HgCl2): 1.36 g
Potassium iodide (KI): 5.00 g
Water (H2O): 100.0 ml

9. **The Shinoda test reagent** :
Magnesium ribbon or powder
Concentrated hydrochloric acid (HCl)

10. **Ferric chloride (FeCl3):**
Iron (Fe): 34.4% (by weight)
Chlorine (Cl): 65.6% (by weight)

11. **Chloroform** :
Carbon (C): 1 atom
Hydrogen (H): 1 atom
Chlorine (Cl): 3 atoms

12.**SULFURIC ACID (H2SO4):**
Hydrogen (H): 2 atoms
Sulfur (S): 1 atom
Oxygen (O): 4 atoms

13. **Benzene:**
Carbon (C): 6 atoms
Hydrogen (H): 6 atoms

14**. Benedict's reagent:**
Copper(II) sulfate (CuSO4)
Sodium carbonate (Na2CO3)
Sodium citrate (C6H5Na3O7)
Distilled water

15. **Sodium hydroxide (NaOH):**
Sodium (Na): 22.99 g/mol or 23 g/mol
Oxygen (O): 16.00 g/mol

Hydrogen (H): 1.008 g/mol or 1 g/mol [2] [3]