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EXAMINING THE ISOLATION OF *STAPHYLOCOCCUS AUREUS* FROM INDUSTRIAL WATER AND IMPACT OF MEDICINAL PLANT AGAINST BACTERIAL ACTIVITY

Akanksha Chaurasia* and Dr. Vinod Kumar Gupta

Rapture Biotech International Pvt. Ltd., Noida, Uttar Pradesh, India.

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***Corresponding Author**
Akanksha Chaurasia
Rapture Biotech International Pvt. Ltd., Noida, Uttar Pradesh, India.

ABSTRACT

A prevalent constituent of the body's microbiota, *Staphylococcus aureus* is a spherically-shaped, Gram-positive bacteria belonging to the Bacillota that is often found on the skin and in the upper respiratory system. A broad range of clinical disorders are caused by the gram-positive bacterium *Staphylococcus aureus*. Both community- and hospital-acquired infections are frequently brought on by this bacterium. Because multi-drug resistant strains like MRSA (Methicillin-Resistant *Staphylococcus aureus*) are becoming more common, treatment is still difficult. *S. aureus* often does not cause infections on healthy skin, but it can cause a number of potentially dangerous illnesses if it gets into the bloodstream or internal tissues. The strain that was taken into consideration came from planting water or mechanically wasting water. Three unique plant extracts—*Clitoria Ternatea* (Aparajita), Pranabjie, and *Andrographis paniculata* (Kalmega)—have antibacterial properties. The germs' antibacterial characteristics suggested that they were resistant to cefixime and methicillin. Aparajita, Pranabjie, and Kalmega extracts showed strong antibacterial activity against *Staphylococcus aureus* strains that were safe to use with methicillin, making them potentially effective antimicrobial agents for treating the strains under consideration. Phytochemical test performed on all the three-plant extract where *Aparajita, Pranabjie* shows positive result in all the qualitative test where as *Kalmega* is in not in favour with all the test. Antibiotic sensitivity test for *Staphylococcus aureus* is negative with *Kalmega* and positive with *Aparajita, Pranabjie* and Antibiotic.

KEYWORDS: *Staphylococcus aureus*, MRSA, Gram-positive, Aparajita, Pranabjie, Kalmega, antibacterial.

INTRODUCTION

In developing countries, there are many factories which have industry waste like sewerage water, Garding water and many more which can cause skin infection. Industrial waste illness can cause by nitrate and phosphate which cause eutrophication, mostly the air which surrounded the industries are highly polluted which can harm our eye sight also and cause skin problem followed by throat and nose problem [Njom, 2017]. Waste water treatment plants play a crucial role in development of antibiotic-resistant bacteria. In many ways sewage water can be reused such as agriculture, aquaculture and some industrial purpose. [Shuval, 2003]

One of the most significant pathogens is *Staphylococcus aureus*, or *S. aureus*. It can cause a range of pyogenic infections, suppuration, abscess development, blepharitis, keratitis, conjunctivitis, and potentially deadly septicaemia [Lowy, 1998]. The early antimicrobial agent was found to be effective against *S. aureus*, which can cause bacteraemia; however, as antibiotic usage increased, staphylococcal resistance quickly emerged [Boucher, 2008]. In hospitals, methicillin-resistant *Staphylococcus aureus* (MRSA) became a significant clinical and epidemiologic issue in the 1980s. Medical facilities of varying sizes are currently dealing with the MRSA issue. MRSA is widespread in many parts of the world today [Taylor, 2024]. MRSA-related infections and outbreaks have become more frequent over time. Since MRSA frequently resists multiple drugs, there are few treatment alternatives available. Novel antimicrobial drugs are therefore required to solve this issue [Rasigade, 2014].

Staphylococcus aureus is commonly found bacteria which is present in the nails and skin of human and in several species of animals. Methicillin-resistant *S. aureus* (MRSA) is currently one of the biggest global dangers to human and animal health [Robinton, 1966]. Few studies have examined the prevalence of *S. aureus* and MRSA in non-clinical settings like wastewater since most wastewater research concentrate on microbiological markers of faecal contamination [Charoenca, 1995]. The choice of therapy for infections of the skin and wounds is influenced by the host factors as well as the disease's severity [Burnham, 2018]. For minor isolated infections in immunocompetent people, cleaning and disinfection are sufficient, occasionally supplemented by a brief surgical procedure and/or a short-term local antibiotic treatment [McDougal, 2006]. Mupirocin, a common topical antibiotic medication

used to treat staphylococcal infections of the skin and decolonize patients at risk prior to surgery, is another instance where things start to go wrong [Alekshun, 2007].

As one of the most prevalent bacteria in the world, *S. aureus* is the source of many human infections, such as bacteraemia, infective endocarditis, infections of the skin and soft tissues (such as impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, infections from prosthetic devices, infections of the lungs (such as pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections [Tong, 2015]. The mec gene, which is part of the broader Staphylococcal chromosomal cassette mec (SCCmec) area of the bacterial chromosome, is carried by MRSA strains. Depending on the type of SCCmec, this gene confers resistance to different drugs.

It's fascinating to hear about the utilization of local plant species in Thailand for treating infectious diseases, especially against challenging pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA). Screening plant extracts for antibacterial activity against MRSA isolates from hospitals can provide valuable insights into potential alternative or complementary treatments. This approach aligns with the growing interest in natural products as potential sources of antimicrobial agents [Voravuthikunchai, 2004]. The study's aim to screen plant extracts previously known for their antibacterial activity against MRSA isolates underscores the importance of evidence-based traditional medicine and the need for novel therapeutic options, particularly in the face of antibiotic resistance. By leveraging the rich biodiversity of plant species found in Thailand, researchers can potentially identify new compounds or combinations of compounds with antimicrobial properties against MRSA [Ninrprom, 2004].

Persicaria pensylvanica also known as Pennsylvania smartweed, highlighting its distribution across the United States and its adaptability to various soil types, excluding drought-prone soils. While it is considered a weed and can cause damage to cotton and soybean crops, it also serves as valuable wildlife food, particularly for waterfowl [Abdi, 2018]. One notable aspect of *P. pensylvanica* is its potential medicinal properties, specifically the production of two anti-cancer compounds known as vanicosides A and B. These compounds are identified as protein kinase C inhibitory glycosides, suggesting a potential role in cancer treatment [Silva, 2014]. Despite these promising attributes, the antimicrobial activity of *P. pensylvanica* has not been thoroughly investigated. The passage mentions established methods for testing

the antimicrobial activity of herbal extracts, such as inhibition zone diameters, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time-kill assays, and fractional inhibitory concentration index (FICI) determination [Abdi, 2018].

Phytochemical studies have identified compounds such as spermidine, rutin, quercetin, tocopherol, and carotenoids from caper plants. These compounds contribute to its antimicrobial, antioxidative, anti-inflammatory, and antiviral activities [Tlili, 2011]. The seed extracts of *Capparis Decidua* exhibit antibacterial, antifungal, and antileishmanial (against the parasite causing leishmaniasis) activities, likely attributed to quaternary ammonium and glucosinolate compounds [Joshi, 2017]. Bearberry (*Arctostaphylos uva-ursi*) and Cranberry (*Vaccinium macrocarpon*) Juice, these plants have been traditionally used to treat urinary tract infections. Both bearberry and cranberry juice have been documented for their effectiveness in this regard [Elfalleh, 2011]. Phenolics, alkaloids, flavonoids, triterpenes, and steroids from *Cameroonian* plants exhibit significant antimicrobial activity. *Crofelemer*, a *proanthocyanidin Oligomer* and the active ingredient of Fulya, is isolated from the plant Croton lechleri, found in the Western Amazonian regions of South America [Kuete, 2010]. Leaf extracts of Myrtus communis and Verbena officinalis demonstrate good antibacterial activity against Staphylococcus aureus, Escherichia coli, and Salmonella typhi [Dzotam, 2017]. Myrtus communis also exhibits remarkable activity against *Pseudomonas aeruginosa*. Carrot seed oil and tea tree oil display antimicrobial activity against Helicobacter pylori and Mycoplasma pneumoniae, respectively [Sierra-Madero, 1997]. Extracts of *Oxalis corniculata, Artemisia vulgaris, Cinnamomum tamala, and Ageratina adenophora* exhibit antimicrobial activities against *Escherichia coli, Salmonela Typhi, Klebsiella pneumoniae, Staphylococcus aureus*, and *Citrobacter koseri* [Wangchuk, 2011]. Extracts *of Berberis vulgaris, Cistus monspeliensis, and Punica granatum* demonstrate high activity against Staphylococcus aureus, Enterococcus faecalis, and Enterobacter cloacae [Bereksi, 2018].

MATERIAL AND METHOD

Media preparation

The identification of bacteria begins with the preparation of Mannitol Salt Agar (MSA) media with gardening water which is used in the industrial waste product. Mannitol Salt Agar (MSA) uses phenol red to indicate the pH of the medium [Cannon, 1986]. The medium turns yellow when the pH falls below 6.9. The shade of phenol red is red in the neutral pH ranges of 6.9 to 8.4, and pink at pH 8.4 [Wang, 2020]. A yellow zone forms around a solitary colony

on MSA as a result of the acid produced by the fermentation of mannitol by a bacterium. Because of peptone breakdown, a nonfermenting bacterium that can tolerate the high salt concentration would show a red to pink patch [Geerling, 1999].

Table 1: MSA Media Composition.

Composition	25ml
Peptone	0.25g
Sodium Chloride	1.875g
D-Mannitol	0.25g
Beef Extract	0.025g
Phenol Red	2-3 drop
Agar	0.375g

After the medium has been produced, it is placed to be autoclaved for 30 minutes at 37°C and petri plate should be left in a laminar air flow and exposed to UV light for 15 minutes. Wipe the plate with ethanol to prevent contamination. Pour the media onto it after 30 minutes of autoclaving, and then expose it to UV light for an additional 15 minutes to eliminate any remaining germs. To ensure that colonies thrive, use a 100µl pipette to pour the collected gardening water sample onto the plate. Then, use a glass spreader to evenly distribute the drop of water across the plate. To observe the bacterial colonies, incubate the plate for 24 hours. If the media's colour changes from red to yellow, *Streptococcus aureus* is present.

Pure bacterial isolated from a single piece of bacteria, a process known as streaking, in which the bacteria form zigzag colonies. In order to accomplish this, we prepared Nutrient Agar Media (NAM) in 25 millilitres of distilled water, autoclaved it for 30 minutes, followed by exposing the plate to UV light for 15 minutes to eliminate all the microbes. After autoclaving, let the medium cool before pouring it into the plate and exposing it to UV light for ten minutes. Sterilize the inoculation loop by heating it to a red hot and 70% ethanol. Using the inoculation loop, take the microbes from the colonies of bacteria and apply a zigzag pattern to the surface of media, then incubate it for 24 hour and observe the result.

Morphological test

Morphological tests involve the examination of the physical characteristics or structures of microorganisms to identify and classify them. These tests can provide valuable information about the shape, size, arrangement, and other external features of bacteria and other microbes [Peters, 2018]. These morphological tests are fundamental in the preliminary identification

and classification of microorganisms and are often used in conjunction with biochemical and molecular tests for accurate microbial characterization [Zang, 2019].

***Gram Straining Method*:** It can be positive and negative or rod shaped and cocci shape bacteria. A variety of reagents are used in gram straining, including decolizer ethanol, safranin, gram iodine, and crystal violet [Zhou, 2020]. The procedure started with a clean slide that was exposed to UV light for ten minutes in order to eradicate any bacteria that might have been on it. Using an inoculation loop, pick a single microbe from the streaked plate, smear it onto a slide, and allow it to dry for 20 minutes in laminar air flow. After the slide has dried, add all of the reagents on it in accordance with the procedure. After heating the slide to a fixed temperature, apply a single drop of crystal violet, wait 45 seconds, and then drain the slide using water. Next, add a couple of drops of iodine to the smear, and after a minute, drain it with water. Additionally, Wash the stain with ethanol or acetone, and then quickly rinse it with water to prevent the remaining microorganisms from dying. Finally, add a single drop of saffranine, then wait 45 seconds before adding water to drain. Before examining the slide under a microscope, let it air dry in a laminar air flow for twenty minutes.

Biochemical Test

Biochemical tests are conducted for identifying distinct species of bacteria. These procedures are labour-intensive and time-consuming, and results take several days to show, despite the fact that they are low-cost and provide both qualitative and quantitative information on the variety of microorganisms present in a sample. Here, we use a number of biochemical tests to determine the form of bacteria and obtain confirmation of it [Abid, 2022].

***Catalyse Test*:** The purpose of this test is to evaluate whether microorganisms produce catalyse enzymes. An experiment first required a clean glass slide that had to be exposed to UV light for ten minutes before bacteria from the streaked plate were added to the slide. Subsequently, apply hydrogen peroxide to the bacteria and observe the results. If bubble formulation occurs, the result is positive whereas no bubble formulation shows the outcome is negative [Iwase, 2013].

***Indole test*:** The experiment demonstrates that certain bacteria may convert the medium-accumulating amino acid tryptophane into indole [Fay, 1974]. To conduct this experiment, the media composition must be prepared in 5mL of distilled water. Add 0.25g of Sodium Chloride (NaCl) and 0.05g of Tryptone in water [Jin-Hyung, 2010]. Once the composition is

ready, autoclave the media at 121°C for 30 minutes. Then, use an inoculation loop inside the test-tube to deliver the bacteria from the culture plate to see the outcome, incubate it for a full day and then add the Kovac reagent in broth on the next day. A positive test result occurs if a pink colour ring forms adhering to the reagent; a negative test result is indicated if no pink colour ring forms.

Nitrate Test*:* The nitrate test is a chemical procedure that detects the presence of nitrate ions in solutions and demonstrates their capacity to combine with sulfanilic acid to make red compounds [Niu, 2021]. The experiment begins with the substrate being prepared in 10 millilitres of distilled water.

Table 2: The table show the composition of Nitrate test in 10ml distilled water.

Composition	10ml
Peptone	0.05g
Meat Extract	0.03g
Potassium nitrate	0.01g
pH	7.0

Before inoculation, autoclave the media for 30 minutes after it has been prepared. subsequently, when autoclaving is complete, inoculate it using an inoculation loop. After that, incubate it for a full day to observe the bacteria grow in the medium. Add the reagents sulfanilic acid and alpha-naphthylamine to determine if the result is positive or negative. If the result is negative, add a small quantity of zinc dust to make it red; otherwise, the result is positive.

***Citrate Test*:** The purpose of the citrate test is to ascertain whether the bacteria can use synthetic ammonium hydrogen phosphate for a source of nitrogen and sodium citrate as their only supply of carbon [Hofwegen, 2016]. Prepare the medium in 10 milliliters of distilled water first.

Table 3: Composition of citrate Agar test in 10ml distilled water.

Composition	10ml
Sodium Chloride	0.05g
Sodium Citrate	0.02g
Ammonium Dihydrogen Phosphate	0.01
Dipotassium Phosphate	0.01g
Magnesium Sulphate	0.02g
Bromothymol Blue	2-3 drops
Agar	0.2g
pH	6.9

To eliminate all contaminated bacteria that have proliferated instead of the necessary microorganisms, autoclave the media for 30 minutes after preparation. When the test tube is taken out of the autoclave, place it in a slanting in laminar air flow to allow the media to solidify at an angle. For solidifying the media leave it for 30 minutes in Laminar Air flow under the blower. After the media solidified, streak it in a zigzag pattern using inoculation loops. After a 24-hour incubation period, check the medium to see if the colour has changed from green to blue. If not, the outcome is negative.

MR/VP Test: The MR-VP test is essentially a pair of distinct or separate tests that use a single broth medium containing glucose to determine the potential kind of glucose fermentation being carried out by bacteria [Barry, 1970]. To conduct this experiment, prepare the media in 10ml of distilled water.

Table 4: Composition of MR/VP test media in 10ml distilled water.

Composition	10ml
Peptone	0.07g
Dextrose	0.05g
Dipotassium Phosphate	0.05g
pH	7

After the media is ready, autoclave it for 30 minutes to eliminate any possible contamination. Next, use an inoculation loop to inoculate bacteria from the culture plate. In order to prevent the bacterial colonies from adhering to the test-tube wall, thoroughly mix the broth. Incubate the media for a full day in order to see the outcome. Make use of the reagent the next day to see the outcome. Put ten drops of the methyl-red reagent in the MR tube, and in the VP test tube, add the KOH and alpha-naphthol.

Protease Test: For the purpose of helping to preserve homeostasis, proteases break down misfolded and damaged proteins by cleaving the features that supply crucial amino acids for protein synthesis [Soleimany, 2022]. The experiment initiates with the media being prepared in 25 millilitres of distilled water.

Table 5: The composition to prepare protease media in 25 ml distilled water.

Composition	25ml
Skim Milk Powder	0.7g
Yeast	0.06g
Tryptone	0.12g
Glucose	0.025g
Agar	0.5g
pH	7

When the substrate has been prepared, autoclave it for 30 minutes. Afterward, clean the Petri plate and expose it to UV light for 15 minutes. Place this test organism onto the plate. Use ethanol to sterilize the inoculation loop and heat it up to a red temperature. Subsequently, streak the organism onto the plate using a straight or zigzag pattern. Incubate the plate at 25℃ or 37℃. Record the result of unknown bacteria.

Lactose/Maltose: A disaccharide called lactose can be found in practically all animal milk. It is made up of beta-D glucose and beta-D galactose, with a glycoside bond connecting glucose's C4 and galactose's C1 [Sendino, 2021]. Maltose is another disaccharide that is created when two alpha-D glucose units are joined at the C1 and C4 positions [Lebot, 2017]. Beginning with two test tubes, each holding five millilitres of distilled water, the experiment introduced one test tube containing 1% lactose and the other containing 1% maltose along with two to three drops of phenol red each. Add 1 drop of NaOH to it to find the dark red colour if the red colour is not visible well. After the media is ready, cover the test tubes with aluminium foil and autoclave them for 30 minutes. Soon after the media has been autoclaved, add 100 microliters of bacterial broth culture then incubate it for 24 hours to see the end result.

Dextrose/Sucrose Test: Dextrose, also known as glucose or blood sugar, is a simple sugar that is chemically identical to glucose and is commonly derived from corn or wheat [Kitsios, 2020]. Sucrose, commonly known as table sugar, is a disaccharide composed of one molecule of glucose and one molecule of fructose bonded together. This bond is called a glycosidic bond [Markov, 2022]. Prepare two test tubes, each containing 5 millilitres of distilled water. Add 1% dextrose (glucose) solution to one test tube and 1% sucrose solution to the other. Also, add two to three drops of phenol red indicator to each test tube. If the red colour of phenol red is not visible well, add 1 drop of NaOH to each test tube to facilitate colour change. Cover the test tubes with aluminium foil to prevent contamination. Autoclave the test tubes at high pressure and temperature for 30 minutes to sterilize the media and kill any existing microorganisms. After autoclaving, allow the media to cool down to room temperature. Add 100 microliters of bacterial broth culture to each test tube. Incubate the test tubes at the appropriate temperature (usually around 37°C for bacterial cultures) for 24 hours. After incubation, observe the colour change in the media.

D-mannitol: D-mannitol is a sugar alcohol often used as a sweetener in sugar-free products, but its role in regulating lipid metabolism in the liver isn't well-established. While it's true

that some sugar alcohols like mannitol can influence metabolic processes, such as insulin sensitivity, their specific effects on lipid metabolism may vary [Sequeira, 2014]. Begin by preparing the media in 5 millilitres of distilled water for the experiment.

Table 6: Composition of D-Mannitol prepared in 5ml distilled water.

Composition	5ml
Tryptone	0.025g
Peptone	0.025g
Yeast Extract	0.005g
Sodium Chloride	0.025g
D-Mannitol	0.05g
Phenol Red	2-3 drop
pH	6.9

To ensure that no microorganisms remain that could contaminate the medium, autoclave it for 30 minutes after preparation. Utilizing nutrient broth culture, inoculate the media and incubate it for a full day to see the outcome.

Sorbitol Test: The sorbitol test is a microbiological test used primarily for the identification of certain strains of bacteria, particularly within the genus Escherichia coli (E. coli) [Liauw, 2019]. To begin the experiment, first make the sorbitol medium in 5 millilitres of distilled water.

Table 7: The table show the composition of Sorbitol in 5ml distilled water.

Composition	5ml
Peptone	0.05g
Yeast Extract	0.005g
Sodium Chloride	0.025g
Sorbitol	0.025g
Phenol Red	2-3 drop

After the media is ready, autoclave it for thirty minutes. Inoculate the broth culture bacteria under laminar airflow, and then incubate the media for a full day. Observe the outcome on the next day. The result is positive.

Urease Test: The urease test is indeed a valuable tool in microbiology for identifying bacterial species that possess the urease enzyme [Dahlén, 2018]. Get the media ready in 10 millilitres of distilled water at the start of the experiment.

Table 8: The table show the composition of Urease test in 10ml distilled water.

Composition	10ml
Peptone	0.01g
Dextrose	0.01g
Sodium Chloride	0.05g
Dipotassium Phosphate	0.02g
Phenol Red	3-4 drop
Urea	0.2g
Agar	0.2g
PH	6.9

When the medium is ready, autoclave it for 30 minutes, then let it cool while positioned slantwise in a laminar airflow to solidify it in that position. Proceed to streak the slant using the inoculation loop that is collected from the culture media. Let it incubate for 48 hours to see its outcome.

Amylase Test: The amylase test is a biochemical assay used to detect the presence and activity of the enzyme amylase in biological samples, such as serum, urine, or bacterial cultures [Chase, 1996]. Amylase is an enzyme that catalyses the hydrolysis of starch and glycogen into simpler sugars like maltose and glucose [Muniraj, 2015]. First, prepare the media in 25 millilitres of distilled water using a specific Amylase Test composition.

Table 9: The table show the composition of Amylase Test in 25ml Distilled water.

Composition	25ml
Peptone	0.125g
Sodium Chloride	0.125g
Yeast Extract	0.075g
Agar	0.5g
Starch	0.25g
pH	7.4

After the media has been prepared, autoclave it for 30 minutes. Up until then, give the Petrie plate a 15-minute UV exposure. After placing the media into the plate and allowing it to solidify for 30 minutes in a laminar airflow, remove the bacteria from the culture plate and use the inoculation loop to streak the bacteria in a straight line. Incubate the plate for 48 hour and then add iodine solution in plate to view clear zone of bacterial growth.

Collecting Plant Extract in Water

Gather the plant extracts named Aparajita, Kalmega, and Pranabjie. These extracts likely contain various bioactive compounds derived from the respective plants [Duraipandiyan,

2006]. Obtain three test tubes and label them accordingly to distinguish between the different plant extracts Measure out 15 millilitres of distilled water using a graduated cylinder or pipette [Voravuthikunchai, 2004]. Add 15 millilitres of distilled water to each of the three test tubes. This will serve as the solvent or medium for the plant extracts. Weigh out 1 gram of each plant sample (Aparajita, Kalmega, and Pranabjie) using a weight balance. Add 1 gram of the respective plant sample to each of the corresponding test tubes containing distilled water. This step involves adding the plant material to the solvent to extract its bioactive compounds. After adding the plant samples to the test tubes, mix the contents thoroughly. This can be done by gently swirling the test tubes or by using a vortex mixer, depending on the volume and consistency of the mixture. Depending on your experimental design, you may choose to incubate the mixtures to allow the bioactive compounds to diffuse into the solvent. This could involve placing the test tubes in a shaker or incubator for a specified period. Filter the sample to get clear substrate.

Phytochemical Test

Phytochemical tests are experiments conducted to detect the presence of various classes of plant compounds, such as alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids [Mbaebe, 2012]. These tests are important in pharmacognosy, ethnobotany, and drug discovery, as they help identify potentially bioactive compounds in plants. By conducting phytochemical tests, researchers can screen plant extracts for the presence of these and other bioactive compounds (Wangchuk, 2011). Let's take a brief look at some of the major classes of plant compounds and their significance.

Saponin*:* Saponins are a diverse group of naturally occurring compounds found in many plants, particularly in the plant families *Asteraceae, Fabaceae, and Solanaceae*, among others. Some of the notable properties and uses of saponins include: Surfactant Properties, Cholesterol-Lowering Effects, Anti-inflammatory Activity and many more. To demonstrate that the saponins are positive, add 2 ml of aqueous plant extract to 6 ml of distilled water in a test tube. Wait 10 to 15 seconds. If form generation is visible [Rai, 2023].

Tannin: The tannin test is a chemical test used to detect the presence of tannins in a substance. Take a test tube. Add a small amount (around 2 mL) of the saponin sample. Add a few drops (around 500 µL) of a dilute solution of Ferric Chloride (usually around 5% concentration). Observe any colour changes in the mixture. Dark black, blue, or green coloration may indicate the presence of tannins [Kaczmarek, 2020].

Flavonoids: The flavonoid test is a chemical test used to detect the presence of flavonoids in a substance [Fattahi, 2014]. Flavonoids are a group of naturally occurring compounds found in various plants, fruits, and vegetables [Joshi, 2013]. They are known for their antioxidant and anti-inflammatory properties and are associated with various health benefits. There are three ways for conducting the test: Sodium Hydroxide Test, Lead Acetate Test, Aluminium chloride test [Elfalleh, 2011].

Sodium Hydroxide Test: The sodium hydroxide test is a common method used to detect flavonoids in a substance [Kancherla, 2019]. Here's how it typically works: Add a solution of 2 ml of sodium hydroxide (NaOH) to 1ml plant sample. The concentration of the NaOH solution can vary depending on the specific protocol being followed. If flavonoids are present in the sample, they can react with the sodium hydroxide to produce a characteristic colour change. This colour change often manifests as a shift from colourless to yellow, although the exact shade of yellow can vary depending on the specific flavonoids present and their concentration.

Lead Acetate: The lead acetate test is a commonly used qualitative test to detect the presence of flavonoids in a given sample [Nortjie, 2022]. Take 1 ml of the plant sample suspected to contain flavonoids and place it in a test tube or a suitable container. Add 1 ml of distilled water to the test tube containing the plant sample. This is done to ensure proper solubility and to create a homogeneous solution. Now, add 1 ml of the lead acetate solution to the test tube containing the plant sample and water mixture. The lead acetate will react with any flavonoids present in the sample, leading to the formation of lead-flavonoid complexes. After adding the lead acetate solution, observe any changes in the colour or appearance of the solution.

Ammonium Test: The ammonia test, also known as the ammonia vapor test, is a simple qualitative test used to detect the presence of flavonoids, particularly flavones and flavanols [Rahimah, 2019]. Take 1 ml of the plant sample. Add 1 ml of diluted ammonia to the plant sample. The diluted ammonia serves to release the flavonoids from the plant material. Mix the plant sample and diluted ammonia thoroughly to ensure proper extraction of flavonoids. After mixing, add 100 microliters (0.1 ml) of sulfuric acid (H_2SO_4) to the mixture. Sulfuric acid is often added to stabilize the colour changes that occur during the test. After adding sulfuric acid, observe any colour changes in the mixture.

Alkaloid Test: The alkaloid test is a chemical test used to detect the presence of alkaloids in a substance. Some commonly used reagents in alkaloid tests include Dragendorff's reagent, Mayer's reagent, and Wagner's reagent. These reagents can help identify the presence of alkaloids based on the colour or precipitate formed during the reaction [Dubale, 2023].

Dragendorff's Test: Dragendorff's reagent is indeed composed of a solution of nitrate or chloride combined with either potassium iodide or iodine dissolved in potassium iodide solution. Take 1 ml of your sample and then add 1 ml of water to the sample. Mix the sample and water thoroughly to ensure homogeneity. Once prepared the sample, add Dragendorff's reagent. Typically, add a few drops of Dragendorff's reagent to the sample mixture and observe any changes. If alkaloids are present in the sample, it can observe the formation of red precipitates or colour changes, indicating the presence of alkaloids.

Mayer's Test: Mayer's reagent is another chemical reagent commonly used in alkaloid testing. Mayer's reagent is typically prepared by dissolving mercuric chloride in a solution of potassium iodide. Take 1 ml of your sample and Add 1 ml of distilled water to the sample, then mix the sample and water thoroughly to ensure homogeneity. Add 200 microliters (µL) of Mayer's reagent to the sample mixture and mix the solution gently. Observe any changes in colour or the formation of precipitates. The appearance of a creamy white or yellow precipitate, indicate the presence of alkaloids in the sample.

Wagner Test: The Wagner test is a simple chemical test used to detect the presence of alkaloids in a given substance. Take 1 millilitre (ml) of the plant sample and add 1 ml of distilled water to the plant sample. This helps in dissolving the alkaloids present in the sample. Ensure thorough mixing of the plant sample with the distilled water to ensure homogeneity. After the sample is properly mixed, add 200 microliters (µl) of Wagner's reagent to the mixture. Wagner's reagent typically consists of a solution of iodine dissolved in potassium iodide. After adding the Wagner's reagent, observe any colour change that occurs in the mixture.

Glycoside: The glycoside test is a chemical test used to detect the presence of glycosides in a given substance. Glycosides are a diverse group of naturally occurring compounds that consist of a sugar molecule (glycone) attached to another molecule (aglycone or genin) through a glycosidic bond [Khan, 2020].

Fehling Solution: Fehling's solution is a chemical reagent used primarily for detecting the presence of reducing sugars, such as glucose and fructose, in a solution. Here's a step-by-step procedure incorporating Fehling's solution for the glycoside test: Take 1 millilitre (ml) of the plant sample suspected to contain glycosides. Add 200 microliters (µl) of Fehling's solution to the plant sample. Heat the mixture gently. The presence of reducing sugars in glycosides will cause them to react with Fehling's solution, resulting in the reduction of copper ions (Cu^2+) to copper oxide (Cu2O), which forms a reddish-brown precipitate. After heating, allow the mixture to cool down to room temperature. Observe any color changes or formation of a precipitate in the mixture.

Sulphuric Acid Test: The sulphuric acid test is a common chemical test used to detect the presence of glycosides in organic compounds. Take 500 microliters of your plant sample and add it to a suitable container. Next, add 500 microliters of distilled water to the container containing the plant sample. This helps to dilute the sample and create a solution that is easier to work with. Add 200 microliters of acetic acid to the mixture. Acetic acid is often used in extraction processes to help solubilize organic compounds, including steroids. Finally, add 100 microliters of sulfuric acid to the mixture. Sulfuric acid is commonly used in steroid tests to facilitate the reaction that helps identify the presence of steroids. After adding all the components, ensure thorough mixing of the solution to ensure uniform distribution of the acids and the sample. Once the sample is prepared, it can be observed that the greenish colour precipitate is formed in test-tube.

Volatile Oil: A volatile oil test, often referred to as steam distillation or steam volatile oil test, is a method used to extract essential oils from plants [Liang, 2020]. Take 1 ml of the plant sample. Add 100 microliters (µl) of sodium hydroxide (NaOH) to the sample. NaOH is a strong base that helps in the saponification of any fats or oils present in the sample. Mix the sample thoroughly after adding NaOH. Shaking the mixture helps in ensuring that the NaOH is well-distributed and facilitates the reaction with any oils present in the sample. Add 100-200 microliters (µl) of diluted hydrochloric acid (HCl) to the mixture. The HCl likely neutralizes the NaOH and adjusts the pH of the solution.

Terpenoid Test: A terpenoid test is a method used to detect the presence of terpenoids in a sample. Terpenoids are a large class of naturally occurring organic compounds derived from terpenes, which are hydrocarbons found in the essential oils of many plants [Jiang, 2016].

This test provides qualitative information about the presence or absence of terpenoids in a sample and can be a useful tool for preliminary screening.

Phlobatannins Test: Phlobatannins are a class of polyphenolic compounds found in plants. They are a type of condensed tannin, which means they are formed by the polymerization of flavan-3-ols. Phlobatannins are often found in foods such as red beans, sorghum, and some fruits [Auwal, 2014]. Take 1 millilitre (1 mL) of the plant solution. Add dilute hydrochloric acid (HCl) to the plant solution. Observe for the formation of a red precipitate. If a red precipitate forms upon the addition of HCl, it suggests the presence of phlobatannins in the plant extract.

Antibiotic Sensitivity Test

The antibiotic sensitivity test, also known as antimicrobial susceptibility testing, is a laboratory technique used to determine the effectiveness of specific antibiotics against a bacterial strain isolated from a patient. Here's a step-by-step guide to carry out the procedure: Preparation of 25 ml NAM Solution:Calculate the required amounts of the components for the NAM (Nutrient Agar Medium) solution. Typically, this includes peptone, beef extract, agar, and water. Dissolve the appropriate amounts of peptone and beef extract in distilled water in a flask or beaker. Stir the mixture until the components are completely dissolved. Add agar to the solution and mix well. Adjust the pH of the solution if necessary. Adjust the final volume to 25 ml with distilled water if needed. Sterilize the NAM solution by autoclaving at 121°C for 15 minutes. Furthermore, Pour the autoclaved NAM solution into sterile Petri dishes. Allow the NAM solution to solidify and form agar plates. Divide each plate into four quadrants using a marker or label. Inoculate each quadrant with the desired sample, Spread nutrient broth in one quadrant (positive control).In the other three quadrants, place plant samples that have been sterilized or treated appropriately to avoid contamination. In the fourth quadrant, add the antibiotic sample Amoxicillin by placing an antibiotic disc or spot. After 24 hours of incubation, observe the plates for bacterial growth. Measure the zones of inhibition around the antibiotic sample to determine the effectiveness of the antibiotic against the bacterial isolates. Compare the growth patterns and zones of inhibition between different samples to assess antibiotic sensitivity. Record and analyse the results.

RESULT AND DISCUSSION

Observing growth of bacterial colonies on an MRS (De Man, Rogosa, and Sharpe) agar plate after 24 hours of incubation indicates that the medium supports the growth of lactic acid

bacteria, commonly used in the fermentation industry and in the study of probiotics. These bacteria are typically found in fermented foods like yogurt, cheese, and sourdough bread. The MRS agar provides a suitable environment for the selective growth of these bacteria due to its specific nutrient composition and pH level.

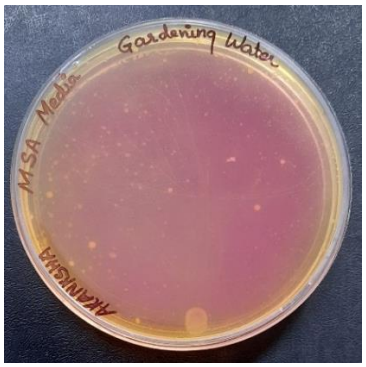


Figure 1: The figure shows the growth of bacteria in MRS media with Gardening water.

Isolated pure bacteria from a single piece of bacteria in Zig-Zag pattern in Nutrient agar media. After incubation observe the growth of bacteria.

Figure 2: The result shows the growth of Staphylococcus Aureus in NAM media.

The result of a Gram stain experiment typically involves observing the colour of the bacterial cells under a microscope after staining. Gram-positive bacteria retain the violet colour of the crystal violet stain and appear purple under the microscope. Gram-negative bacteria do not retain the violet stain and instead take on the colour of the safranin counterstain, appearing pink or red under the microscope. Observing the staining pattern can provide valuable information about the morphology and cell wall characteristics of the bacteria being studied. Additionally, the Gram stain result can be an important initial step in the identification and classification of bacterial species.

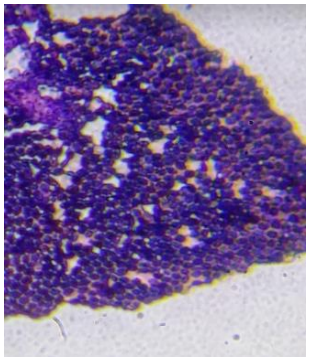


Figure 3: The result of gram staining is positive and it is spherical shaped.

They are seen moving long distances across the field of view the it is motile and if they remain in one place and do not show movement other than Brownian motion then they are non-motile. The results of handing drop.

There are many different biochemical tests which used to identified particular bacteria spp with positive result.

Figure 4: The figure show the positive result for the test in S. aureus.

Figure 5:The indole test has positive result for S. aureus.

The bubble formulation occurs shows the result is positive whereas no bubble formulation shows the outcome is negative.

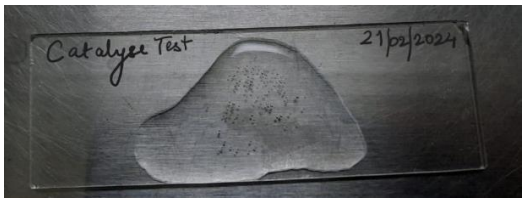


Figure 6: The test has bubbles formation, this shows positive result.

Figure 7: The result is positive for Nitrate test after adding zinc dust.

Figure 8:The D-mannitol test for staphylococcus aureus is positive.

Figure 9: The test show the growth of bacteria after 24 hour, which is positive result.

If a clear zone is observed around the bacterial colony, it indicates that the bacteria produced amylase and hydrolysed the starch. If no clear zone is present, the bacteria did not produce amylase, and the starch remains intact.

Figure 10: The test show the light zone around the bacterial colonies. The result is positive.

Antimicrobial and Phytochemical Studies on medical plant for treatment of Staphylococcus Aureus.

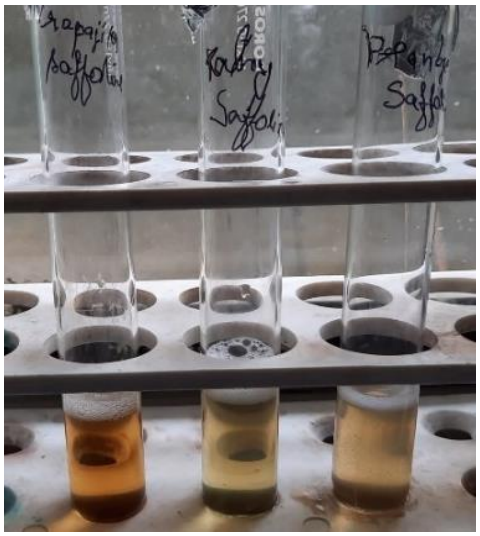


Figure 11: The form formation are visible, this show positive result of saponin test.

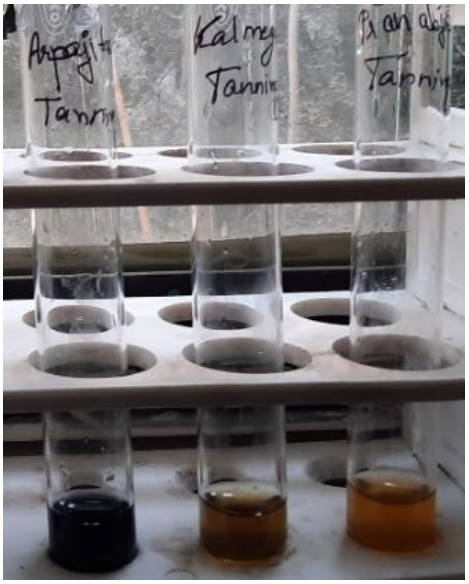
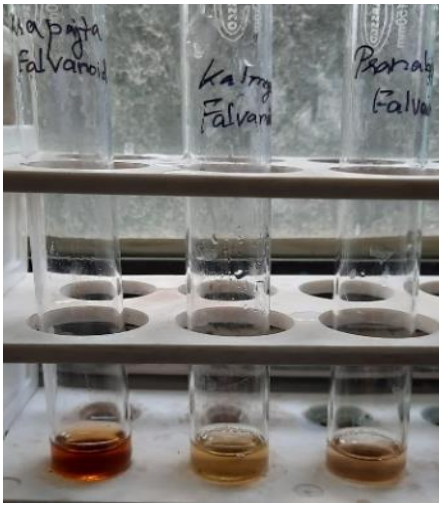
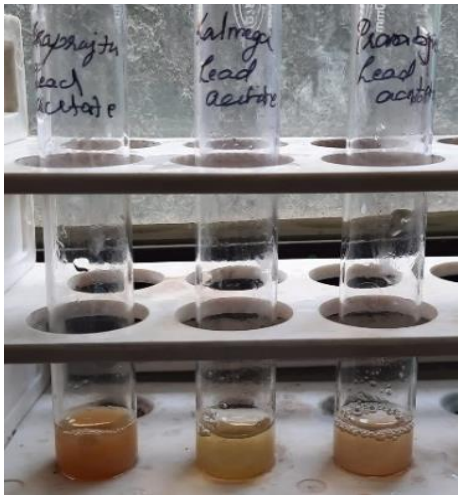


Figure 12: The result show positive test of Tannin in plant sample.

A positive result would typically be indicated by the formation of a precipitate or a colour change, which suggests the presence of flavonoids in the plant sample. If a precipitate forms or if there's a noticeable colour change (usually yellow to brown or dark brown), it indicates a positive result for the presence of flavonoids. However, if there's no significant change in colour or appearance, it suggests that flavonoids may not be present in the sample or may be present in very low concentration.



(a) Sodium hydroxide test.

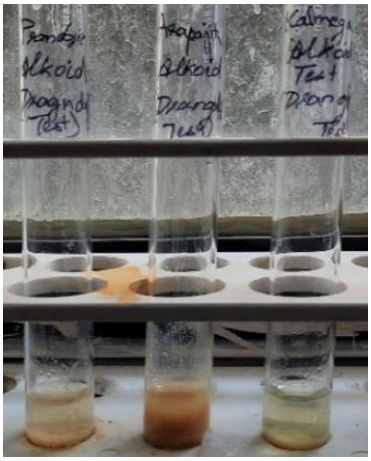


(b) Lead Acetate.

(c) Ammonia Test.

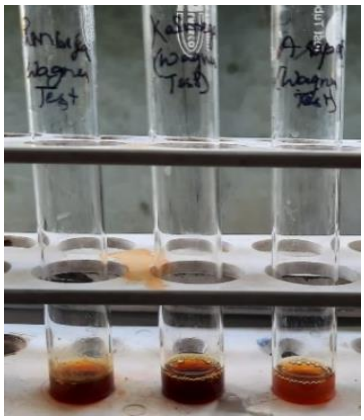
Figure 13: All three test show positive result for Flavonoid test in three different methods.

The appearance of a creamy white or yellow precipitate, indicate the presence of alkaloids in the sample. If alkaloids are present in the plant sample, a coloured precipitate may form, indicating a positive result for alkaloids.



(a) Dragndroff,s Test

(b) Mayer Test.



(c) Wagner Test

Figure 14: All three show the positive result for Alkaloid Test in three methods.

A positive result for glycosides is indicated by the formation of a reddish-brown precipitate. The presence of a reddish-brown precipitate confirms the presence of reducing sugars in the sample, which suggests the presence of glycosides.

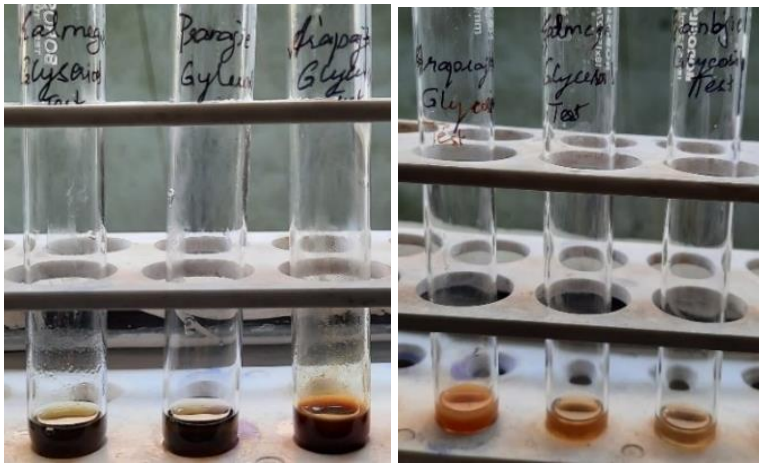


Figure 15: The result shows the positive result for Glycoside test in two different ways.

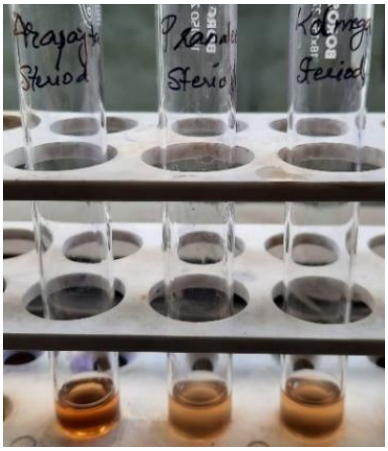


Figure 16: The figure show positive result for steroid test.

Figure 17: The result show positive result for volatile oil in three plant sample.

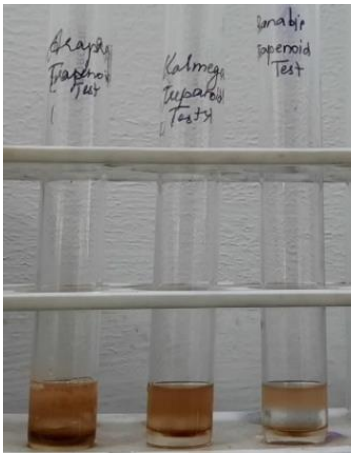


Figure 18: The result show reddish brown ring which indicate positive result for Terpenoid test.

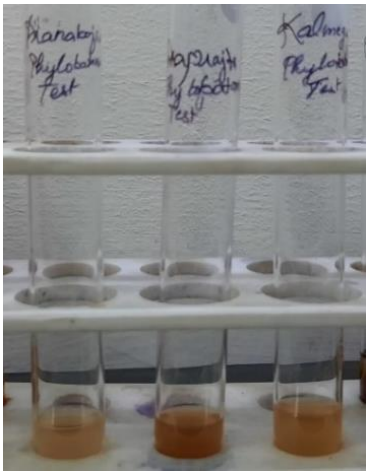


Figure 19: The result show red precipitate which indicate positive result for Phalobatannies test.

The table show the result of present of phytochemical test in three different plant sample.

Phytochemical test	Araprajita	Pranabjie	Kalmega
Saponin Test	+	+	+
Tannin Test	+ + +	+ +	Maybe
Flavonoid Test	+ +	+ +	++
Alkaloid test	Absent	+	++
Glycoside test	+ + +	+ + +	++
Steroid Test	+	+	+
Volatile oil	+	Maybe	+
Terpenoid Test	++	+	+
Paleobotanies test	+	+	Maybe

After incubation, the plates are examined to see how well the bacteria have grown in the presence of each antibiotic. The presence or absence of bacterial growth around each antibiotic disk indicates whether the bacteria are susceptible, intermediate, or resistant to that particular antibiotic. The results of the sensitivity test are typically reported as a list of antibiotics (Amoxicillin) along with their corresponding interpretations (susceptible, intermediate, or resistant).

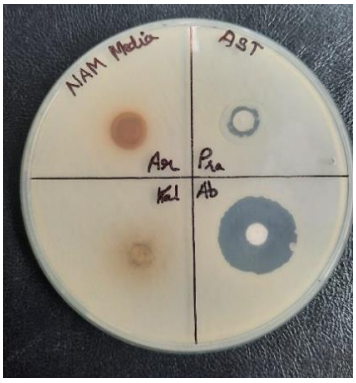


Figure 20: The figure show positive result for Ar and Pr and Ab for AST.

Table 20: Antibiotic sensitivity measurement for different substant.

Substant	Zone of Inhibition
Aparajita (Ar)	8.5mm
Pranabjie (Pr)	9.5 mm
Kalmega (Kal)	No inhibition
Antibiotic (Ab)	20 mm

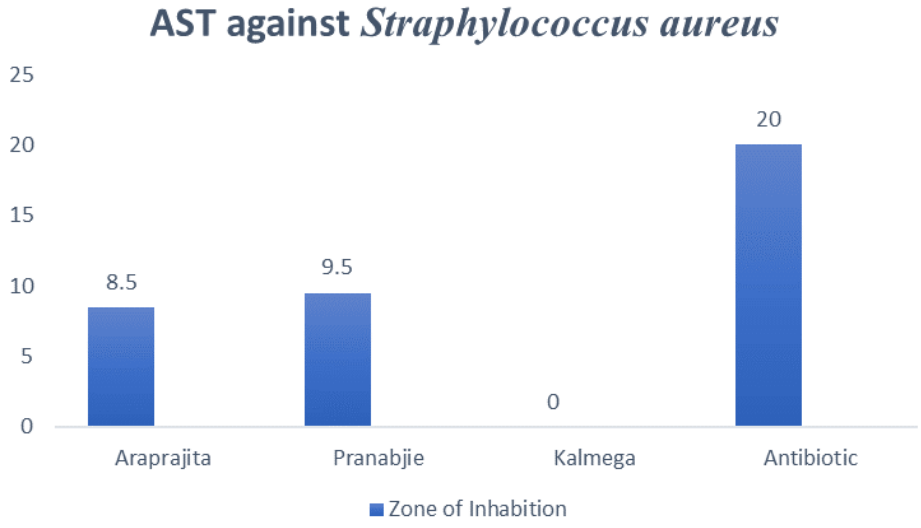


Figure 21: AST against *Staphylococcus aureus.*

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CONFLICT OF INTEREST

The author (s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCE

1. Abdi, R. D., Gillespie, B. E., Vaughn, J., Merrill, C., Headrick, S. I., Ensermu, D. B., D'Souza, D. H., Agga, G. E., Almeida, R. A., Oliver, S. P., & Kerro Dego, O. Antimicrobial Resistance of Staphylococcus aureus Isolates from Dairy Cows and Genetic Diversity of Resistant Isolates. *Foodborne pathogens and disease*, 2018; *15*(7): 449–458.
2. Abid, S., Farid, A., Abid, R., Rehman, M. U., Alsanie, W. F., Alhomrani, M., Alamri, A. S., Asdaq, S. M. B., Hefft, D. I., Saqib, S., Muzammal, M., Morshedy, S. A., Alruways, M. W., & Ghazanfar, S. Identification, Biochemical Characterization, and Safety Attributes of Locally Isolated *Lactobacillus fermentum* from *Bubalus bubalis* (buffalo) Milk as a Probiotic. *Microorganisms*, 2022; *10*(5): 954.
3. Alekshun, M. N., & Levy, S. B. Molecular mechanisms of antibacterial multidrug resistance. *Cell*, 2007; *128*(6): 1037–1050.
4. Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). *Veterinary research forum: an international quarterly journal*, 2014; *5*(2): 95–100.
5. Azeredo H.M.C., Waldron K.W. Crosslinking in polysaccharide and protein films and coatings for food contact—A review. *Trends Food Sci. Technol.*, 2016; 52: 109–122.
6. Balamuguran G, Selvarajan S. Preliminary phytochemical Investigation and Anthelmintic activity of Indigofera tinctoria linn. *Int J Drug Dev Res.*, 2009; 1: 157–160.
7. Barry, A. L., Bernsohn, K. L., Adams, A. P., & Thrupp, L. D. Improved 18-hour methyl red test. *Applied microbiology*, 1970; *20*(6): 866–870.
8. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab (Lond)*, 2005; 2: 5.

9. Bereksi, M.S., Hassaine, H., Bekhechi, C., & Abdelouahid, D.E. Evaluation of Antibacterial Activity of some Medicinal Plants Extracts Commonly Used in Algerian Traditional Medicine against some Pathogenic Bacteria. *Polymer Journal,* 2018; *10:* 507-512.
10. Boucher, H. W., & Corey, G. R. Epidemiology of methicillin-resistant Staphylococcus aureus. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, 2008; *46(5):* S344–S349.
11. Burnham JP, Kollef MH. Treatment of severe skin and soft tissue infections: a review. *Current opinion in infectious diseases*, 2018; *31*(2): 113–119.
12. Cannon MC, Nuttall FQ, Krezowski PA, Billington CJ, Parker S. The serum insulin and plasma glucose responses to milk and fruit products in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia,* 1986; 29: 784–791.
13. Charoenca, N., & Fujioka, R.S. Association of staphylococcal skin infections and swimming. *Water Science and Technology,* 1995; *31:* 11-17.
14. Chase CW, Barker DE, Russell WL, Burns RP. Serum amylase and lipase in the evaluation of acute abdominal pain. Am Surg., 1996; 62(12): 1028-33.
15. Dahlén, G., Hassan, H., Blomqvist, S., & Carlén, A. Rapid urease test (RUT) for evaluation of urease activity in oral bacteria in vitro and in supragingival dental plaque ex vivo. *BMC oral health*, 2018; *18*(1): 89.
16. Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *Journal of experimental pharmacology*, 2023; *15:* 51–62.
17. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *Complement Altern Med.*, 2006; 6: 35–41.
18. Dzotam Kamgue, Joachim & Kuete, Victor. Antibacterial and Antibiotic-Modifying Activity of Methanol Extracts from Six Cameroonian Food Plants against Multidrug-Resistant Enteric Bacteria. BioMed Research International, 2017; 1-19.
19. Elfalleh, W., Tlili, N., Nasri, N., Yahia, Y., Hannachi, H., Chaira, N., Ying, M., & Ferchichi, A. Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (Punica granatum) fruits. *Journal of food science*, 2011; *76*(5): C707–C713.
20. Fattahi, S., Zabihi, E., Abedian, Z., Pourbagher, R., Motevalizadeh Ardekani, A., Mostafazadeh, A., & Akhavan-Niaki, H. Total Phenolic and Flavonoid Contents of

Aqueous Extract of Stinging Nettle and In Vitro Antiproliferative Effect on Hela and BT-474 Cell Lines. *International journal of molecular and cellular medicine*, 2014; *3*(2): 102–107.

21. Fay, G. D., & Barry, A. L. Methods for detecting indole production by gram-negative nonsporeforming anaerobes. *Applied microbiology*, 1974; *27*(3): 562–565.
22. Iwase, T., Tajima, A., Sugimoto, S., Okuda, K., Hironaka, I., Kamata, Y., Takada, K., & Mizunoe, Y. A simple assay for measuring catalase activity: a visual approach. *Scientific reports*, 2013; *3:* 3081.
23. Jiang, Z., Kempinski, C., & Chappell, J. Extraction and Analysis of Terpenes/Terpenoids. *Current protocols in plant biology*, 2016; *1:* 345–358.
24. Jin-Hyung Lee, Jintae Lee, Indole as an intercellular signal in microbial communities, *FEMS Microbiology Reviews*, July, 2010; 34(4): 426–444.
25. Joshi R.K. A Perspective on the Phytopharmaceuticals Responsible for the Therapeutic Applications. In: Keservani R.K., Sharma A.K., Keservani R.K., editors. *Recent Advances in Drug Delivery Technology*. IGI Global; Hershey, PA, USA, 2017; 208–228.
26. Joshi, S., Ray, P., Manchanda, V., Bajaj, J., Chitnis, D.S., Gautam, V., Goswami, P.N., Gupta, V., Harish, B.N., Kagal, A., Kapil, A., Rao, R., Rodrigues, C., Sardana, R., Devi, K.S., Sharma, A., & Balaji, V. Methicillin resistant Staphylococcus aureus (MRSA) in India: Prevalence & susceptibility pattern. *The Indian Journal of Medical Research*, 2013; *137:* 363 - 369.
27. Kaczmarek B. Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials-A Minireview. *Materials (Basel, Switzerland)*, 2020; *13*(14): 3224.
28. Kancherla, N., Dhakshinamoothi, A., Chitra, K., & Komaram, R. B. Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (In Vitro). *Maedica*, 2019; *14*(4): 350–356.
29. Khan, H., Pervaiz, A., Intagliata, S., Das, N., Nagulapalli Venkata, K. C., Atanasov, A. G., Najda, A., Nabavi, S. M., Wang, D., Pittalà, V., & Bishayee, A. The analgesic potential of glycosides derived from medicinal plants. *Daru: journal of Faculty of Pharmacy, Tehran University of Medical Sciences*, 2020; *28*(1): 387–401.
30. Khan, Z. A., Siddiqui, M. F., & Park, S. Current and Emerging Methods of Antibiotic Susceptibility Testing. *Diagnostics (Basel, Switzerland)*, 2019; *9*(2): 49.
31. Kuete, V., & Efferth, T. Cameroonian medicinal plants: pharmacology and derived natural products. *Frontiers in pharmacology*, 2010; *1:* 123.

32. Lebot V. Rapid quantitative determination of maltose and total sugars in sweet potato (*Ipomoea batatas* L. [Lam.]) varieties using HPTLC. *Journal of food science and technology*, 2017; *54*(3): 718–726.
33. Li, H., Li, L., Chi, Y., Tian, Q., Zhou, T., Han, C., Zhu, Y., & Zhou, Y. Development of a standardized Gram stain procedure for bacteria and inflammatory cells using an automated staining instrument. *Microbiology Open*, 2020; *9*(9): e1099.
34. Liang, Y., Zou, J., Zhang, X., Shi, Y., Tai, J., Wang, Y., Guo, D., & Yang, M. Preparation and quality evaluation of a volatile oil microemulsion from Flos magnoliae and Centipeda minima. *Molecular medicine reports*, 2020; *22*(6): 4531–4540.
35. Liauw, S., & Saibil, F. Sorbitol: Often forgotten cause of osmotic diarrhea. *Canadian family physician Medecin de famille canadien*, 2019; *65*(8): 557–558.
36. Lowy F. D. Staphylococcus aureus infections. *The New England journal of medicine*, 1998; *339*(8), 520–532.
37. Markov D. D. Sucrose Preference Test as a Measure of Anhedonic Behavior in a Chronic Unpredictable Mild Stress Model of Depression: Outstanding Issues. *Brain sciences*, 2022; *12*(10): 1287.
38. McDougal L.K., Steward C.D., Killgore G.E., Chaitram J.M., McAllister S.K., Tenover F.C. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: Establishing a national database. *J. Clin. Microbiol*, 2003; 41: 5113–5120.
39. Moran, G. J., Krishnadasan, A., Gorwitz, R. J., Fosheim, G. E., McDougal, L. K., Carey, R. B., Talan, D. A., & EMERG Ency ID Net Study Group. Methicillin-resistant S. aureus infections among patients in the emergency department. *The New England journal of medicine*, 2006; *355*(7): 666–674.
40. Muniraj T, Dang S, Pitchumoni CS. PANCREATITIS OR NOT?--Elevated lipase and amylase in ICU patients. J Crit Care., 2015; 30(6): 1370-5.
41. Niu, C., Zhai, T., Zhang, Q., Wang, H., & Xiao, L. Research Advances in the Analysis of Nitrate Pollution Sources in a Freshwater Environment Using $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3$. *International journal of environmental research and public health*, 2021; *18*(22): 11805.
42. Njom Nlend, A. E., Motaze, A. N., Ndiang, S. T., & Fokam, J. Predictors of Virologic Failure on First-line Antiretroviral Therapy Among Children in a Referral Pediatric Center in Cameroon. *The Pediatric infectious disease journal*, 2017; *36*(11): 1067–1072.

43. Nortjie, E., Basitere, M., Moyo, D., & Nyamukamba, P. Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review. *Plants (Basel, Switzerland)*, 2022; *11*(15): 2011.
44. Orozco-Topete R., Sierra-Madero J., Cano-Dominguez C., Kershenovich J., Ortiz-Pedroza G., Vazquez-Valls E., Garcia-Cosio C., Soria-Cordoba A., Armendariz A.M., Teran-Toledo X., et al. Safety and Efficacy of Virend for Topical Treatment of Genital and Anal Herpes Simplex Lesions in Patients with AIDS. *Antivir. Res.*, 1997; 35: 91–103.
45. Peters J.-C.; Marker R.; Pan Z.; Breen J.-A.; Hill J. O. The Influence of Adding Spices to Reduced Sugar Foods on Overall Liking. *J. Food Sci.*, 2018; 83: 814–821.
46. Rahimah, S. B., Djunaedi, D. D., Soeroto, A. Y., & Bisri, T. The Phytochemical Screening, Total Phenolic Contents and Antioxidant Activities *in Vitro* of White Oyster Mushroom (*Pleurotus Ostreatus*) Preparations. *Open access Macedonian journal of medical sciences*, 2019; *7*(15): 2404–2412.
47. Rai, S., Kafle, A., Devkota, H. P., & Bhattarai, A. Characterization of saponins from the leaves and stem bark of *Jatropha curcas* L. for surface-active properties. *Heliyon*, 2023; *9*(5): e15807.
48. Robinson, B. W. A study of anxiety and academic achievement. *Journal of Consulting Psychology*, 1966; *30*(2): 165–167.
49. Robinton, E.D.; Mood, E.W. A quantitative and qualitative appraisal of microbial pollution of water by swimmers: A preliminary report. *J. Hyg.*, 1966; *64:* 489–499.
50. Sendino, T., Sandúa, A., Calleja, S., González, Á., & Alegre, E. Lactose tolerance test as an alternative to hydrogen breath test in the study of lactose malabsorption. *Advances in laboratory medicine*, 2021; *1*(4): 20200102.
51. Sequeira, I. R., Lentle, R. G., Kruger, M. C., & Hurst, R. D. Standardising the lactulose mannitol test of gut permeability to minimise error and promote comparability. *PloS one*, 2014; *9*(6): e99256.
52. Shah, F. A., Kitsios, G. D., Zhang, Y., Morris, A., Yende, S., Huang, D. T., O'Donnell, C. P., & McVerry, B. J. Rationale for and Design of the Study of Early Enteral Dextrose in Sepsis: A Pilot Placebo-Controlled Randomized Clinical Trial. *JPEN. Journal of parenteral and enteral nutrition*, 2020; *44*(3): 541–547.
53. Shuval H. Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. *Journal of water and health*, 2003; *1*(2): 53–64.

54. Soleimany, A. P., Martin-Alonso, C., Anahtar, M., Wang, C. S., & Bhatia, S. N. Protease Activity Analysis: A Toolkit for Analyzing Enzyme Activity Data. *ACS omega*, 2022; *7*(28): 24292–24301.
55. Suhrabi Z, Taghinejad H. A Comparative Study on the Efficacy of Ibuprofen and Celecoxib on the Intensity of Perineal Pain Following Episiotomy: A Randomized Clinical Trial. *Iran Red Cres Med J.*, 2013; 15(12): e9980.
56. Taylor, T. A., & Unakal, C. G. Staphylococcus aureus Infection. In *StatPearls*. StatPearls Publishing, 2023.
57. Tlili N., Elfalleh W., Saadaoui E., Khaldi A., Triki S., Nasri N. The Caper (*Capparis* L.): Ethnopharmacology, Phytochemical and Pharmacological Properties. *Fitoterapia*, 2011; 82: 93–101.
58. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev., 2015; 28(3): 603-61.
59. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G., Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical microbiology reviews*, 2015; *28*(3): 603–661.
60. Van Hofwegen, D. J., Hovde, C. J., & Minnich, S. A. Rapid Evolution of Citrate Utilization by Escherichia coli by Direct Selection Requires citT and dctA. *Journal of bacteriology*, 2016; *198*(7): 1022–1034.
61. Van Nieuwenhoven MA, Geerling BJ, Deutz NEP, Brouns F, Brummer RJM The sensitivity of the lactulose/rhamnose gut permeability test. *European Journal of Clinical Investigation*, 1999; 29: 160–165.
62. Voravuthikunchai SP, Lortheeranuwat A, Ninrprom T, Popaya W, Pongpaichit S, Supawita T. Effective medicinal plants against enterohaemorrhagic Escherichia coli O157: H7. J Ethnopharmacol, 2004; 94: 49–54.
63. Voravuthikunchai, S., Lortheeranuwat, A., Jeeju, W., Sririrak, T., Phongpaichit, S., & Supawita, T. Effective medicinal plants against enterohaemorrhagic Escherichia coli O157:H7. *Journal of ethnopharmacology*, 2004; *94*(1): 49–54.
64. Wang Y.J., Peng J.F., Cao X.F., Xu Y., Qu J.H. Isotopic and chemical evidence for nitrate sources and transformation processes in a plateau lake basin in Southwest China-ScienceDirect. *Sci.TotalEnviron*, 2020; **711**: 134856.
65. Wangchuk, P., Keller, P. A., Pyne, S. G., Taweechotipatr, M., Tonsomboon, A., Rattanajak, R., & Kamchonwongpaisan, S. Evaluation of an ethnopharmacologically selected

Bhutanese medicinal plants for their major classes of phytochemicals and biological activities. *Journal of ethnopharmacology*, 2011; *137*(1): 730–742.

66. Zhang P.; Hai H.; Sun D.; Yuan W.; Liu W.; Ding R.; Teng M.; Ma L.; Tian J.; Chen C. A high throughput method for total alcohol determination in fermentation broths. *BMC Biotechnology*, 2019; 19: 30–38.