In Vitro Antibacterial Approach of *Syzigium Cumini* Leaves Extract against Diarrhea Causing Bacteria by Using Different Biochemical Test

1. **Hajra Sohail*,** Lahore College for Women University
2. **Usman Minhas*,** Pakistan Council of Scientific and Industrial Research (PCSIR)
3. **Hafiza Ayesha Andleeb*,** M.Phil. Microbiology University of Lahore
4. **Eeza Shafique,** University of Central Punjab
5. **Dr Amina Mahmood**, University of Lahore
6. **Ayesha Asif**, University of Central Punjab
7. **Raazia Waseem**, Lahore College for Women University
8. **Ayesha Ali**, Lahore College for Women University
9. **Faiza Saleem (Corresponding Author)**, Lahore College for Women University, Faiza.saleem@lcwu.edu.pk

Abstract

An evergreen, glabrous tree native to Asia's tropical and subtropical climates is called *Syzigium cumini* (Family: Myrtaceae). Plants are abundant in bioactive substances, such as *(anthocyanin, quercetin, glycoside, kaempferol, antimellin and myrecetin*). By employing the agar well diffusion method, the high antibacterial activity of leaves against several bacteria that cause diarrhoea will be assessed in this study. Since diarrhoea is one of the leading causes of mortality and morbidity in children under the age of five, it has been spreading at an alarming rate around the world. *S. cumini* leaf extract had effective potential against *B. subtilis, Salmonella typhi, Salmonella typhi, E. coli,* and *S. aureus*. Along with multi-resistant antibiotics including, *Amoxilin*, *Azithromycin*, *Erythromycin*, and *Tetracycline*, it also demonstrated a synergistic impact. As a result, it will be used as an antibacterial and anti-diarrheal component in numerous pharmaceutical and health goods.

Keywords : evergreen, pharmaceutical, component, antibacterial

Tob Regul Sci. ™ 2023;9(1): 1526-1541
DOI: doi.org/10.18001/TRS.9.1.105

Introduction

Syzigium cumini (*S. cumini*) is a member of the Myrtle family (*Myrtaceae*). It is commonly found in milder climates across the United States of America, Eastern Africa, South America, and the Asian subcontinent. The common names of these evergreen tropical trees, such as Jamun, java plum, black plum, blackberry, and jambolana, are known all around the globe. (Devi *et al.*, 2020).

In Asia, particularly in Pakistan, India, Sri Lanka, Nepal, and Bangladesh, *S. cumini* is a native, minor crop. The tree typically reaches a height of 30 to 40 feet. It is a cross-fertilized plant with a long lifespan that can produce fruits for 60–70 years. Every year, *S. cumini* trees produce raspberries that are oblong or ellipsoid. The fruit has a sweet flavor and is used to make wine, juices, jellies, and other nutritious beverages. Scientific investigations have represented that *S. cumini* extracts have a range of medicinal properties, including antioxidant, antibacterial, anti-diarrheal, anti-cancer, anti-inflammatory, anti-diabetic, antiHIV, and anti-ulcerogenic properties. These extracts come from different kinds of plant parts, including flowers, buds, barks, leaves, flowers, and woods. *S. cumini* has multiple therapeutic and medicinal properties. All of its parts utilized in various ways, including seeds for the treatment of bleeding gums, leaves for the prevention of diarrhea and dysentery, and bark for antibacterial purposes. *S. cumini* plant components with different therapeutic characteristics are utilized to make herbal medications as they are regarded less harmful than synthetic ones. It can be use in the treatment of skin wounds because the extract from *S. cumini* leaves exhibits antibacterial and antifungal action against several pathogenic microorganisms (Shaheen *et al.*, 2019).

An examination of the antibacterial and toxic properties of the methanolic extract of *S. cumini* leaves was conducted. Both gram-positive and gram-negative bacteria growth was suppressed by the ethanolic leaf extract. An aerobic, Gram-positive soil bacterium is called *Bacillus subtilis*, has a single-cell membrane, which makes secreting proteins easier than in *Escherichia coli*. In the other infections, *B. subtilis* is linked to bacteraemia, endocarditis, pneumonia, and septicaemia. The rod-shaped bacteria known as *Klebsiella* belong to the *Enterobacteriaceae* family. Microbiologically, *Klebsiella* organisms are classified as facultative anaerobic, gramnegative, non-motile bacteria. There are *Klebsiella* organisms in soil, water, and plants, and some strains are thought to be a natural component of the human gastrointestinal tract's flora. *K. pneumonia*, *meningitis*, *urinary tract infection*, and bloodstream infection are all associated with it. Gram-positive *Staphylococcus aureus* bacteria are cocci-shaped and frequently grouped together in grape-like" clusters. Infections caused by *S. aureus*, one of the most prevalent bacteria in humans, include bacteraemia, infective endocarditis, skin and soft tissue infections, osteomyelitis, septic arthritis, infections of prosthetic devices, and pulmonary infections (Nolt and ondusko, 2018).

The only host for the gram-negative, rod-shaped, flagellated *Salmonella typhi* bacterium is the human body. Typhoid fever is the result. *Pseudomonas aeruginosa* is a Gram-negative, aerobic rod bacterium belonging to the Pseudomonadaceae family. It is incredibly hazardous for individuals suffering from serious burns, cancer, AIDS, tuberculosis, and soft tissue infections in the urinary tract, respiratory system, dermis, and blood. Tetracycline and Amoxilin are the two most efficient antibiotics that were employed in this investigation (Baker *et al.*, 2018).

Materials And Methods

3.1. Study Design

This study is an analytical observational cross-sectional. It lasted six months, starting in January, primarily focused on the antimicrobial activity of *S. cumini* leaf extracts against pathogenic bacterial strains. The Pakistan Council of Scientific and Industrial Research (PCSIR) complex, Laboratories Lahore and Lahore College for women university (LCWU) collaborated on the project.

3.2. Samples Collection

The samples were from the hospital. The hospital lab grew bacterial strains commonly found in stool samples from diarrheal patients on various media to facilitate easy characterization.

3.3. Culturing of Samples

A 24-hour incubation period at 37°C was followed by numerous biochemical tests to identify the species of colonies isolated from the material.

3.3.1. Culture Media

All agar and broth media were allowed to cool to 50°C in a water bath before being poured (approximately 25–30mL) into clean Petri plates. All media plates were set to 4°C and allowed to cool down at room temperature.

3.4. Isolation of Bacterial Strain

Swabs were constantly used on top of agar plates. For 24 hours, plates were put at 37°C in an incubator. Colonies exhibiting a particular appearance on culture and biochemical tests were isolated and streaked on brand-new, sterilized agar plates to obtain a well-isolated bacterial culture. Pure cultures were left in an incubator overnight at 37 °C.

3.5. Streak Plate Technique

The streaking was performed to get pure culture using a sterile inoculation wire-loop.

3.6. Identification of Bacterial Strains

The identification of microorganisms is based on numerous variables, including colony form, cell development, cell wall composition, nutritional needs, substrates employed in specific reactions, enzymes produced, and end products released. Bacterial strains are characterized by examining their morphology, the results of gram stain tests, and biochemical tests like the catalase and oxidase test.

3.6.1 Gram Staining and Cell Morphology

Gram staining to classify bacteria based on the physical and chemical characteristics of their cell walls. The strong peptidoglycan layer in the cell wall of gram-positive bacteria shows purple colour while gram negative shows pink colour (*Smith et al.,* 2005).

3.6.2. Biochemical Reactions

The only reliable way to identify the bacteria is through biochemical tests. Based on the biochemical functions that each type of bacteria performs, the tests are used to distinguish between them. Oxidase testing and catalase testing are two of the most significant and significant

biochemical tests used to distinguish between *E. coli*, *S. aureus*, *Pseudomonas*, *Salmonella typhi*, *Klebsiella sp.*, and *Bacillus subtilis*.

3.6.2.1 Oxidase Test

Oxidase is an enzyme produced by bacteria that uses oxygen as a hydrogen receptor to transform it into water or hydrogen peroxide. There are different reagents available, including the Kovacs Oxidase Reagent, Gordon and McLeod's Reagents which may be used for the test to find out if the bacteria's oxidase enzymes are present. (Shields *et al.*, 2010).

3.6.2.2. Catalase Test

The catalase enzyme helps toxic hydrogen peroxide (H_2O_2) break down into water and oxygen. When bacteria are added to a solution containing 3% H_2O_2, which leads to the rapid generation of oxygen bubbles, the enzyme can be identified using a catalase test.

3.6.2.3. Methyl Red Test

The methyl red test can be used to identify bacteria that use the mixed acid pathway to convert glucose into different types of acids (lactic acid, acetic acid, or formic acid). If bacteria are producing acid, the colour of the methyl red indicator will change to red; otherwise, the broth mixture will remain yellow (McDevitt et al., 2009).

3.6.2.4. Voges-Proskauer Test

The Voges-Proskauer test can be used to identify acetyl methyl carbinol (acetoin), which can be produced as a result of glucose fermentation.

3.6.2.5. Indole Ring Test

The indole ring test helps locate bacteria with the capacity to manufacture the enzyme tryptphanase. Amino acids in the bacterial broth changed into indole gas by this enzyme.
Kovac's reagent will be added to observe the gas.

3.6.2.6. Citrate Utilization Test

With the help of the permease enzyme, which turns citrate into pyruvate and promotes growth in culture media, this test can help identify bacteria that can use citrate as an energy source.. In this test, bromothymol blue is employed as an indicator; when the pH is raised to 7.6, it changes the indication colour from green to blue (Kaur *et al.*, 2021).

3.7. Preservation of Isolates

The length of time an isolation must be depends on its intended use.

3.7.1. Short Term Storage

When it was necessary to preserve bacterial colonies for a period, such as a few days or weeks, cultivated plates were properly and firmly wrapped with sterilized parafilm before being covered in a polythene sheet and kept in the refrigerator at 4°C.

3.7.2. Medium-Term Storage

On slants, bacterial colonies were kept for a few months. According to the manufacturer's recommendations, 5 to 6 ml of agar media were made, placed into glass tubes with caps, and sterilized in an autoclave at 12°C. After being streaked and incubated at 37°C for 24 hours, the isolated colony was wrapped firmly in parafilm and put in a refrigerator at 4°C.

3.7.3. Long-Term Storage

A few well-isolated bacterial characteristic culture colonies were placed in cryogenic vials filled with glycerol at a 30:70 ratio for long-term storage. The vials were kept at minus (-) 80°C until they were needed.

3.8. Antimicrobial Susceptibility Detection

The Kirby-Bauer disc diffusion method assesses the antimicrobial sensitivity and resistance of microorganisms. The pathogenic bacterium distributes over Mueller Hinton agar (MHA), and filter paper discs soaked in antibiotics are placed on the media. Finally, the culture plates are incubated at the proper temperatures. After that, growth is seen all around the disc. The efficacy of that antimicrobial agent to suppress the bacteria depends on the presence or absence of microorganism development (Hudzicki *et al.*, 2009).

3.9. Plant Extraction

The process of removing the required bioactive components from their natural source is called extraction. According to the extraction principle, several extraction procedures are applied, such as the distillation method and solvent extraction. The most popular extraction technique is solvent extraction, in which the compounds dissolve in the solvent as it permeates the solid matrix, causing the diffusion of the solid matrix's bioactive components, which can be collected One of the most significant and popular techniques for removing chemicals from different plant sources is microwave-aided extraction (Redfern et al., 2014).

3.9.1. Plant Sample Preparation

S. cumini leaves were used for the investigation. The leaves were cleaned with distilled water and let to dry for a week in the shade. The dried sample was then reduced in size by being crushed and ground, which increased the surface area of the samples. The size separation was carried out to improve the extraction process. The sample was ground into powder, and then uniform-sized particles were stored for extraction (Karimi *et al.*, 2019).

3.10. Antibacterial Activity of *Syzygium Cumini* Leaves Extract

3.10.1. Culture Medium and Inoculation Preparation

At 120°C for 20 minutes, the petri plates and nutritional medium were sterilized. Laminar airflow was used for the media pouring process. A sterile petri plate was filled with about 25ml of a medium, and they were left to solidify. After the agar plates have suitably hardened, cotton swabs were used to remove the bacterial strains onto the medium.

3.10.2. Agar Well Diffusion Method

S. cumini leaf extracts in ethanolic, methanolic, and aqueous forms were examined for antibacterial activity using the agar well diffusion method described by (Heggers *et al.*, 1990). Under aseptic conditions, sterile nutrient agar plates were produced and inoculated using the spread plate method. The agar has four 4mm-diameter wells cut out of it. Each well received 100µl of crude leaves, 100µl of ethanolic extract, 100µl of methanolic extract, 100µl of aqueous extract, and a positive control of organic solvent (ethanol, methanol). Six different bacterial strains, including *E. coli*, were the targets of the antibacterial action. *Salmonella typhi*, *Klebsiella*, *S. aureus*, *Pseudomonas*

and *Bacillus subtilis*. The diameter of the inhibition zone was used to measure the antibacterial activity of leaf extracts against a particular bacterial strain (in mm).

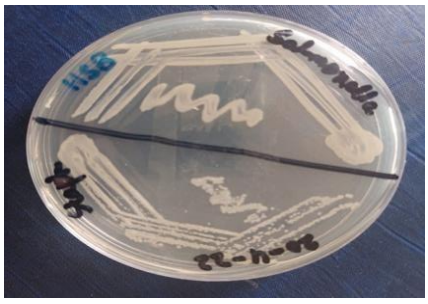
3.11. Synergistic Antibacterial Activity of *Syzygium Cumini* Leaves Extract with Antibiotics

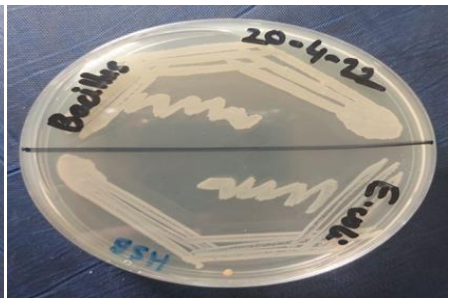
Synergistic effect is the one in which one agent enhances the effect of the other and they act more efficiently together as compared to the effect of individual agent.

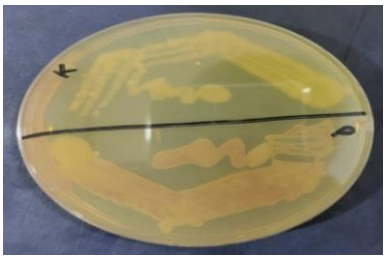
Results

4.1. Confirmatory Identification of Isolates

All the bacterial strains collected and preserved for the study was cultured on the nutrient agar plates and incubated in an incubator set at 37 ℃ for 24 hours. The colonies of bacterial strains which appeared in the agar plate after incubation were identified and categorized based on color, size and appearance. Some colonies had large, white appearance, other had greyish white, small, creamy and sticky appearance. Such as *E. coli* colonies appeared off-white or bluish black with metallic green shiny texture, *B. subtilis* colonies appeared rough, blurry white or yellow with irregular edges. Whereas *Pseudomonas* colonies were blue-green to yellow in color with skinny appearance, *S. aureus* colonies appeared golden yellow, circular, and pinpoint on the agar plates, *Salmonella typhi* colonies were blue to black in color with black centers and *Klebseila* colonies appeared slightly pink to white with sticky texture (Figure 4.1. Colonies of *E. coli, B. subtilis, Pseudomonas, S. aureus, Salmonella typhi* and *Klebseila* on nutrient agar plates). Further analyses of bacterial colonies were performed by using different techniques such as Gram staining and biochemical tests to confirm the isolated bacterial strains. **Figure 4.1:** Identification of Bacterial Colonies







a) ***Salmonella sp., S.aureus*** **(b)** ***B. subtilis, E. coli*** **(c)** ***Klebsiella sp., Pseudomonas sp.***

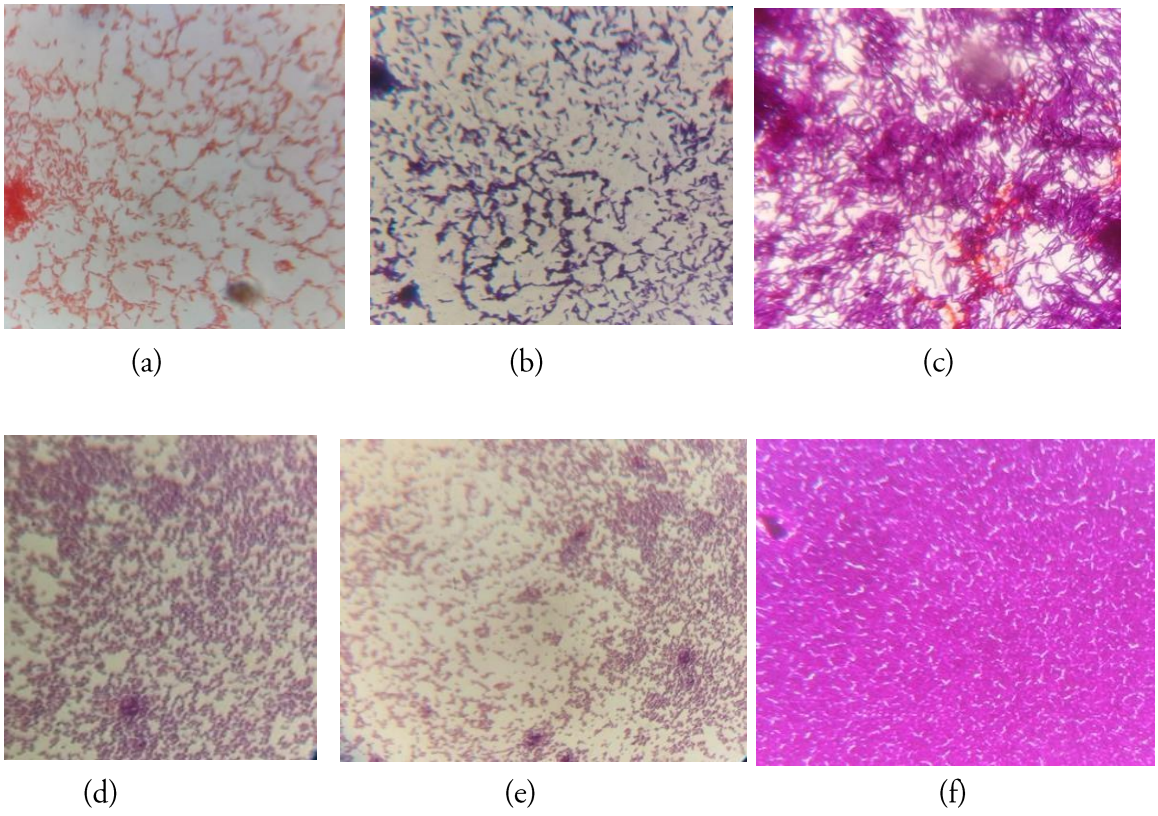
4.2. Identification of Bacterial Strains

Identification of strains was done through colony morphology, microscopic study and biochemical testing. Morphological characteristics gave the idea of the shape and cell wall properties. Biochemical tests show the ability of the microorganisms to utilize their resources for their survival.

4.2.1. Microscopic Study and Cell Morphology

Morphological analyses of bacterial strains was done using Gram staining method. With the help of standard microscope the color, shape and size of bacteria were observed. The bacteria which were stained purple to blue indicating the presence of large amount of peptidoglycan in their cell

wall and matched the morphological characteristics of Gram-positive bacteria i.e. *B. subtilis* and *S. aureus*. Whereas the bacteria which stained pink to red indicating the thin peptidoglycan layer in their cell wall and matched with the morphological characteristics of Gram-negative bacteria i.e. *Salmonella typhi, E. coli, Klebseila* and *Pseudomonas* (Figure 4.2.
Morphological analysis of bacterial strains under microscope).



(a) (b) (c)

(d) (e) (f)

Figure 4.2: Morphological Analysis of Bacterial Strains (a) *Salmonella sp.*, (b) *S.aureus*, (c) *B.subtilis*, (d) *Pseudomonas sp.*, (e) *E.coli* and (f) *Klebseila sp*. Under Microscope

4.2.2. Biochemical Test

Biochemical tests are used to identify the microorganisms. Different biochemical tests such as oxidase test, catalase test, indole ring test, methyl red test, Voges-Proskauer test and citrate utilization test were used to identify and confirm the bacterial strains.

4.2.2.1. Oxidase Test

All bacterial strains are oxidase negative, produce no color except *Pseudomonas* strain that have ability to produce oxidase enzyme and change into purple color (Figure 4.3).

4.2.2.2. Catalase Test

All the bacterial strains produced catalase enzymes to catalyze the decomposition of harmful hydrogen peroxide and formed bubble at 3% H_2O_2 solution (Figure 4.4.)

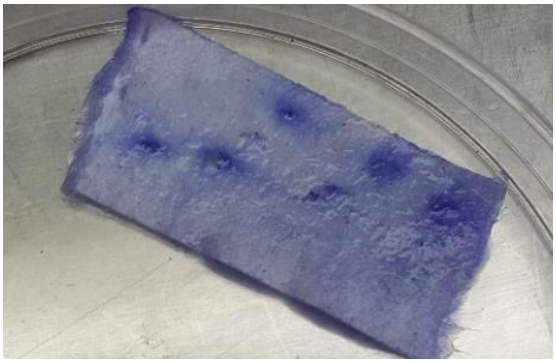


Figure 4.3: Oxidase Test (Positive Result) of Pseudomonas sp. Strain

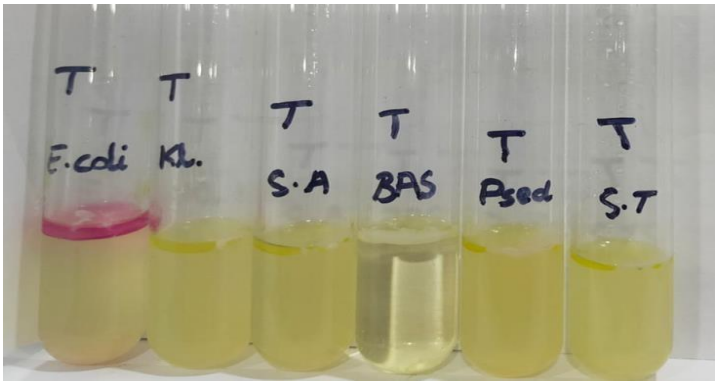
(a) (b) (c) (d) (e) (f)

Figure 4.4: Catalase Test (Positive Result) of Bacterial Strains

(a) *E.coli* (b) *S.aures* (c) *B.subtilis* (d) *Klebseila sp.* (e) *Salmonella sp.* and (f) *Pseudomonas sp.*

4.2.2.3. Indole Ring Test

E. coli is only produce indole gas and generates red color ring on the top of media whereas all the other bacterial strains are negative to the test and not produce indole gas (Figure 4.5. indole ring production in the media after adding Kovac's reagent confirms the presence of IR test positive bacteria in media).



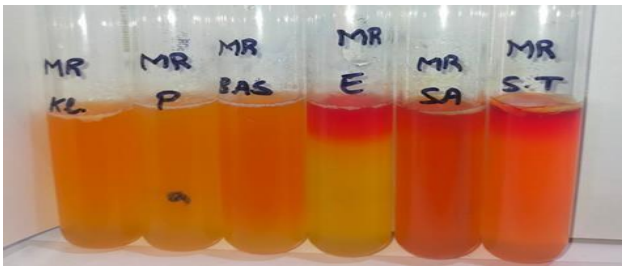
(a) (b) (c) (d) (e) (f)

Figure 4.5: Illustration of Indole Ring Test

(a) *E.coli* (Showed Positive Result) (b) *Klebseilla sp.* (c) *Salmonella sp.* (d) *B.subtilis* (e) *Psuedomonas sp.* (f) *S.aures* (Showed Negative Result)

4.2.2.4. Methyl Red Test

The bacterial strains that are positive to methyl red test have ability to convert sugars into various acids by fermentation which results in the reduction of the pH of the medium to below 4.5 i.e. *E. coli, Salmonella typhi* and *S. aureus*. Acids can be distinguished using methyl red indicator which changes its color to red if acid is present in the media. Pseudomonas, *B. subtilis* and Klebseila are negative to methyl red test (Figure 4.6. Color change in the media from cloudy yellow to dark orange after the addition of methyl red indicating the occurrence of MR test positive bacteria in the media).



(a) (b) (c) (d) (e) (f)

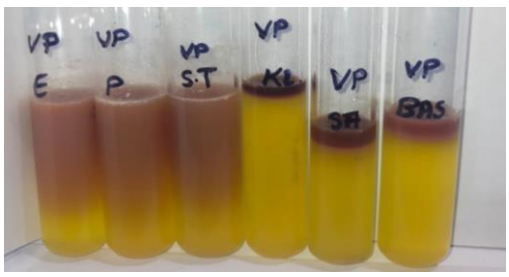
Figure 4.6: Illustration of Methyl Red Test

(a) *Klebseilla sp.* (b) *Psuedomonas sp.* and (c) *B. subtilis* (Showed Negative Result)

(b) *E.coli* (e) *Salmonella sp.* and (f) *S.aures* (Showed Positive Result)

4.2.2.5 Voges-Proskauer Test

B. subtilis, Klebseila and *Salmonella typhi* tested positive for VP which was detected by the color change of the reaction mixture. Whereas *E.coli, S.aureus* and *Pseudomonas* sp. are negative to VP test.



a) b) c) d) e) f)

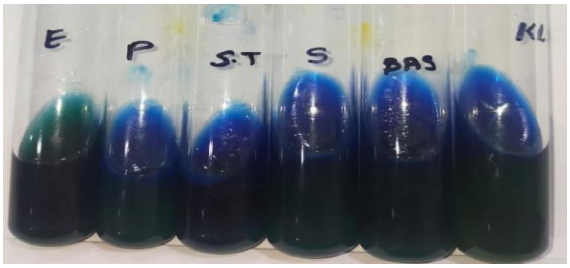
Figure 4.7: Illustration of Voges-Proskauer Test

***(a)E.coli* (b) *Psuedomonas sp.* and (c) *S.aures* (Showed Negative Result)**

***(d) Klebsiella sp.* (e) *Salmonella sp.* and (f) *B.subtilis* (Showed Positive Result)**

4.2.2.6. Citrate Utilization Test

All bacterial strains are positive to citrate utilization test, produce permease enzyme and show green color in slants. Whereas *E.coli* is the only bacteria that gives negative result and show no color change (Figure 4.7. color change of cultural media slant from green to blue indicates the existence of bacteria have ability to produce permease enzyme).



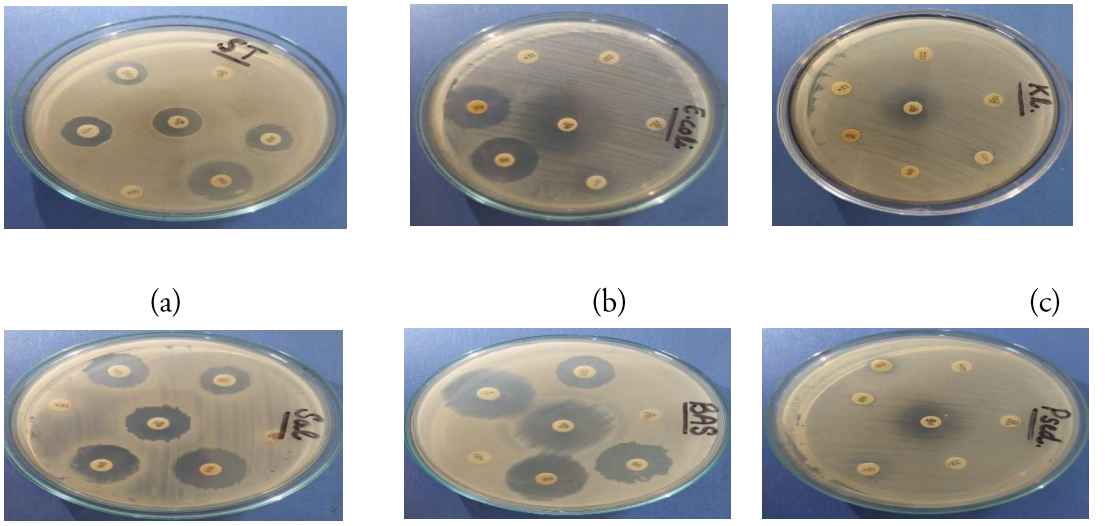
(a) (b) (c) (d) (e) (f)

Figure 4.8: Illustration of citrate utilization test

(a) *E.coli* (showed positive result) (b) *Klebsiella sp.* (c) *Psuedomonas sp.* (d) *B.subtilis* (e) *Salmonella sp.* and (f) *S.aures* (showed positive result). (a) to (f), from left to right

4.3. Antibiogram

Antibiotics susceptibility test is used to get the information about whether the bacteria are resistant or sensitive to the antibiotics available. The antibiotics used to profile the susceptibility/ resistance pattern of 6 pathogenic bacterial strains (i.e. *E. coli, B. subtilis, Pseudomonas, Salmonella typhi, Klebseila* and *S. aureus*) were Tetracycline, Amoxilin, Azithromycin, Cefoxitin, Vancomycin, Erythromycin and Penicillin. (Figure 4.8. Clear zones around the antibiotics in Kirby's disc diffusion method indicates the bacterial strains being sensitive to the antibiotics). The antimicrobial resistance against pathogenic strains categorized into three different categories i.e. resistance, intermediate and sensitive. Number of resistant bacteria against 7 antibiotics with their zone of inhibition was given in table 4.1. Among the antibiotics used, Penicillin and Cefoxitin was the least effective antibiotics against which approximately 98% bacteria were exhibited resistance. The most effective antibiotics used in present study is Tetracycline with 66.7% and Amoxilin with 50% bacteria exhibiting susceptibility to the antibiotics. Other antibiotics was not much effective as <55% bacterial strains showed sensitivity to them (Figure 4.9. Antibiogram of pathogenic strains obtained using Kirby's Disc Diffusion method).



(a) (b) (c)

(d) (e) (f)

Figure 4.9: Zone Of Inhibition of Different Antibiotic Discs on Muller-Hinton Agar Plates

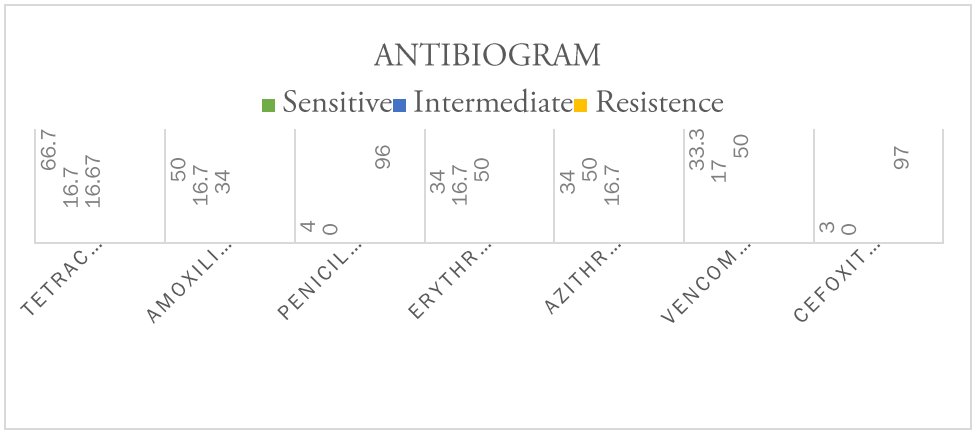
Organisms	Diameter of Zone of Inhibition (Mm)						
	TE (30µg)	AX (25µg)	AZM (15µg)	FOX (30µg)	E (15µg)	P (10µg)	VA (30µg)
E.coli	20	22	30	0	14	0	0
B.subtilis	23	23	30	0	27	0	17
Pseudomonas	10	0	20	0	0	0	0
Klebseila	0	0	20	0	0	0	0
S.aureus	20	15	13	0	15	0	11
Salmonella	24	23	20	0	20	0	14

* Bacterial strains (a) *S.aures* (b) *E.coli* (c) *Klebseilla sp.* (d) *B.subtilis* (e) *Salmonella sp.* and (f) *Psuedomonas Sp.*

* Antibiotics used are Tetracycline (30µg), Amoxilin (25µg), Azithromycin (15µg), Cefoxitin (FOX), Erythromycin (15µg), Penicilin (10µg) and Vencomycin (30µg)

Table 4.1: Pathogenic Strains Found Resistant, Intermediate and Sensitive to Antibiotics Represents antibiotics zones of inhibition from which Tetracycline and Amoxilin showed maximum antimicrobial activity against all the pathogens. Tetracycline gives >20mm (ZOI) against *Salmonella, S.aureus, E.coli* and *B.subtilis* whereas Amoxilin gives >23mm (ZOI) against *B.subtilis, salmonella* and *E.coli.* Penicillin and Cefoxitin were least effective to bacterial strains as all the bacteria were 99% resistant to them and zone of inhibition were obtained. Other antibiotics showed both resistant and intermediate results as <55% bacteria were sensitive to them.

Figure 4.10: Comparison of Antibiotics concentration on The Basis of Their Susceptibility Pattern

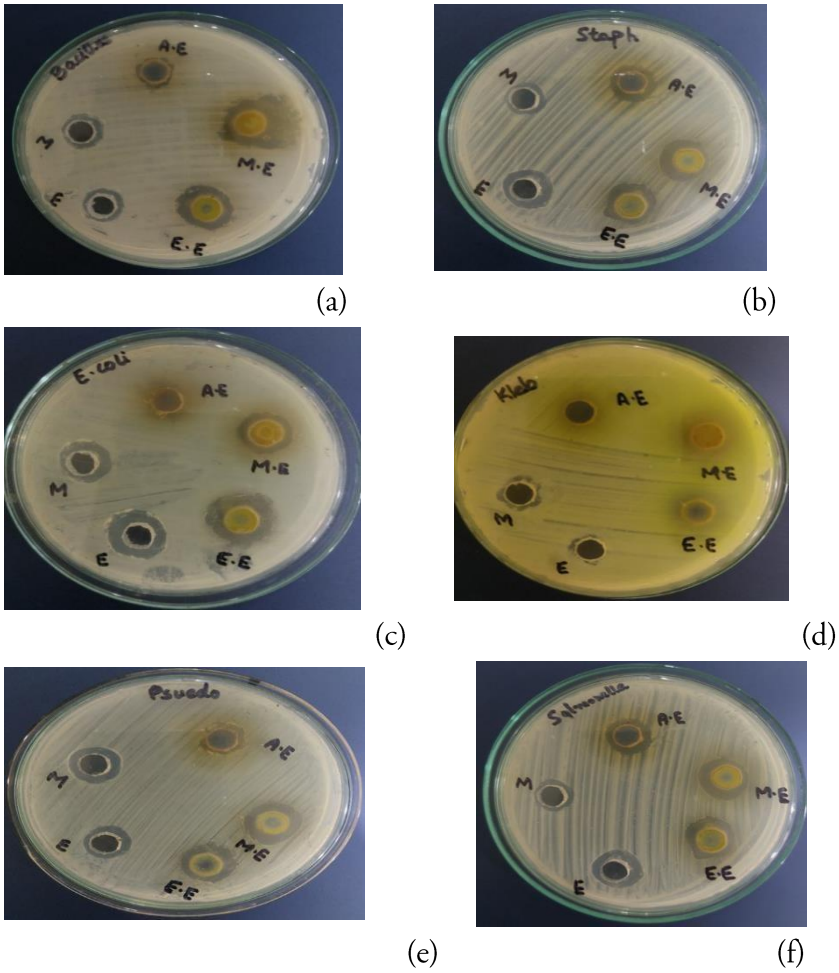


4.4. Antimicrobial Activity of *Syzygium Cumini* Leaves Extracts

Antibacterial activity provides sufficient protection against microorganisms as well as transmission of disease. It is used to get the information whether the bacteria are resistant, sensitive or intermediate to available extracts. The antimicrobial activity of *S. cumini* leaves extracts (ethanolic, methanolic and aqueous) were observed against diarrheal causing bacterial strains i.e. *E.coli, B.subtilis, Pseudomonas, Salmonella, S.aureus* and *Klebseila.* Ethanolic extract of Syzygiumcumini leaves showed resistance against all bacterial strains. The zone of inhibition (ZOI) of ethanolic, methanolic and aqueous extracts of *S. cumini* leaves were given in Table 4.2.

Table 4.2: Antimicrobial Activity of *S.cumini* Leaves Extracts by Using Well-Diffusion Method

Organisms		Diameter of Zone of Inhibition (mm)			
	Ethanolic Extract	Methanolic Extract	Aqueous Extract	Ethanol (Control)	Methanol (Control)
E.coli	16	13	10	13	12
B.subtilis	15	16	10	12	11
Pseudomonas	15	16	12	11	10
Salmonella	14	13	12	10	10
Klebseila	15	12	10	13	12
S.aureus	14	14	12	12	12



(a) (b) (c) (d) (e) (f)

Figure 4.11: Antibacterial Activity of *S.cumini* Leaves Extracts against Bacterial Strains (a) *B.subtilis* (b) *S.aures* (c) *E.coli* (d) *Klebsiella sp.* (e) *Psuedomonas sp.* and (f) *Salmonella sp.*

4.4 Synergistic effect of *Syzygium cumini* leaves extract

S.cumini leaves extract have ability to showed synergy when combined with multi- resistant antibiotics.

Table 4.3: Synergistic effect of *Syzygium cumini* leaves extract in combination with antibiotics

Organisms	of inhibition (mm)								
	SLE	AZT		ER T		AM X		TE T	
	Alone	Alone	Combined	Alone	Combined	Alone	Combined	Alone	Combined
E.coli	10	15	38 (S)	13	18 (A)	17	30 (S)	12	22 (AT)
B.subtilis	10	13	40 (S)	12	15 (A)	14	23 (A)	14	22 (A)
Salmonella	10	15	37 (S)	13	16 (A)	13	22 (A)	13	20 (A)
S.aureus	14	16	16 (I)	13	26 (A)	13	20 (A)	16	21 (A)

* Tetracycline (TE), Amoxilin (AX), Azithromycin (AZM), Erythromycin (E)

* Synergistic (S), Antagonistic (A), Indifferent (I), Additive (AT)

Table 4.3 Represents combination effects of *S.cumini* leaves extract with different antibiotics. Azithromycin along with *S.cumini* leaves showed synergistic effects toward all bacterial strains except *S.aureus* which showed indifferent effect. Erythromycin showed antagonistic effect against all pathogenic bacteria. While amoxilin showed mixed effects like antagonistic against *B.subtilis, S.aureus* and *Salmonella* whereas synergistic against *E.coli*. Tetracycline gave antagonistic effects towards most bacteria except *E.coli* which showed additive effect

DISCUSSION

In underdeveloped nations, diarrhoea is one of the leading causes of "morbidity" and "mortality." The definition of diarrheal disease is an irregular increase in the fluidity, frequency, and volume of faeces when compared to a person's usual discharge. Every year, diarrheal infections cause about 2.5 million deaths, of whom 50–70% are children under the age of five. *S. cumini* extracts were highly effective against the gram-positive and gramnegative bacteria that cause diarrhoea (Ahmad *et al.*, 2019). In this investigation, ethanolic, methanolic and aqueous *S. cumini* extracts showed significant antibacterial activity against isolated bacterial strains associated with diarrhoea. *S. cumini* leaf extracts were found to be antibacterial when tested against bacterial strains. Six strains (including *B.subtilis* and *S.aureus*) were gram-positive, and only two were gram-negative (i.e., *Salmonella typhi*, *Salmonella paratyphi*, *Pseudomonas and E.coli*). According to another study, *S. cumini* leaves have antibacterial efficacy against strains of bacteria that are multi-resistant, such as *Klebseila*, *Pseudomonas*, and *S. aureus*. *Candida krusei* was inhibited by *S. cumini* hydro-alcoholic crude extract with a maximal zone of inhibition of 14.7mm (Oliveira *et al.*, 2007). According to the results of this study, the primary substance discovered, 13.2% of which was α- Pinene, demonstrated several biological properties, including antibacterial, antioxidant, anticoagulant, gastro-protective, and anticancer properties. Antibiotic-resistant bacteria are becoming a serious global healthcare issue. Effective first-line medicines were replaced with second or third-line antibiotics, which were more costly, time-consuming, and dangerous treatments for bacterial infections. Plants can act as antimicrobial agents and boost the effectiveness of antibiotics (*Moussaoui et al.*, 2016).

Conclusion

This research showed the use of *S. cumini* leaf extracts in collaboration with multi-resistant drugs to combat bacteria that cause diarrhoea. The study demonstrated the *S. cumini* leaf extract's antibacterial capabilities. Additionally, the synergistic combination of the antibiotics and the extract enhances this feature. According to the study, several beneficial chemicals in the extract from *S. cumini* leaves showed antibacterial activity, particularly against *Pseudomonas sp*, *B. subtilis* and *E. coli*. *S. cumini* leaves are generally beneficial against microorganisms that cause diarrhoea.

References

[1] Ahmed R, M Tariq, M Hussain, A Andleeb, MS Masoud, I Ali and A Hasan. 2019. Phenolic contents-based assessment of therapeutic potential of *Syzygium cumini* leaves extract. *Journal of Sciences*. **14(8): 13-18.**

[2] Das MK and S Mandal. 2016. *Syzygium cumini* and *Mangifera indica* Seed Extracts.

[3] De Lacerda Leite GM, M de Oliveira Barbosa, MJP Lopes, G de Araújo Delmondes, DS Bezerra, IM Araújo, and MR Kerntof. 2021. Pharmacological and toxicological activities of α-humulene and its isomers: a systematic review. *Trends in Food Science and Technology*. **32(3): 255-274.**

[4] Devi A, VS Dahiya, SK Upadhyay, R Singh, I Sharma, and P Kamboj. 2020. Antimicrobial Activity and Phytochemical Constituents in seed, leaf and bark extract of *Syzygium cumini (L.). Plant Archives.* **20(2): 39-65.**

[5] Heggers JP, ES Carino, JA Sazy, JL Theissen, R Mann, R Rutan and DN Herndon. 1990. A topical antimicrobial test system (TOPITEST): Nathan's Agar Well revisited. *Surgical Research Communications.* **8(2): 109-116.**

[6] Hudzicki J. 2009. Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*. **15(3): 55-63.**

[7] *Journal of Infectious Diseases and Preventive Medicine*. **4(2): 1-2.**

[8] Karimi S, F Lotfipour, S Asnaashari, P Asgharian, Y Sarvari and S Hazrati. 2019. Phytochemical analysis and anti-microbial activity of some important medicinal plants from north-west of Iran. *Iranian Journal of Pharmaceutical Research: IJPR.* **18(4):**

[9] **1871.**

[10] Karkey A, GE Thwaites, and S Baker. (2018). The evolution of antimicrobial resistance in Salmonella Typhi. *Current opinion in gastroenterology*. ***34*(1): 25-30.**

[11] Kaur R, N Sidhu and GK Mavi. 2021. Estimation and removal of heavy metal from industrial sludge by bacterial strain. *Journal of Entomology and Zoology Studies*. **9(1): 1045-1050.**

[12] McDevitt S. 2009. Methyl red and Voges-Proskauer test protocols. *American Society for Microbiology*. **2(1): 8.**

[13] Moussaoui F and T Alaoui. 2016. Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants. *Asian Pacific Journal of Tropical Biomedicine*. **6(1): 32-37.**

[14] Ondusko DS and D Nolt. (2018). Staphylococcus aureus. *Pediatrics in review*. ***39*(6): 287-298.**

[15] Redfern J, M Kinninmonth, D Burdass and J Verran. 2014. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *Journal of Microbiology and Biology Education.* **15(1): 45-46.**

[16] Shaheen, R., Nisar, S., Kowalski, R., and Jilani, M. I. 2019. Essential oil isolates of Indian Black Berry Leaves: A review. *International Journal of Chemicals and Biochemical Sciences.*

[17] **16(19): 11-16.**

[18] Shields P and L Cathcart. 2010. Oxidase test protocol. *American Society for Microbiology*.

[19] **2(1): 1-9.**

[20] Smith, AC and MA Hussey. 2005. Gram stain protocols. *American Society for Microbiology*. **1(14): 113-144.**