Anti-bacterial activity of *Syzygium cumini* (L.) Skeels leaves extract.

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Abstract

It has been determined that *Syzygium cumini L.*, also known as jamun and a member of the Myrtaceae family, has anticancer properties. Bacterial infection a significant problem around the globe. Therefore, the primary objective of this work is to use S. cumini leaf extract as antibacterial agent. Fresh plant samples of the *S. cumini* species were collected Al-Zafaraniyah - Botanical Garden. Methanol was used for extraction. The effectiveness of the extract was evaluated as antibacterial agent by using agar well diffusion with different concentration of extract against two Gram negative bacteria (*Escherichia coli* , *Pseudomonas aeruginos*a) and two Gram positive (*Streptococcus pneumonia* , *Staphylococcus aureus*). The zones of inhibition were measured by using a caliper micrometer against the back of the petri plates . Leaf extract significantly exhibit the most potent as antibacterial with Inhibition zone diameter range 22.1mm -11.5mm against Gram positive and 21.8mm- 12.4mm against Gram negative. The minimum inhibitory concentrations (MICs) of extract were determined. The MIC for Gram positive bacteria was 104 µg/ml while the MIC for Gram negative bacteria was 208 µg/ml . The synergistic effect of *S. cumini* leaves extract was investigated with six antibiotics against the higher resistance isolates which appeared increase the antibiotic effect .

Key words : *Syzygium cumini , Myrtacea , Antibacterial activity .*

Introduction

There are around 150 genera and 3,600 species in the Myrtaceae plant family, which is found in the tropics and subtropics. (1). Jamun, also referred to as *Syzygium cumini* L., is a significant and widely cultivated member of this family (2). For many years, South Asian traditional medicine has employed the fruits, leaves, and seeds of S. cumini to treat diabetes mellitus. The leaves are employed as a diuretic and astringent. (3). They have antioxidant, hypolipidemic, anti-inflammatory, antipyretic, psychopharmacological, and hypoglycaemic effects. (4). *S. cumini's* fruits, stems, and leaves have all been researched for their antibacterial properties,

The antimicrobial activity of the *Syzygium cumini* leaves hydroalcoholic extract may be due to tannins and other phenolic constituents. *Syzygium cumini* is known to be very rich in gallic and ellagic acid polyphenol derivatives . *Syzygium* species have been reported to possess antibacterial activity (5). Aqueous and methanol extracts of *Syzygium* species have been shown to inhibit the growth of some fungal microorganism implicated in skin diseases, such as *Candida albicans*, and *Trichophyton rubrum* and *Staphylococcus aureus* (6).

Bacteria are microorganisms that are simple in structure and vary in shape and size. They are single-celled and considered prokaryotic. Bacteria are found in all different environments in the air, water, and soil, as they play an important role in life cycles on the surface of the Earth. Pathogenic bacteria differ among themselves in the extent of their virulence according to the virulence factors they possess and their ability to bypass the host's defenses, as well as their ability to resist antibiotics.

Among the most important genera of pathogenic bacteria that are resistant to many antibiotics *are E. coli, S. aureus, P. aeruginosa*, and others (7).

the objectives of the present study to evaluate the *in vitro* antibacterial properties of *S. cumini* against Gram positive and Gram negative bacterial.

Material and Methods

1: Collect plant samples

Fresh plant samples of the *S. cumini* species were collected for a period from 12/7/2023 until 8/1/2024, from Baghdad Governorate (Al-Zafaraniyah - Botanical Garden). Samples were placed in cardboard boxes Write on it the name of the plant, the place of collection, and the date of collection. Then the samples were washed with water Regular twice, then with filtered water once, then placed on paper to dry for (3-5) Days in the shade and at room temperature with constant stirring to prevent any rotting of parts The plant was obtained to obtain dry samples, which were then ground using an electric grinder (blender To obtain dry plant powder, then it was collected in clean glass containers until use (8).

2: preparation of Plant extraction

The plant extract was prepared based on the method [9] with some minor modifications. The solvent was used Aqueous-Alcoholic (Methanol) 50% for the purpose of preparing plant extract .

The extraction process was done as follows: (10).

- Dry leaves of the *S.cumini* were ground using an electric grinder to obtain Powder.
- Place 80 gm. of leaves powder in a 1 liter volumetric flask in a mixture consisting of (water, methanol (400/400 ml)) and close the opening of the container tightly with aluminum foil to avoid evaporation of the mixture and air oxidation.
- The flasks were placed in a water bath at 37 Cº for 2 hour at high speed.
- The extracts were filtered in two stages, the first using medical gauze and the second using a kit Layers of Whatman type (1) filter paper.
- Place the filtrate in the centrifuge at 2500 rpm for 10 minutes.
- Place the filter in a clean glass dish and then place it in the Oven at 40 Cº for 2-5 days. To be dried well.
- Scrape the extract after it dries completely, then store it in clean containers until use.

3: Collect pathogenic bacteria

Four pathogenic bacteria , 2 gram-positive and 2 gram-negative, were collected from microbiology laboratories at the University

of Babylon\ College of Science\ Department of Biology. The diagnosis has been confirmed by VITEK-2 Compact System.

4: Identification of bacteria by VITEK-2 Compact System

Suspension of bacteria was prepared according to the manufacturer's instructions. An adequate number of colonies was obtained by transferring an overnight pure culture and suspending it in 3.0 ml of sterile saline in (polystyrene) test tube. Adjustment of turbidity to 0.5 McFarland was made. Employing a Densi-Chek turbidity meter. Finally, the vitek-2 chamber with the specimen suspension tubes was loaded with the Gram negative -ID, and Gram positive -ID cassette (10).

5: Inocula Preparation (Turbidity Standard)

To prepare the inocula , colonies from overnight culture of bacteria isolates were transferred to 5 ml tube of normal saline to obtain culture with 1.5×108 CFU/ml by adjusting to 0.5 McFarland standard (11).

6: Antibiotic Susceptibility Test (AST)

Lists of antibiotic susceptibility testing were created using documents and breakpoints from the Clinical Laboratory Standards Institute (CLSI , 2020) the European Committee on Antibacterial Susceptibility Testing (EUCAST) and the United States Food and Drug Administration (FDA). This study included 10 antibiotics was determined by disc-diffusion method , which are Azithromycin , Nitrofurantoin , Imipenem , Amikacin , Nalidixic acid , Ciprofloxacin , Doxycycline , Amoxicillin / calvulanic acid , Trimethoprime / sulphamethoxazole , Ampicillin against (*E.coli* , *p. aeruginosa* , *St. pneumonia* , *S. aureus*). (12).

7: Antibacterial activity of leaves extract

This method was done on Muller Hinton agar (13).

- Turbidity of each bacterial isolates compared to McFarland 0.5 standard to get the right concentration for each of them .
- A 0.1 ml of each bacterial isolates were added to petri dish containing Muller Hinton agar and spread by spreader and left the dishes for an 1 hr.
- Wells were made by using cork borer (2 mm diameter) as it was equal distance between the well and the other.
- The extract were dissolved to get various concentrations (1000 as stock and by dilution got the three concentration(500 , 250 , 125) mg/ ml.
- A 40 microliter of each concentration were added to each well and incubated in the incubator at 37 ° C for 24 hr . inhibition zones were measured by a ruler .

8: Minimum Inhibitory Concentrations (MICs) Determination:

The minimum inhibitory concentrations (MICs) were determined by a serial dilution technique. MICs were defined as the lowest concentration of an antimicrobial that

inhibits growth of a microorganism after their incubation for overnight (14) . The MIC for Gram positive bacteria was 104 µg /ml while the MIC for Gram negative bacteria was 208 µg /ml . as showed in table (4).

9: Synergistic effect

The antagonistic activity of antibiotic and extract combination were determined by the modified disc diffusion method according to the NCCLS guidelines.

The MHA plates were seeded with the above antibiotic disc impregnated with exract (104 µg /ml) for (*St.pneumonia* and *S. aureus*) and (208 µg /ml) for (*P. aeruginosa and E.coli*) along with plain antibiotic disc taking as positive control , the MHA plates were kept at 4C° for 1 hr to allow the proper diffusion , after that kept at 37 C° for 24 hr . The zones of inhibition were measured by using a caliper micrometer against the back of the petri plates (15].

Results and Discussion

1: Morphological characteristics of

***Syzygium cumini* (L.) Skeels .**

The species *S. cumini* is a large evergreen tropical tree, 25-30 meters high, with thick, brown bark. One of the fast growing trees. The leaves of the plant are simple, opposite . The leaf blade is broadly elliptic and may be oblong-ovate, or elliptical in shape , with a length of 6-12 cm. The leaf is smooth and sometimes somewhat leathery. The leaf petiole is 3 cm long. The flowers are white - pink or greenish-white, about 1 cm. They are arranged in branched groups at the ends of the stem. The calyx leaves are funnel-shaped or cup-like, (4 mm) long, with 4 lobes (teeth). Petals: small fused petals that fall together as a small disk, white or light purple. Stamens are multiple and longer than the calyx leaves. Fruits are oval in shape, 1.5-3.5 cm long, dark purple or almost black, and dark red. The fruit contains a single seed approximately 2 cm long. . (15).

Taxonomic Tree : (16)

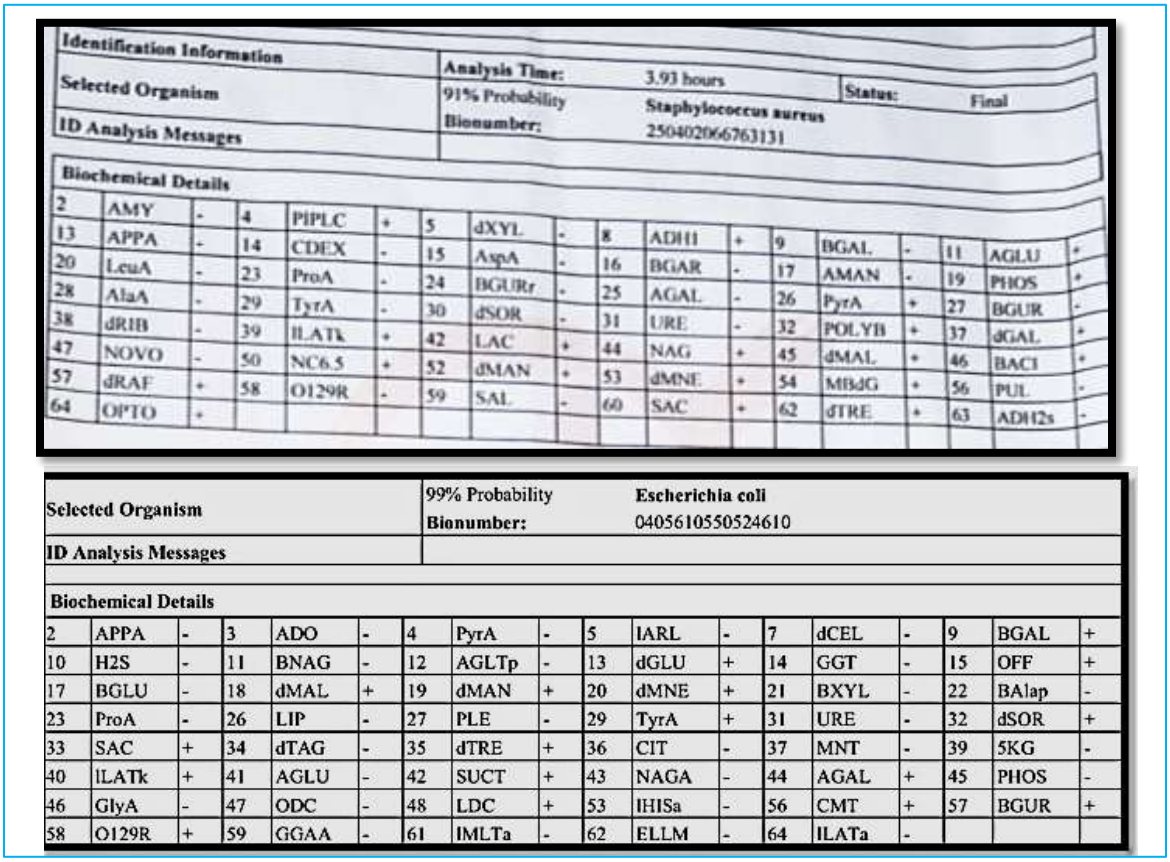
Kingdom: Plantae
Phylum : Spermatophyta
Subphylum : Angiospermae
Class : Dicotyledonae
Order : Myrtales
Family : Myrtaceae
Genus : *Syzygium*
Species : *Syzygium cumini*

2 : Bacterial isolates according to Vitek 2 Advanced Expert System

In VITEK2 Advanced Expert System system (bioMerieux, Marcy l'Etoile, France),the isolates were identified as *S. aureus* with probability of (91) % , *E .coli* 99% , *St. pneumonia* 99% and *P.aeroginosa* with probability of 89% . table (1). Biological characteristics of all bacterial isolates were done also by VITEK2 compact system , figure 1 .These results are similar to (16) who found that *S. aureus* was the most common pathogens ,followed by *P. aeruginosa* .

Table (1): Identification of pathogenic bacteria depended on the colonial morphology and Vitek 2 system .

Bacterial species	No. of isolates	Probability in Vitek 2 system
Streptococcus pneumonia	15	99 %
Staphylococcus aureus	20	91 %
Escherichia coli	10	99 %
Pseudomonas aeruginosa	10	89 %
TOTAL	55	-------



Identification Information			Analysis Time:	3.93 hours		Status:	Final
Selected Organism			91% Probability	Staphylococcus aureus			
ID Analysis Messages			Bionumber:	2504020666763131			
Biochemical Details							
2 AMY -	4 PIPLC +	5 dXYL -	8 ADH1 +	9 BGAL -	11 AGLU +		
13 APPA -	14 CDEX -	15 AspA -	16 BGAR -	17 AMAN -	19 PHOS +		
20 LeuA -	23 ProA -	24 BGURr -	25 AGAL -	26 PyrA +	27 BGUR -		
28 AlaA -	29 TyrA -	30 dSOR -	31 URE -	32 POLYB +	37 dGAL +		
38 dRIB -	39 ILATk +	42 LAC +	44 NAG +	45 dMAL +	46 BACI +		
47 NOVO -	50 NC6.5 +	52 dMAN +	53 dMNE +	54 MBdG +	56 PUL -		
57 dRAF +	58 O129R -	59 SAL -	60 SAC +	62 dTRE +	63 ADH2s -		
64 OPTO +							

Selected Organism			99% Probability	Escherichia coli			
ID Analysis Messages			Bionumber:	0405610550524610			
Biochemical Details							
2 APPA -	3 ADO -	4 PyrA -	5 IARL -	7 dCEL -	9 BGAL +		
10 H2S -	11 BNAG -	12 AGLTp -	13 dGLU +	14 GGT -	15 OFF +		
17 BGLU -	18 dMAL +	19 dMAN +	20 dMNE +	21 BXYL -	22 BAlap -		
23 ProA -	26 LIP -	27 PLE -	29 TyrA +	31 URE -	32 dSOR +		
33 SAC +	34 dTAG -	35 dTRE +	36 CIT -	37 MNT -	39 5KG -		
40 ILATk +	41 AGLU -	42 SUCT +	43 NAGA -	44 AGAL +	45 PHOS -		
46 GlyA -	47 ODC -	48 LDC +	53 IHISa -	56 CMT +	57 BGUR +		
58 O129R +	59 GGAA -	61 IMLTa -	62 ELLM -	64 ILATa -			

Figure (1) : Picture clear biochemical characteristics of all bacterial isolates were done also by VITEK2 compact system.

3 : Antibiotic Susceptibility Test (AST)

This study included 10 antibiotics was determined by disc-diffusion method , which are Azithromycin , Nitrofurantoin , Imipenem , Amikacin , Nalidixic acid , Ciprofloxacin, Doxycycline , Amoxicillin/calvulanic acid, Trimethoprime / sulphamethoxazole , Ampicillin against (*E.coli* , *p.aeruginosa* , *St.pneumonia* , *S. aureus*). The pathogenic bacterial isolates used for the study showed high resistance to most of the antibiotics used, as shown in Table 2 .

Table (2) : Antibiotic Susceptibility Test for pathogenic bacteria.

Antibiotic	***Antibiotic susceptibility of pathogenic bacteria* (%)**			
	E.coli	***P.aeruginosa***	***St.pneumonia***	***S. aureus***
AZM	R	S	S	R
AK	R	I	S	S
NA	I	R	S	S
CIP	R	I	R	R
F	R	S	R	R
IPM	S	I	S	S
DO	I	R	R	S
SXT	I	R	R	I
AMC	I	R	I	S
AM	R	R	S	R

The emergence of antibiotics resistance in the majority of pathogenic bacterial strains is a cause of utmost concern in infectious bacterial diseases. Therefore, there is an inevitable need to identify the effective antibacterial agents which are more effective against microbial ailments with minimal side effects on host cells (17) .

the phenotypic prevalence of resistance to Azithromycin and Ampicillin was reported in more than 30% of cases, which is which matches perfectly with the results of this study (18) ,while it was not consistent with the study of (18). in Kuwait , and (19) . in Qom, and (20) in Tabriz ,who demonstrated that highest antibiotic resistance was found in Nalidixic acid, which included .

4 : Antibacterial activity of *S. cumini* leaves extract

In well diffusion method various concentrations (2000, 1000 ,500 mg\ml) from methanol leaves extract were test against 4 pathogenic bacteria . It was found that at concentration 500 mg\ml gave the highest inhibition effectiveness and all concentrations were significant at P-value 0.05 as showed in table 4 and figure 2.

leaves extract may bind into proteins, thereby inhibiting the assembly of proteins. This, in turn, suppresses the formation of inhibition zone lead to inhibit cytokinesis and bacterial proliferation[ ]. The results shown in Table (3) show that the concentration of 2000 mg/ml) has the highest effect on pathogenic bacteria, and all concentrations were significant at P-value 0.05. This suggests that the observed differences in bacterial growth inhibition between different extract concentrations were statistically significant.

Table (3) : Antibacterial effect of methanol *S. cumini* leaves extract .

Concen. mg\ml / Bacteria type	Inhibition zone diameter mm.				
	2000	**1000**	**500**	**C+**	**C-**
S. aureus	22.1±2.5 **b**	20.6±2.9 **b**	18.3±1.1 **b**	17.7±4.4 **b**	0±0 a
St.pneumonia	21.1±3.8 **c**	20.8±1.8 **c**	11.5±0.9 **b**	10.4±2.2 **b**	0±0 a
E. coli	21.8±1.8 **c**	21.4±0.9 **c**	15.7±1.9 **b**	15.4±2.5 **b**	0±0 a
P. aeruginosa	20.4±1.9 **d**	20.6±1.3 **d**	12.4±1.4 **b**	15.4±1.5 **c**	0±0 a

- **Different letters refer to significant difference at p≤0.05.**

One study published in the Journal of Microbiology and Biotechnology in 2017 evaluated the antibacterial effects of *S. cumini* leaves extract against clinical isolates of *S. aureus* and *P.aeruginosa* (21). The results demonstrated that *S. cumini* leaves extract exhibited significant inhibitory activity against the tested strains. Another study published in the Journal of Natural Products in 2019 found *S. cumini* leaves extract was effective in inhibiting the growth of *S. aureus* biofilms, which are communities of bacteria that can be particularly resistant to antibiotics (22). It is believed that *S. cumini* leaves extract can disrupt the bacterial cell membrane, interfere with DNA replication and protein synthesis, and inhibit enzymes necessary for bacterial survival (23).

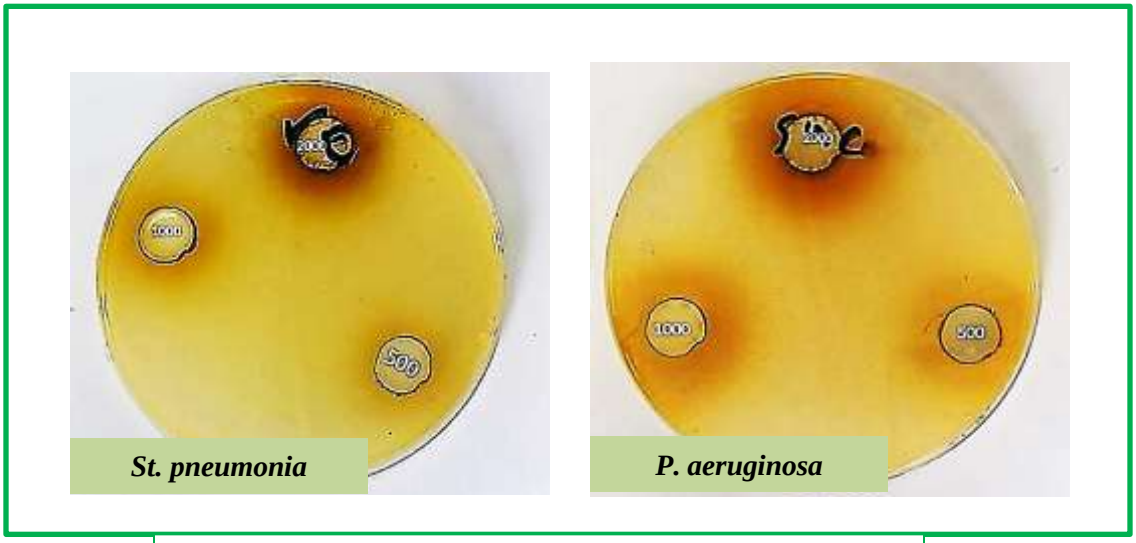


Figure 2: Antibacterial effect of *S.cumini* . leaves extract on some pathogenic bacterial strains..

5 : Minimum Inhibitory Concentrations Determination

The minimum inhibitory concentrations (MICs) were determined by a serial dilution technique. MICs were defined as the lowest concentration of an antimicrobial that inhibits growth of a microorganism after their incubation for overnight. The MIC for Gram Negative bacteria was 208 µg/ml. The MIC for Gram positive bacteria was 104 µg/ml , as showed in Table 4.

Table (4) : Minimum Inhibitory Concentrations and (MICs) of *S. cumini* leaves extract .

Test organisms	Minimum Inhibition Concentration (MIC) (µg/ml)
E. coli	208
P. aeruginosa	208
S. aureus	104
St.pneumonia	104

By measuring the minimum inhibitory concentration of the extract against the pathogenic bacteria, it was found that the Gram-positive bacteria were more sensitive to the extract, as they require the inhibition of the positive bacteria to 104 µg/ml, while they require the inhibition of the negative bacteria was 208 µg/ml. The difference between the sensitivity of Gram-positive and Gram-negative bacteria may be due to the difference between the bacteria in terms of wall structure and the presence of the peptidoglycan layer, in addition to the nature of Gram-negative bacteria and their containment of a high percentage of fats, which delays the absorption and assimilation of substances.

6 : Synergistic effect of *S. cumini* leaves extract with antibiotic .

The synergistic effect of *S. cumini* leaves extract was investigated with six antibiotics against the higher resistance isolates which appeared increase the antibiotic effect as showed in figure (3). The MHA plates were seeded with the above antibiotic disc impregnated with exract (104 µg /ml) for (*St.pneumonia* and *S. aureus*) and (208 µg /ml) for (*P. aeruginosa and E.coli*) along with plain antibiotic disc taking as positive control , the MHA plates were kept at 4C° for 1 hr to allow the proper diffusion , after that kept at 37 C° for 24 hr . The zones of inhibition were measured by using a caliper micrometer against the back of the petri plates (24).

S. cumini leaves extract increased the effectiveness of antibiotics and increased the efficiency of their work by causing modifications in the structure of the antibiotic to make it more effective**.**

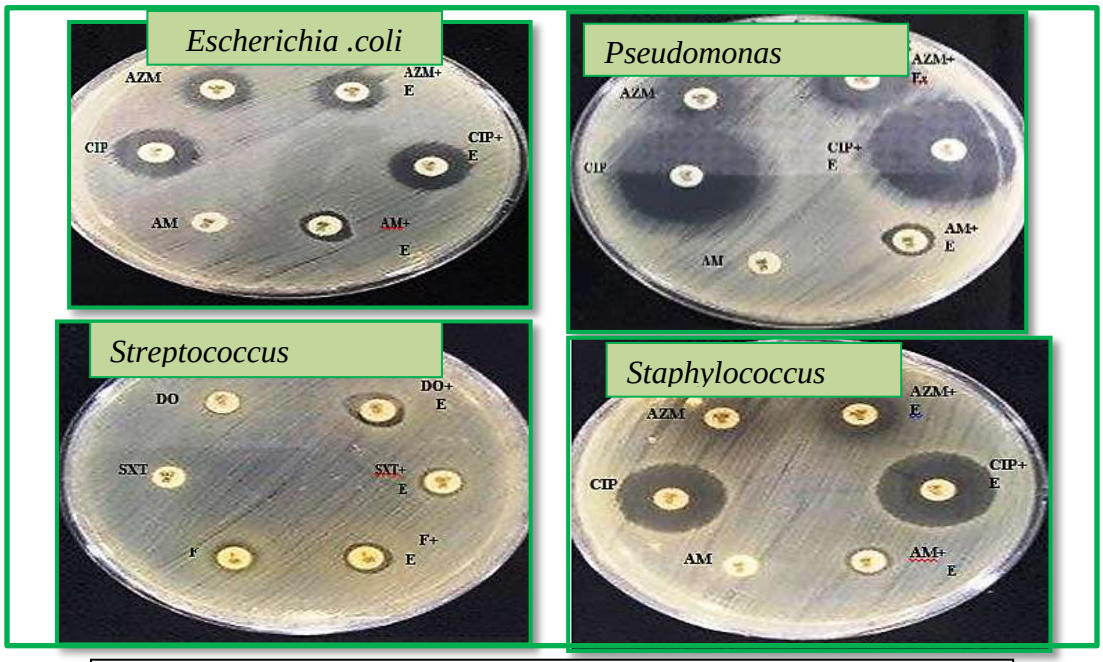


Figure 3 : The synergistic effect of *S. cumini* leaves extract was investigated with six antibiotics against the higher resistance isolates

Conclusion

The Aqueous-alcoholic extract.of the leaves of ***S. cumini*** is of great importance as an antidote to pathogenic bacteria, both positive and negative, of the Gram stain. The use of medicinal plants can contribute to solving the problem of antibiotic resistance .

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