World Journal of Pharmaceutical Science and Research

www.wjpsronline.com

Research Article

ISSN: 2583-6579
SJIF Impact Factor: 6.916
Year - 2026
Volume: 5; Issue: 8
Page: 464-481

ANTI MRSA ACTIVITY OF POMEGRANATE RIND EXTRACT IN COMBINATION WITH METAL SALT AND VITAMIN C”

Anuja K. U.*, Jayesh V. N.

Faculty of Triveni Institute of Pharmacy, Eranellur, Kecheri, Thrissur, 680501.

Article Received: 11 June 2026 | | ***Article Revised: 02 July 2026*** | | ***Article Accepted: 23 July 2026***

***Corresponding Author: Anuja K. U.**
Faculty of Triveni Institute of Pharmacy, Eranellur, Kecheri, Thrissur, 680501.
DOI: https://doi.org/10.5281/zenodo.21705742

How to cite this Article: Anuja K. U., Jayesh V. N. (2026) ANTI MRSA ACTIVITY OF POMEGRANATE RIND EXTRACT IN COMBINATION WITH METAL SALT AND VITAMIN C”. World Journal of Pharmaceutical Science and Research, 5(8), 464-481.

ABSTRACT

Antibiotic resistance (AR) is a serious and growing global health threat that compromises the effectiveness of life-saving medications. Antibiotics function by targeting various bacterial processes such as cell wall synthesis, protein synthesis, nucleic acid synthesis, and metabolic pathways. However, misuse, overuse, and improper prescription of these drugs have accelerated the emergence of resistant strains. Bacteria naturally evolve through mutations and genetic material transfer, leading to mechanisms that render antibiotics ineffective. The World Health Organization has identified AR as one of the top ten global public health threats. A key example of antibiotic resistance is Methicillin-Resistant Staphylococcus aureus (MRSA), a strain resistant to many commonly used antibiotics. MRSA infections typically occur through skin contact and can cause symptoms such as painful red bumps, fever, and swelling. In severe cases, it can lead to pneumonia, bloodstream infections, and even death. The resistance mechanism of MRSA involves the acquisition of the mecA gene, which encodes a penicillin-binding protein (PBP2a) with low affinity for β-lactam antibiotics. In response, research is focusing on alternative therapies including natural products. Pomegranate rind extract (PRE), especially when combined with metal salts like copper sulfate and stabilized with vitamin C, has demonstrated promising antibacterial activity against MRSA. Additionally, silver nitrate has been studied for its potent antimicrobial properties due to its ability to disrupt bacterial DNA and protein synthesis. This study highlights the urgent need for continued surveillance, judicious antibiotic use, and the exploration of novel antimicrobial agents to combat the growing challenge of antibiotic resistance.

KEYWORDS: Antibiotic resistance, MRSA, mecA gene, Pomegranate rind extract, Silver nitrate, Antimicrobial agents.

INTRODUCTION

ANTIBIOTIC RESISTANCE

Antibiotics are medicines that fight against infection in humans. Antibiotics work by interference with cell wall synthesis, interference with nucleic acid synthesis, inhibition of protein synthesis inhibition of a metabolic pathway, inhibition of membrane function or inhibition of ATP synthase.

Antibiotic effectiveness is a common pool resource that can be prematurely depleted through resistance. Antibiotic resistance occurs when bacteria evolve mechanism that protects them from the effects of antibiotics. This makes certain infections difficult to treat. Antibiotic resistance occurs when bacteria change so that medicines can't kill them or stop their growth.

Antibiotic resistance is considered as 10th major global threat according to WHO reports. The global rise in antibiotic resistance poses a significant threat, diminishing the efficiency of common antibiotics against widespread bacterial infections. The Global Antimicrobial Resistance and Use Surveillance System (GLASS) report highlights alarming resistance rate among prevalent bacterial pathogens. Antibiotic resistance threatens the very core of modern medicine and the sustainability of an effective, global public health response to the enduring threat from infectious diseases. Antibiotic resistance is dangerous because it reduces treatment options for people who are sick. It may also delay effective treatment.[1]

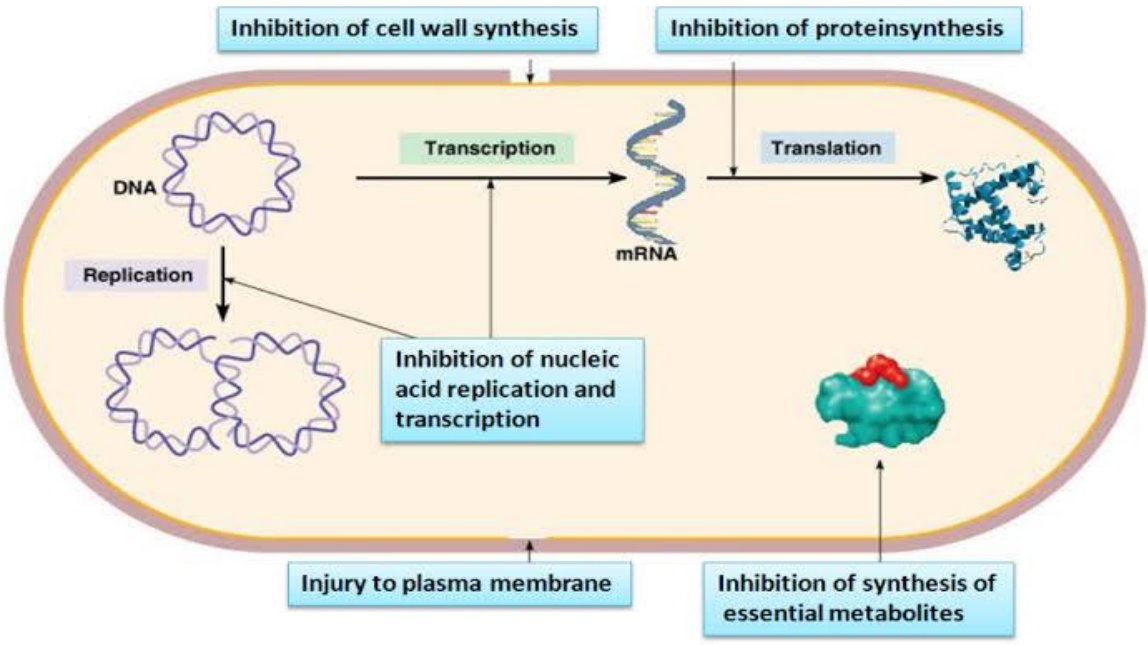

Fig.no. 1: Mechanism of antibiotic resistance.

Alert to this crisis, May 2015 World Health Assembly adopted a global action plan on antimicrobial resistance. The main five objectives of this plan are:

- To improve awareness and understanding of antimicrobial resistance through effective communication, education and training,
- To strengthen the knowledge and evidence base through surveillance and research.
- To reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures,

- To optimize the use of antimicrobial medicines in human and animal health, and
- To develop the economic case for sustainable investment that takes account of needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other inventions.

With this plan we can ensure the quality of use of medicines in different infectious diseases.[2]

CAUSES OF ANTIBIOTIC RESISTANCE

Microorganisms, such as bacteria are living organisms that adapt over time. Their main objective is to replicate, survive, and spread as rapidly as possible. As a result, microbes adjust to their surroundings and evolve in ways that guarantee their continued existence.

If something stops their ability to grow, such as an antibiotic, genetic modifications may arise, making the bacteria immune to the medication and allowing them to survive.

It is the natural process of bacteria to develop drug resistance. However, several elements remain currently at stake in the multifaceted etiology of antibiotic resistance. This involves antibiotic overuse and abuse, inexact diagnosis and improper antibiotic prescribing, patient sensitivity loss and self-medication, bad healthcare environments, poor personal hygiene, and widespread agricultural use.[3]

- **Microbial (natural) reasons**

AMR is primarily caused by alterations within the bacteria and may occur in a variety of ways:

- **Genetic mutation**

Changes in few base pairs may occur during bacterial replication (point mutations), resulting in the replacement of one or a few amino acids in a critical target (enzyme, cell wall, or cell structure), as well as control genes or chromosomal structural, resulting in new resistant strains. The newly developed defense may render antibiotics ineffective, which were meant to be able to handle the organism for years.

- **Genetic material transfer**

Resistance from another species or genus may be accumulated by a formerly susceptible strain. Most of the antibacterial resistance genes are carried on plasmids and other types of mobile genetic elements, which may and do spread to bacteria of different genus and species. Drug-resistant bacteria may pass on a copy of their genes to other non-resistant CC bacteria. The non-resistant bacteria accumulate the new DNA and develop drug resistance.

- **Selective pressure**

Selective pressure may be defined as the environmental conditions that allow the survival and proliferation of organisms with novel mutations or newly developed characteristics. When treated by an antimicrobial, microbes are either destroyed or if they resistance genes they survive.

These survivors will multiply, and resistant microbes that are newly developed, will rapidly overtake the microbial population as the dominant form.

- **Inaccurate diagnosis**

While diagnosing an infection, healthcare professionals sometimes rely on unreliable or inaccurate knowledge, prescribing an antibiotic "just in case" or a wide-spectrum antibiotic when a particular narrow spectrum antibiotic might be more appropriate.

- **Inappropriate prescription of antibiotics**

When doctors are unclear if an infection is exacerbated by bacteria or a virus, they may prescribe antibiotics. Antibiotics, on the other hand, do not act against viral infections, and resistance may develop.

- **Self-medication**

In Southeast Asian region of the world, antibiotics are widely used without physician's prescription. Self-medication with antibiotics (SMA) is linked to the possibility of improper drug usage, which puts patients at risk for adverse drug reactions, masking signs of underlying diseases, and development of drug resistance in microbes.

- **Inadequate and overuse of antibiotics**

If an individual does not finish a course of antibiotics, develop resistance to that antibiotic. Again, in the year 1945, the discoverer of antibiotics, Alexander Fleming, had issued a public warning against the overuse of antibiotics, as he had realized the dangers associated with the inappropriate use of these drugs. Taking antibiotics too often for the wrong reasons can develop modifications within the bacteria that antibiotics do not work against them

- **Poor hospital environment**

Thousands of patients, staff, and visitors arrive at hospitals every day, each with their own set of microbiome and colonizing bacteria on their clothing and on inside their bodies. Bacteria can spread if hospitals do not have adequate procedures and protocols in place to help maintain spaces clean. As a result, the emergence and spread of AMR is aided.[4]

METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

Staphylococcus aureus is a very common germ. About one out of every three people have germ on their skin or their nose. This germ does not cause problem for most people.

MRSA is a type of staphylococcus that can be resistant to several antibiotics. Anyone can get a MRSA infection or carry MRSA. The risk increases for people with hospitalizations or nursing home stays, skin-to-skin contact with others and exposures to crowded and unhygienic places.

The symptoms of a *S. aureus* infection including MRSA, depend on the part of the body that is infected. Broken skin, such as where there are scrapes or cuts, is often the site of a MRSA infection. Most *S. aureus* skin infections, including MRSA appear as a bump or infected area on the skin that might be.[6]

Table no. 1; Symptoms.

SYMPTOMS OF S. AUREUS INFECTION	
1	Red bump
2	Swelling
3	Pain
4	Warm to touch

5	Fever
6	Lumps filled with pus

MRSA infection can cause serious problems in and outside of healthcare settings, including:

- Pneumonia
- Bloodstream infections
- surgical site infections
- sepsis (the body's extreme response to an infection),
- Death

Although anyone can get MRSA, some groups have a higher risk:

- Athletes
- Daycare and school students
- Military personnel in barracks
- Who receive inpatient medical care.
- People who have surgery or medical devices inserted in their body.

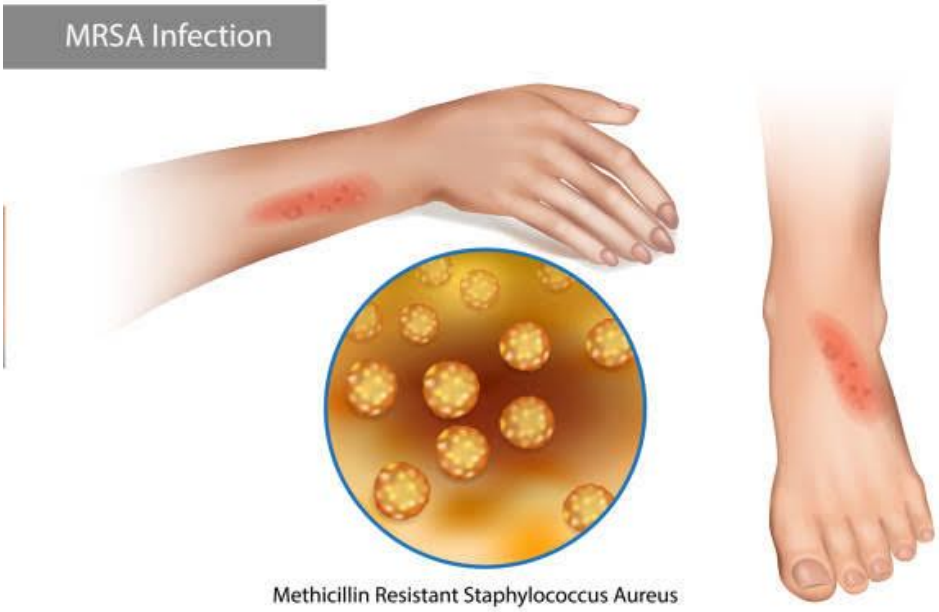

Fig.no. 2: MRSA infection.

MRSA spreads in the community through contact with infected people, wounds, or things that have touched infected skin and are carrying the bacteria. Some people who carry MRSA can go on to get a MRSA infection. Healthcare providers often prescribe antibiotics to treat this MRSA infection. Some types of *S. aureus* infections need surgery to drain infected areas. While MRSA can be resistant to several antibiotics, meaning these drugs cannot cure the infections, there are antibiotics available to treat MRSA infections.

Currently, there are seven common antibiotics used against MRSA, which are: vancomycin, daptomycin, linezolid, sulfamethoxazole and trimethoprim, clindamycin and tigecycline.[8]

MECHANISM OF MRSA

- Horizontally transferred DNA element – SCC mec.
- Site specific recombination.
- mecA gene encodes PBP2a.
- PBP2a=78KDa PBP-capable of cell wall synthesis.
- PBP2a has low affinity for all B-lactams

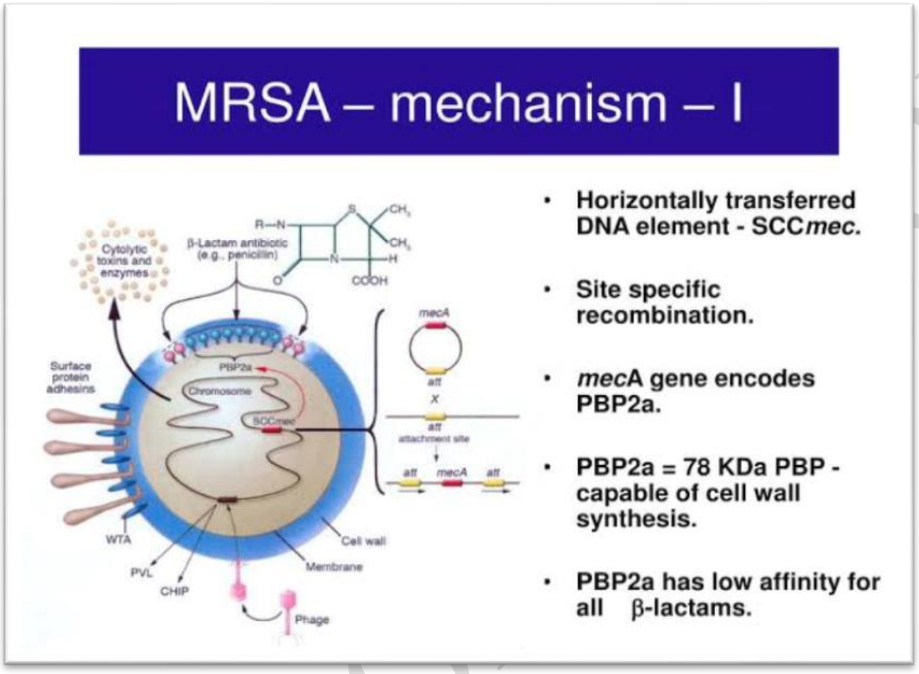

Fig. no. 3: Mechanism of MRSA.

ANTI MRSA ACTIVITY OF POMEGRANATE RIND EXTRACT

A wide range of natural products have been screened for activity against *S. aureus*, including the pomegranate (Punica granatum). Various extracts of pomegranate in ethanol, chloroform, ethyl acetate, butanol and water all exhibited activities against MRSA. Out of these PRE (pomegranate rind extract) is having antimicrobial properties and has been used to treat many infections in humans.

Considerable emphasis is being placed on combating multi drug resistant bacteria in the clinical setting. These proposed infection control measures encompasses the study of hospital cleaning measures ,prevention of transmission by health care workers and the development of new antimicrobial agents .During the year beginning in April 2005,methicillin resistant Staphylococcus aureus(MRSA) bacteremia cases in England totaled 7,087.Methicillin sensitive Staphylococcus aureus are also resistant to multiple antibiotics ,however these isolates respond better to treatment than MRSA counterparts .The community acquired MSSA can produce the Panton -Valentine Leukocidin (PVL) toxin which is of particular concern as infections with these organisms have resulted in increased levels of morbidity and mortality.[9]

Recently, studies have been redirected towards evaluating traditional medicines as sources of antimicrobial agents. A wide range of natural products have been screened for activity against S aureus, including the pomegranate (Punica-

granatum). In a study by Machado et al various extracts of pomegranate (in ethanol, chloroform, ethyl-acetate, butanol and water) all exhibited activities against MSSA and MRSA. A further study by Braga et al demonstrated the inhibition of growth and release of enterotoxin by some S aureus isolates.

Numerous metals containing antimicrobial agents have been reported despite advanced protective mechanisms for the detoxification of heavy metal ions being found in many bacteria. The enhancement of the ant bacteriophage and antimicrobial activities exhibited by pomegranate rind extract (PRE) by the addition of metal ions has been reported.

Stewart et al reported remarkable anti bacteriophage activities for the combination of pomegranate rind extracts with ferrous salts. However, this combination was found to exhibit activity for a short period probably owing to instability.

Previous work carried out by the current authors demonstrated that PRE, in combination with different metals, showed an anti-bacterial effect against a range of laboratory strains of both gram positive and negative bacteria. This study showed that PRE with the addition of CuSO4 demonstrated inhibitory effects against three gram-negative strains of bacteria: Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli. Against S. aureus this combination reduced the bacterial population, however, the addition of a stabilizing agent (vitamin c) to the combination resulted in no detectable growth after 30 minutes.[16]

The aim of the study was to explore the potential role for the PRE/Cu (SO4) with the addition of a stabilizing agent against clinical isolates of S. aureus. [10]

ROLE OF VITAMIN C

Vitamin C is a water- soluble vitamin .it is needed for normal growth and development. Due to its function as an antioxidant and its role in immune function vitamin c has been promoted as a means to help prevent and /or treat numerous health conditions Vitamin c is needed for the growth and repair of tissues in all parts of your body. It is used to form an important protein called collagen, used to make skin, tendons, ligaments, and blood vessels. It is used to heal wounds and form scar tissue. It aids in the absorption of iron. Vitamin c contributes to strengthening immunity, increasing resistance by supporting the function of various cells throughout the immune system. It supports epithelial barrier function against pathogens and promotes oxidative scavenging activity of the skin.[12]

WHY SILVER NITRATE IS USED AS METAL SALT

Silver nitrate is used as a caustic, antiseptic, and astringent agent. The Ag+ ion is precipitated by the chloride in tissue fluids, so that it does not readily penetrate. AGNO3 is effective in-vitro against Neisseria gonorrhea and Staphylococcus aureus in a concentration about 0.1%. It is more effective against gram-positive bacteria. Silver is more potent antimicrobial agent against a variety of microorganisms and once element entered the microbial cell, it accumulates as silver nanoparticles with large surface area causing cell death. At the same time, the bacterial cell becomes a reservoir for silver. Bacterial kill occurs as the ions deposit themselves into the cell walls and vacuoles of bacteria, damaging the cell structures including the cell envelope, cytoplasmic membrane and the cell-membrane`s contents. Once inside the cell, silver ions bind to DNA and RNA molecules, causing them to condense. This makes it more difficult for ribosomes to read or transcribe DNA and RNA. Thus inhibits the protein synthesis and cell division. At high concentration it kills bacteria and at lower concentrations it induces them to synthesize silver nanoparticles.[14] Penicillin G is used as a Standard drug in this experiment.

REVIEW OF LITERATURE

1. Derek F J Brown and et.al; illustrated the guidelines for the laboratory diagnosis and susceptibility testing of MRSA. He considered the detection of screening samples and reduced susceptibility.
2. Simon W J Gould and et.al; describes the anti MRSA activity of pomegranate rind extract singularly and in combination with cupric sulphate. PRE alone showed limited efficacy against MRSA strains. Exposure to copper ions alone resulted in moderate activity.
3. Declan Naughton and et.al; studied the use of pomegranate for MRSA treatment. He was in a search of new antibiotic because of the rise in infections resistant to the drug. After a number of experiments found that combining two ingredients: pomegranate, vitamin C have more potent effect. The test was conducted using microbes from hospital patients.
4. Alison F Kelly and et.al; investigated the anti MRSA activity of pomegranate rind extract. It has been reported to have antimicrobial activity against a wide range of gram positive and negative bacteria. The study was to demonstrate the antimicrobial activity of PRE with metal salt and vitamin C. PRE solutions prepared by blending rind sections with distilled water prior to sterilization and screened with disc diffusion method.
5. Anthony Coates and et.al; professor of medical microbiology at St George in London, studied the anti MRSA activity of pomegranate rind extract. He used the natural sources as most antibiotic come from the nature. It is very valid to look at the natural sources. they used the natural sources as the patients are less likely to experience any major side effect.
6. G.K Jayaprakash S Negi and et.al; made several studies and reported that the pomegranate peel extract has anti MRSA activity and activity against multi drug resistant Salmonella typhimurium.
7. Vildan Celiksoy and et.al; made research and his findings supported the fact that pomegranate extract has unusual and potent broad-spectrum activities against gram positive and gram-negative bacteria. He also found that these activities are attributed to the presence of phenolic compounds and ellagic acid.
8. Ali A Ali Redha and et.al; had made the phytochemical investigation of pomegranate rind extract and aril extract and concluded that the extract is rich in hydrolysable ellagitannins like punicalagins and punicalins.
9. Amal Al Rashidi and et.al; made a time-kill assay study on the synergistic bactericidal activity of pomegranate rind extract and zinc against MRSA, Staphylococcus epidermidis, E. coli.
10. Erin M Mc Carrel et.al, investigated the antibiotic activity of pomegranate rind extract. The screening assay demonstrated that PRE exhibited activity against the gram organism with no effect in gram negative organism. After 24 hours zone of inhibition was only observed for ps. Aeruginosa. In addition of copper salt to PRE solution extended the activities resulting in no growth observed against E coli, ps. Aeruginosa.

AIM

The aim of the study is to demonstrate the anti MRSA activity of pomegranate rind extract in combination with metal salt and vitamin c.

OBJECTIVES

To evaluate:

- Natural products as a source of antimicrobial agent.
- Anti- MRSA activity of pomegranate rind extract in combination with metal Salt and Vitamin C.

MATERIALS REQUIRED

Table no.2: Materials required.

SI.NO.	MATERIALS REQUIRED
1	Pomegranate rind extract
2	Vitamin C
3	Penicillin G
4	MRSA strips
5	Muller Hinton agar medium

Table no. 3: Equipment required.

SI.NO.	EQUIPMENTS REQUIRED
1	Mortar and pestle
2	Beaker
3	Funnel
4	Stirrer
5	Filter paper
6	Petri dish
7	Hot air oven
8	Autoclave

Table no. 4: Reagents required.

SI.NO.	REAGENTS REQUIRED
1	Silver nitrate
2	Ringer lactate
3	Distilled water

PERFORMANCE OF THE EXPERIMENT

PREPARATION OF POMEGRANATE RIND EXTRACT

PRE were prepared by blending 15 grams of finely sectioned pomegranate rind with 45 ml distilled water for 10 minutes. The crude extract was filtered through muslin followed filtration. Then autoclave at (121 degree Celsius for 15 minutes) or filter sterilization using a 0.2 micrometer filter (Millipore), then store at -20 degree Celsius. A sample of the PRE was freeze dried to determine the dry weight content which was found to be 0.0437 g/l.[15 -17]

Fig.no. 4: Preparation of pomegranate rind extract.

AGAR WELL DIFFUSSION ASSAY

Overnight cultures of the strains of MRSA were prepared on nutrient agar plates. The bacterial isolates were suspended in Ringer's solution to a turbidity equivalent to 0.5 McFarland (1.5x108 CFU/ml) and 100 microliter was then spotted

on to Mueller-Hinton agar plates. The PRE (10 microliter) was then introduced into well made on agar plates. The plates were incubated at 37 degrees Celsius for 24 hours prior to recording zones of inhibition. Penicillin G was used as standards.[18]

Fig. no. 5: Prepared agar plates.

ANTI MRSA ACTIVITY OF PRE/METAL SALT COMBINATIONS

Overnight cultures on nutrient agar were prepared as previously described. An aliquot of PRE (330 microliter) was added to 700 microliter of the freshly prepared solutions (4.8 Mm) of metal salts and the final solution was protected from light. The appropriate bacterial dilution was prepared in Ringer's solution and 50 microliters were placed in a sterile Eppendorf micro-centrifuge tube with a 100 microliters aliquot of PRE/metal salt solution. After exposure for 30 minutes at room temperature, the activity of the PRE/salt solution was neutralized by adding an equal volume of 2% (v/v) Tween-80 in Lambda buffer. Serial dilution was prepared in Ringer lactate solution and 10 microliters of each dilution was spotted onto nutrient agar plates and incubated for 24 hours at 35 degrees Celsius. Each assay was conducted in triplicate.[21]

ANTIBACTERIAL ACTIVITY OF PRE/METAL SALT COMBINATION PLUS VITAMIN C

The assay was carried out as described above with the following addition: vitamin C was added to the metal salt solution immediately prior to mixing with the PRE. Aliquots of vitamin C were added to give final metal ion: vitamin C ratios (and vitamin C concentrations) of 1:1 (4.8 mM), 1:5 (24 mM), and 1:20 (96 mM). A 700 microliters aliquot of the solution of metal salt/vitamin C was then added to PRE prior to assay.[23]

PREPARATION OF DIFFERENT DILUTIONS OF PRE/METAL SALT COMBINATION PLUS VITAMIN C

Three different dilutions of PRE + silver salt + vitamin C combination was prepared as follows:

PRE+Ag salt+vitamin C(1:1), PRE+Ag salt+vitamin C(1:5), PRE+Ag salt+vitamin C (1:20)

Fig. no. 6: Different dilutions of sample solution.

PHYTOCHEMICAL DETERMINATION OF POMEGRANATE RIND EXTRACTS

Pomegranate is an ancient fruit native to Persia which has been cultivated in the Mediterranean region through years. Pomegranate which belongs to the family of Punicaceae, botanically named Punica granatum. Pomegranate trees are considered as shrubs or small trees and can occur either as single blossoms or clusters of several blossoms. Pomegranate is a berrylike fruit with a leathery rind coating sweet juicy aril.

Pomegranate is rich in bioactive molecules. Pomegranate peels constitute approximately 60% of the total weight of the pomegranate fruit. The pomegranate peel is considered as an agro-waste, but it can be potential source of anti-oxidant, anti-bacterial activity.[34]

The amount of polyphenol is rich in this fruit. Phytochemical compounds such as gallotannins, ellagic acid, gallic acid, punicalins, and punicalagins present in pomegranate rind. Punica granatum is a rich source of bioactive compounds and contain a variety of secondary metabolites.

In this study, the potential phytochemicals present in the pomegranate rind extract were carried out. Phytochemical investigation included, phenols, tannins, flavonoids, glycosides, alkaloids, carbohydrates, terpenoids, proteins and amino acids.[35]

PRELIMINARY PHYTOCHEMICAL SCREENING

Table no. 5: preliminary phytochemical screening.

TEST	PROCEDURE	OBSERVATION	INFERENCE
TEST FOR CARBOHYDRATES			
Molisch' s test	To few drops of extract ,2 ml Molisch' s reagent is added. Add 2ml of conc. Sulfuric acid along the sides of test tube.	A reddish ring formed at the junction of two layers.	Presence of carbohydrate
Fehling's test	To few drops of extract, 2ml of Fehling's reagent A and B is added and boil for 5 minutes.	Brick red precipitate	Presence of reducing sugar
TEST FOR ALKALOIDS			
Mayer's test	To a few drops extract, 2 drops of Mayer's reagent is added by side of the test tube.	Green color precipitate formed	Presence of alkaloids
Wagner's test	To a few drops extract, 2 drops of Wagner's reagent is added by and shake well.	A reddish- brown precipitate is formed.	Presence of alkaloids

TEST FOR TANNINS			
Ferric chloride Test	Add 5% ferric chloride solution drop by drop to 2 to 3 ml of the extract and observe the color produced.	Bluish-black color observed.	Presence of tannins
Gelatin test	To a solution of the extract, aqueous solution of gelatin and Nacl solution is added.	A white buff colored precipitate is formed.	Presence of tannins
Vanillin-hydrochloride test	To sample solution, add vanillin-hydrochloride solution.	A pink color is observed.	Presence of tannins
TEST FOR FLAVONOIDS			
Acid test	To few ml of extract, few drops of dilute sulfuric acid is added.	Orange color develops	Presence of flavonoids
TEST FOR TERPENOIDS			
Acetic anhydride test	To a few drops of extract, 2 ml of acetic anhydride and conc. Sulfuric acid is added.	Formation of blue-green ring.	Presence of terpenoids
TEST FOR AMINO ACIDS			
Ninhydrin test	To a few drops of extract, few drops of ninhydrin solution is added in a test tube.	A characteristic blue color is observed.	Presence of amino acid
TEST FOR PROTEINS			
Biuret test	Test solution was treated with 10% sodium hydroxide and two drops of 0.1% copper sulphate solution.	Formation of violet color.	Presence of proteins
Millon's test	To a few ml of extract, few drops of Millon's reagent was added.	Formation of white precipitate	Presence of proteins
TEST FOR GLYCOSIDES			
Liebermann's test	To 2ml of extract, 2ml of chloroform and 2ml of acetic anhydride is added.	Formation of blue-green ring is observed.	Presence of glycosides

RESULT OF PRELIMINARY IDENTIFICATION TEST

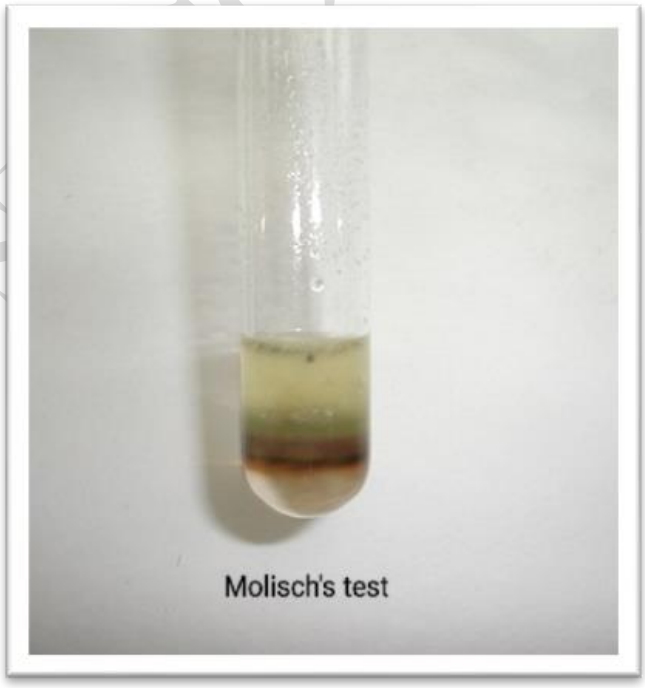

Fig. no. 7: Result of test for carbohydrates.

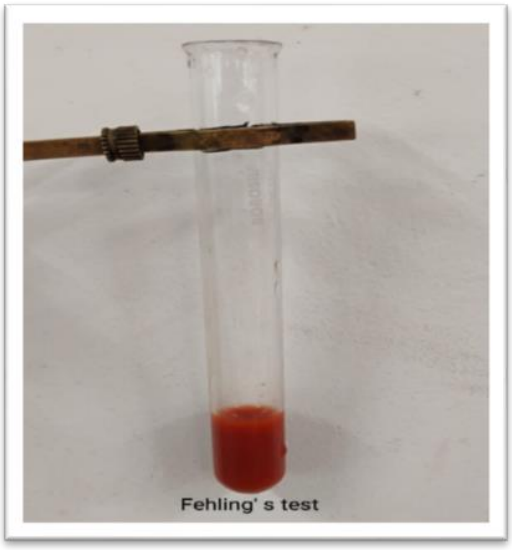

Fig. no. 8: result of test for alkaloids.

Fig. no. 9: Result of test for tannins.

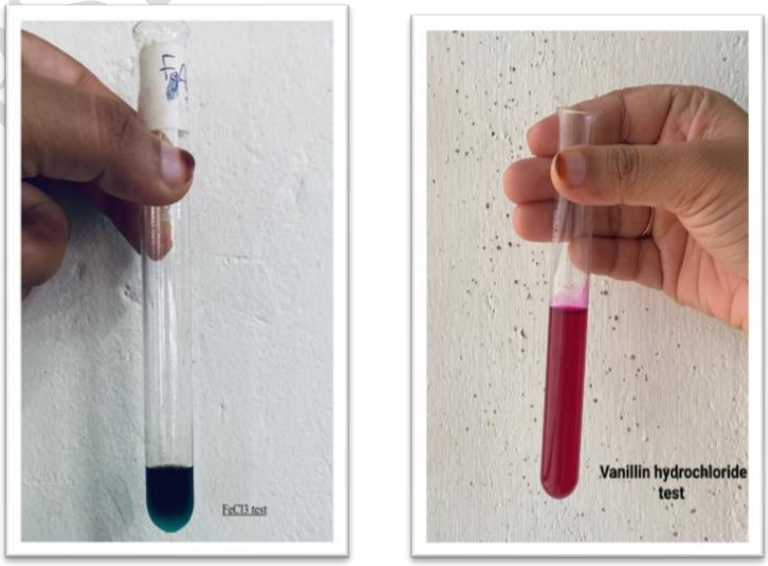

Fig. no: 10: result of the test for terpenoids.

Fig. no: 11: result for test for amino acid.

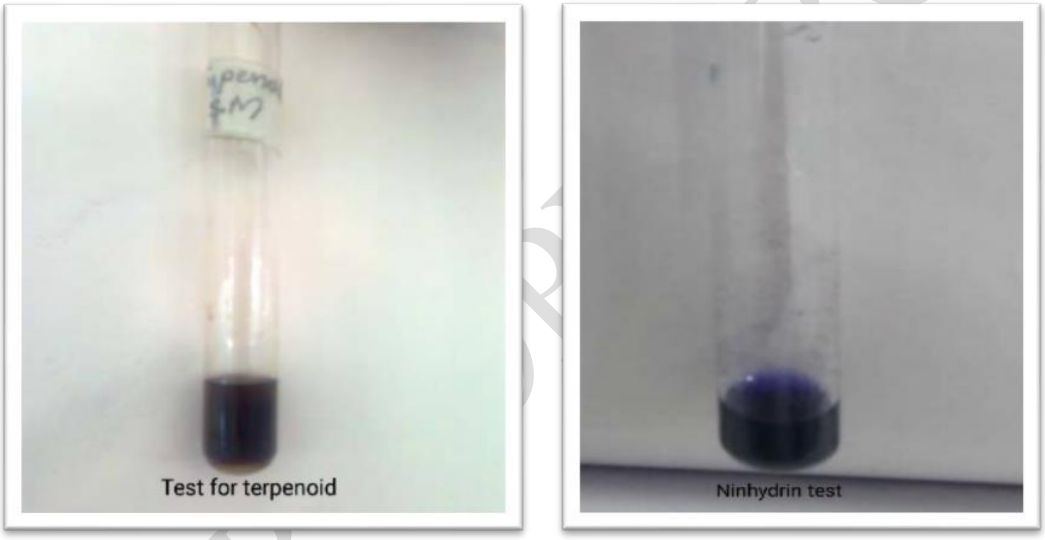

Fig. no: 12: result of test for proteins.

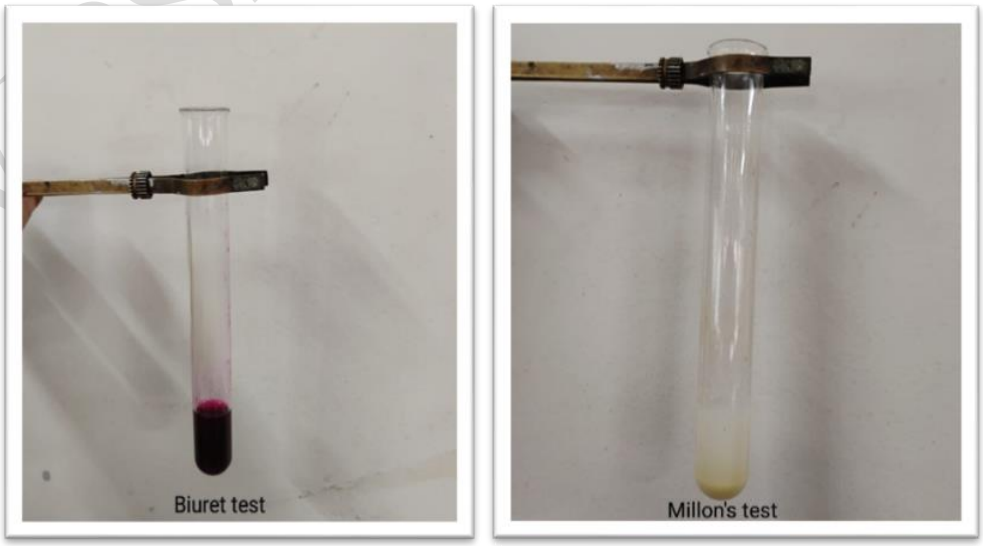

Fig. no: 13: result for the test for glycosides.

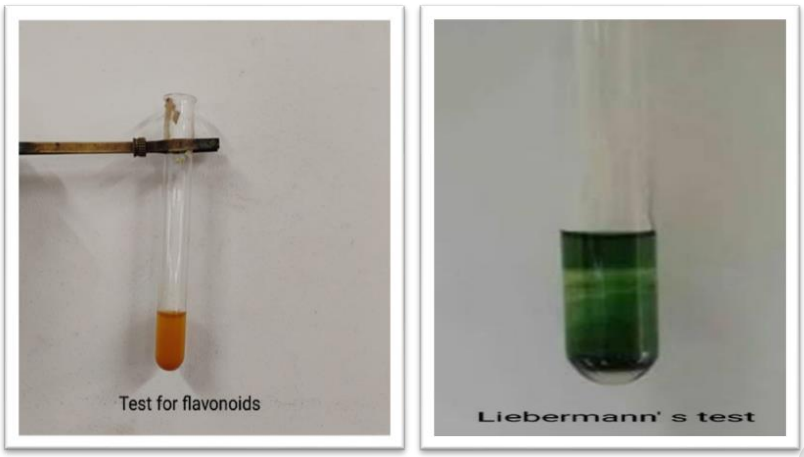

Fig. no: 14: result for the test for flavonoids.

RESULT OF ZONE OF INHIBITION

ZONE OF INHIBITION BASED ON COMBINATION

Fig. no. 15: zone of inhibition on the basis if combination.

Table no.6

SI.NO	SAMPLES	ZONE OF INHIBITION (mm)
1.	Penicillin G	28
2.	PRE +silver salt +vitamin C	29
3.	PRE + silver salt	26
4.	PRE	25

ZONE OF INHIBITION BASED ON DILUTION

Fig. no. 16: zone of inhibition based on dilution.

Table no. 7

SI. NO	SAMPLES	ZONE OF INHIBITION (mm)
1	Penicillin G	28
2.	T1	29
3.	T2	27
4.	T3	25

T1[PRE+Ag salt+vitamin C(1:1)]

T2[PRE+Ag salt+vitamin C(1:5)]

T3[PRE+Ag salt+vitamin C (1:20)]

SUMMARY AND CONCLUSION

The sample extract showed positive result for the chemical test for carbohydrates, alkaloids, tannins, terpenoids, flavonoids, amino acids, proteins and phenols.

Zone of inhibition on agar medium of penicillin G was found to be 28mm. Zone of inhibition on agar medium of PRE + silver salt +vitamin C was found to be 29 mm. Zone of inhibition on agar medium of PRE + silver salt was found to be 26 mm. Zone of inhibition on agar medium of pomegranate rind extract was found to be 25 mm.

Zone of inhibition on agar medium of different dilution of PRE + silver salt +vitamin C combination (T1, T2, T3) was found to be 29 mm, 27 mm, and 25 mm respectively.

Hence, in this study, we showed that the PRE + silver salt + vitamin C combination have shown enhanced anti-MRSA activity.

So, this study showed the potential of PRE in combination with metal salt and vitamin C as an alternative for the treatment of MRSA infection.

OUTCOMES

- Alternative treatment for MRSA infection.
- Utilization of natural resources.
- Less side effects.
- Enhanced efficacy due to the use of combination.

REFERENCES

1. Stuart B Levy: Factors impacting on the problem of antibiotic resistance; volume 49; Journal of antimicrobial chemotherapy; page 25-30.
2. Chinmayee A Nadgir and Dalia A Biswas; Antibiotic resistance and its impact on disease management, 2023 April.
3. C Lee Ventola; Antibiotic resistance crisis; 277-283.
4. Martin Vestergaard, Dorte Frees, Hanne; Antibiotic resistance and MRSA problem, 22 March 2019.
5. Ameer Khusro b, B M Redwan Matin Zidan a, Saikat Mitra: Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects.
6. Abdul H. Siddiqui; Janak Koirala. Methicillin-Resistant Staphylococcus aureus, April 2, 2023.

7. Bindu VP Jayachandran, C D Elsey, 2016, Evaluation of Ant staphylococcal Activity of Ellagic Acid Extracted from Punica granatum Fruit Peel on MRSA, 245 – 253.
8. Derek F. J. Brown, David I. Edwards, Peter M. Hawkey, Donald Morrison, Geoffrey L. Ridgeway, Kevin J. Towner: Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA)
9. Erin M Carrel, Waffa El Sankary, Mark D Fielder, antimicrobial activity of pomegranate rind extract enhancement by addition of metal salts and vitamin C, 2008; page 64.
10. Abdulaziz Alqahtani, Alzhraa Ali Mohamed, Irfan Ahmad, Yasser Alraey, Mohammed Asiri, Jonathan Kopel, Mohammed Merae Alshahrani, Pomegranate peel extract is an effective agent against MDR bacterium Cellular and Molecular Biology, 2023; 69(5): 6-11.
11. Hany M Yehia, Manal F Elkhadragy, antimicrobial activity of pomegranate rind peel extract, 2011; 22: 3664-3668.
12. Amal AL Rashidi, Mohammed Jafar, Niamh Higgins, Ciara Mulligan, Carmine Varricchio, Ryan Moseley, Vildan Celiksoy, David MJ Houston, Charles M Heard, 1889; A time-kill assay study on the synergistic bactericidal activity of pomegranate rind extract and Zn (II) against Methicillin-resistant Staphylococcus aureus, page 11.
13. Nicholas A Turner, Batu K Sharma-Kuinkel, Vance G Fowler Jr; MRSA; An overview of basic and clinical research.
14. Sangeeta Joshi, Pallab Ray, Vikas Manchanda, Jyoti Bajaj, D.S. Chitnis, Vikas Gautam, Anju Kagal; MRSA in India: Prevalence and susceptibility pattern; 363-369.
15. Simon WJ Gould, Mark D Fielder, Anti-microbial activities of pomegranate rind extract: enhancement by cupric sulphate against clinical isolates of S. aureus, MRSA and PVL positive CA-MSSA, July 2009; Volume 9.
16. Yu-Yue Qin, Zhi-Hong Zhang, Lin Li, Wei Xiong, Tian-Rui Zhao, Jian Fan, Antioxidant effect of pomegranate rind powder extract, pomegranate juice, and pomegranate seed powder extract as antioxidants in raw ground pork meat, Food science and biotechnology, 2013; 22: 1063-1069.
17. P Panichayupakaranant, S Tewtrakul, S Yuenyogsawad: Antibacterial activity of Pomegranate, Orange and Lemon peel extracts against food-borne pathogens and spoilage bacteria In vitro and on poultry skin, Food Chemistry, 2010; 123(2): 400-403.
18. Salma Luaiby Dahash, Salah Burhan Al-Windy, Azhar Hatif Al-Kuraishi, Nawar Raad Hussien, Marwa Salih Al-Niemi, Hayder Mutter Al-Kuraishy, Ellagic acid-rich Pomegranate extracts synergizes moxifloxacin against methicillin resistance staphylococcus aureus (MRSA), 2021; S88-S92.
19. Hadi Ebrahim Najad, Maryam Ebadi, Ladan Mansouri-Najand, The anti-planktonic and anti-biofilm formation activity of Iranian pomegranate peel hydro-extract against Staphylococcus aureus, Iranian Journal of Veterinary Science and Technology, 2020; 12(1): 1-9.
20. Mohammad Alnees, Lubna Kharraz, Mahdi Awwad, Duha Najajra, Wafaa Menawi, Nour Dalu, Ghofran Kilani, Rozan Mesad, Haya Othman, Hala Hamayel, Abdalaziz Darwish: Antimicrobial activity of pomegranate peel extract against various type of microorganism, Journal of Medical and Health Sciences, 2023; 4(4).
21. Balwinder Singh, Jatinder Pal Singh, Amritpal Kaur, Narpinder Singh: Antimicrobial potential of pomegranate peel: A review of International Journal of Food Science & Technology, 54(4): 959-965.

22. Elisabete MC Alexandre, Sara Silva, Sónia AO Santos, Armando JD Silvestre, Maria F Duarte, Jorge A Saraiva, Manuela Pintado: Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction, Food research international, 2019; 115: 167-176.
23. Simon W J Gould, Mark D Fielder, Alison F Kelly and Declan P Naughton, Anti-microbial activity of pomegranate rind extract enhancement by metal salt against S. aureus, 2009; 10: 1186-1472.
24. Wedad M Abdelraheem, Marwa M.M Refaie, Assessment of antibacterial and anti-biofilm effects of vitamin C against pseudomonas aeruginosa clinical isolate, 2022.
25. Soraya Mousavi, Stefan Bereswill, immunomodulatory and antimicrobial effects of vitamin C, 73-79.
26. Johanna Kallio, Mari Jaakkola, vitamin C inhibits staphylococcus aureus growth and enhances the inhibitory effect of quercetin on growth of e. coli in vitro, PubMed, oct 2012; 78(17).
27. Pavillard R, Harvey K, Douglas D, Hewston A, Andrew j, Collopy B, et al. Epidemic of hospital -acquired infection due to methicillin -resistant Staphylococcus aureus in major Victorian hospital, 1982; 1: 451-4.
28. Lucet JC, Paoletti X, Demontpion C, Degrave M, Vanjak D, Vincent C, et al. A Domicile study group. Carriage of methicillin -resistant Staphylococcus aureus in home care setting prevalence, duration, and transmission to the household members, 2009; 169: 1372-8
29. Srinivasan S, Sheela D, Shashikala Mathew R, Bazroy J, Kanungo R. Risk factors and associated problems in the management of infections with methicillin resistant Staphylococcus aureus. India J Med Microbial, 2006; 24: 182-5
30. Daskalaki M, Otero JR, Chaves F. Molecular characterization of resistance to mupirocin in methicillin -resistant Staphylococcus aureus isolates in a tertiary hospital in Spain, 2009; 63: 826-8
31. Celiksoy, V; Heard, C.M. The antimicrobial potential of pomegranate. In pomegranate; Info Tech Open: London, UK, 2021; Available online: https://cdn.intechopen.com/pdfs/75059.pdf (accessed on 13 December 2021).
32. Evaluation of Anti staphylococcal Activity of Ellagic Acid Extracted from Punica granatum Fruit Peel on MRSA, Int. J. Curr. microbial. Appl., 2016; Sci 5: 245-253.
33. Mohamed Taha Yassin, Ashraf Abdel-Fattah Mostafa, Abdulaziz Abdulrahman Al Askar, In Vitro Evaluation of Biological Activities and Phytochemical Analysis of Different Solvent Extracts of *Punica granatum* L. (Pomegranate) Peel, Plants, 2021; 10(12): 2742.
34. Rajashekhar Pinnamaneni, preliminary phytochemical investigation of peel of pomegranate Punica granatum, 2018; 11(8).
35. G Karthikeyan, A. K. Vidya, phytochemical analysis, anti- oxidant, and anti- bacterial activity of pomegranate peel., 2019; 0501: 22.