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Original Research Article

Harnessing Nature: The Potential of Pomegranate Peel Ethanol Extract as an Alternative Treatment for Antibiotic-Resistant Foodborne Illnesses

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Abstract: The rising incidence of foodborne illnesses brought on by harmful bacteria emphasizes how urgently alternative treatment solutions are needed to counteract these microbial threats. The objective of this study is to assess the ethanol extract of pomegranate peel's antibacterial, antidiabetic, anti-inflammatory, and anti-biofilm properties in addition to conducting a thorough GC-MS analysis of its bioactive components. Food samples from tainted sources are first collected for the study, and then pathogenic bacteria are isolated and identified using biochemical assays. In order to create a baseline for therapeutic efficacy, the bacteria's resistance to commercial antibiotics is evaluated. Pomegranate peel is solvent extracted concurrently to produce ethanol-based extracts, which are subsequently tested for antibacterial activity against the identified food pathogens. . Alpha-amylase inhibition, a sign of possible antidiabetic qualities, is measured in order to assess the extract's antioxidant, antidiabetic, and anti-inflammatory actions. The ethanol extract's anti-biofilm properties, which are essential for controlling bacterial adhesion and biofilm formation, are further investigated in this work. To find the bioactive substances in the extract and gain a better knowledge of its medicinal potential, photochemical screening and GC-MS analysis are used. Lastly, the effectiveness of the pomegranate peel extract in cleaning tainted food is evaluated, indicating its potential for use in public health and food safety. This study demonstrates the pomegranate peel's many medicinal uses and raises the possibility that it could be used as a natural remedy to fight foodborne infections and associated illnesses.

Keywords: Pomegranate peel extract, food borne pathogens, Alpha-amylase inhibition, anti-biofilm properties, photochemical screening, GC-MS analysis.

INTRODUCTION

Food processing generates substantial amounts of biodegradable waste, notably fruit peels, which, if improperly managed, can lead to environmental pollution and economic losses (Singh et al., 2022). Pomegranate peel, constituting up to 50% of the fruit, is rich in bioactive compounds such as tannins, phenolic acids, flavonoids, and anthocyanins, exhibiting potent antioxidant, antimicrobial, anticancer, and anti-inflammatory properties (Jalal et al., 2018; Usanmaz et al., 2016). India, as the world's leading pomegranate producer with 234,000 hectares under cultivation, significantly contributes to global pomegranate by-product generation (Valero-

Mendoza et al., 2023). Scientific studies have demonstrated that pomegranate peel extract (PPE) effectively inhibits foodborne pathogens and extends the shelf life of perishable food items, including fish and fishery products, which are particularly susceptible to spoilage due to high levels of polyunsaturated fatty acids (Barnossi et al., 2021; Charalampia et al., 2017). With growing concerns over the health risks associated with synthetic antioxidants, such as urinary bladder and stomach cancers (Ito et al., 1986), and the rise of antibiotic resistance (Lambert, 2005), there is an increasing demand for natural, plant-derived preservatives. Consequently, pomegranate peels, often discarded as agricultural waste, present a valuable, eco-friendly resource for developing natural preservatives and functional ingredients in the food, cosmetic, pharmaceutical, and aquaculture sectors (Kahramanoglu et al., 2019; Cao et al., 2002; Barnossi et al., 2021).

SAMPLE COLLECTION

Pomegranate peels was collected from different juice vendors in Korampallam, Thoothukudi, Tamil Nadu, India. The pomegranate peels were manually cleaned and washed to remove dirt and unwanted materials. They were cut into 1x1 inch size, placed on a tray, and dried in a tray drier at a constant temperature of 40°C. Dried peels were ground and sieved into powder by mechanical mixer. The coarse powder was then stored in air tight sealed container and placed at 4°C. Plate 1 showed the Pomegranate peel. (PLATE 1)

PREPARATION OF EXTRACTS

Twenty grams of peel powder was mixed with 180 ml of polar solvent of ethanol. The conical flasks were tightly stoppered with plugs of absorbent cotton and this was wrapped with aluminium foil as a precautionary measure. The conical flasks were placed in a shaker pre-set at 30°c at 130 rpm for 72 hours for solvent extraction to complete. After that, the flasks were removed from the shaker and the filter it using Whatman No.1 filter paper and was poured into Petri plates to allow for ethanol vaporization. After that, ethanol extract was used for further studies.

ISOLATION OF FOOD PATHOGENS

Contaminated food samples were collected from home and transported to our laboratory using sterile containers. The samples were processed in our lab using the preparation of selective media such as Mannitol salt agar, SS agar, EMB agar, Campylobacter isolation agar. One gram of contaminated sample was swabbed on the selective media. The plates were incubated with 37° C for 48 hrs. (PLATE 2)

IDENTIFICATION OF FOOD PATHOGENS

All isolates were subjected to Gram's staining, motility, morphological characteristics and bio chemical tests according to Bergy's manual. (TABLE 1) (PLATE 3)

ACTIVITY OF SELECTED COMMERCIAL ANTIBIOTICS AGAINST ISOLATED FOOD PATHOGENS

Assay of antibiotic activity against the food pathogen was performed by Kirby-Baeur disc diffusion method. The Muller Hinton agar plates were prepared and the organism was swabbed over it using a sterile cotton swab. The antibiotic discs such as Streptomycin, Chlorampenicol, Ampicillin and Tetracyclin were placed on the surface of the agar plates and then, the plates were incubated at 37^0C for 24hrs. After incubation, the zone of inhibition was measured in mm. (TABLE 2) (PLATE 4)

EVALUATION OF ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF POMEGRANATE PEEL

INOCULUM PREPARATION

Loopfull inoculum from each bacterial isolate were inoculated into nutrient broth and incubated at 37 °C for 24 hours. Adjust the turbidity with McFarland quantity of std (0.5) to get a uniform suspension containing 1.5×108 CFU / ml.Evaluation of Antimicrobial Activity. Antimicrobial activity of plant extracts was performed using the agar-well difusion bioassay. The antimicrobial activity of ethanol extract of *Pomegranate* peel against food pathogens was performed by agar well diffusion method. Mueller Hinton Agar (Hi-Media) was prepared, autoclaved, and poured into sterile petriplates. The ethanol extract of pomegranate peel was dissolved in DMSO at the ratio of 1:1. Briely, 100µL of fresh culture (approximately10^6 CFU/mL) was uniformly spread onto Mueller-Hinton agar (MHA) plates by sterile swab dipped with food pathogen. Then, inoculated plates were allowed to dry at room temperature for 20min. Ater that, wells of 6mm in diameter were made in the agar using a sterilized well cutter and 100 µL of different concentration of ethanol extract of PE 100,200,300,400µl/well was poured in different wells. DMSO was used as negative control. Plates were incubated at 37◦C for 24 h. Antibacterial activity was evidenced by the presence of clear inhibition zone around each well. The diameter of this zone was measured in mm and recorded. (TABLE 3) (PLATE 5)

MINIMAL INHIBITORY CONCENTRATION OF ETHANOL EXTRACT OF POMEGRANATE PEEL (EPP)AGAINST FOOD PATHOGENS (MIC)

Determination of minimum inhibitory

concentrations (MIC's) of the Ethanol Extract of Pomegranate peel is defined as the lowest concentration of the extract that inhibits the bacterial growth after 24 h. of incubation. The most effective extracts which exhibiting a strong antibacterial activity at 400 µl/ml was manipulated to determine their MIC using broth dilution method and evaluate their efficiency in controlling bacterial strains causing food poisoning /food infection causing pathogens.

In Broth dilution technique, different concentration of EPP were prepared in ranging from 100 to 500 µl/ml. From each concentration of EPE extract was mixed with one milliliter of nutrient broth. Ten microliter of each standardized broth cultures (1.5 $X10^6$ CFU/ml) was cultivated on the broth containing various concentrations of the EPP extract. Control was maintained without EPP extract. The tubes were then incubated at 37^0C for 24 hrs according to growth requirement of each organism and observed for any visible bacterial growth. And measured the bacterial growth by take OD value at 360nm. MIC was the lowest concentration of extract that resulted in no visible growth on the broth. The broths were incubated according to growth requirement of each organism. The lowest absorbance of turbidity in the broth dilution was the evidence of bactericidal activity. (TABLE 4)

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATIONS (MBC'S) OF EPP EXTRACT

The one µl sample was taken from lowest concentrations of the EPP extract exhibiting invisible growth from MIC broth dilution and subcultures onto sterile Nutrient agar (NA) plates by spread plate method. The plates were incubated at 37^0 C for 24h.After incubation, then examined for bacterial growth in corresponding to lowest concentrations of the EPP extract. MBC was taken as the lowest concentrations of the EPP extract that did not exhibit any bacterial growth on the freshly inoculated Nutrient agar plates.(TABLE 5) (PLATE 6)

PRELIMINARY ASSAY OF ANTIDIABETIC ACTIVITY OF ETHANOL EXTRACT OF POMEGRANATE PEEL

The antidiabetic assay was performed by Adding 1ml of 1% starch to control and test sample tubes.Then 0.5 ml of amylase enzyme was added to all the tubes. Tubes were subjected to Incubation at 37ºC for 30 min. After incubation 1ml DNSA reagent was added to all the tubes. Then allow the tubes for heating at 95 ºC for 15 min in water bath. Atlast Read the absorbance at 510 nm. (PLATE 7)

% of Inhibition = [Absorbance Control-Absorbance Test)/Absorbance control] ×100

ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT OF POMEGRANATE PEEL

Antioxidant activity of ethanol extract of *Pomegranate* peel was done by Phosphomolybdenum assay. For the conduction of the phosphomolybdenum assay, the method was followed by Prieto *et al.,* (1999). An aliquot of 0.1mL of sample solution of different concentrations (25–400µg/mL) treated with 1mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C in a water bath for 90 min. The samples were cooled to room temperature and their absorbance was recorded at 765 nm. Ascorbic acid was used as the positive control. Antioxidant capacity was estimated by using following equation. (TABLE 6) (PLATE 8)

Antioxidant activity % = [(Absorbance control − Absorbance sample)/Absorbance control] × 100

ANTI BIOFILM ACTIVITY OF ETHANOL EXTRACT OF *POMEGRANATE* PEEL

Test tube containing 1ml of Nutrient Broth was prepared and sterilized at 121°C for 15 minutes. Then the tubes were inoculated using a test microorganisms known to form biofilms. Add the 100 µl, 200 µl, 300 µl, 400 µl of the prepared concentration of *Pomegranate* extracts solution to the test tubes separately, ensuring each tube receives the desired concentration of the extract. Include the control tube without the extract. Place the inoculated test tubes in an incubator set at 37°C for 48-72 hours. After the incubation, the modified crystal violet staining (CVS) assay was performed to quantify the biofilm biomass. (TABLE 7,8) (PLATE 9)

CRYSTAL VIOLET STAINING (CVS) ASSAY

The tubes were carefully emptied and tubes were washed at least two times with sterile distilled water to remove unattached or loosely attached cells. The tubes were air -dried for 20 mins. The adhered cells stained with 1ml of 0.1% crystal violet solution for 10 minutes at room temperature. Excess stain was rinsed off by washing the plates at least two times with water. Thereafter, the biofilm biomass was evaluated semiquantitatively by re-solubilizing the crystal violet stain bound to the adherent's cells with 150 µl of 100% ethanol to destain the wells. The absorbance of the tubes was read at 590 nm using a Spectrophotometer after careful and gentle shaking. The OD value (OD590nm) of the sample was determined and results expressed as percentage (FIGURE 1)

Inhibition using the equation below

Percentage (%) inhibition = OD Negative control – OD Sample] / OD Negative control × 100

ANTI-INFLAMMATORY ACTIVITY OF

ETHANOL EXTRACT OF POMEGRANATE PEEL

PREPARATION OF REACTION MIXTURE

Bovine serum albumin solution was prepared by adding 1g of Bovine serum albumin(BSA) in a 100ml of distilled water. In a test tube add 1ml of bovine serum albumin (BSA) solution and 0.2 ml, 0.4ml, 0.6ml, 0.8ml of the sample solution separately, then make up to 2ml using the distilled water. Include control tubes without the sample extract can be added.

INCUBATE THE SAMPLES

Allow the mixture to incubate at 60°C for 10 minutes in a water bath to allow any potential anti-inflammatory activity to occur. (PLATE 10)

PERFORM THE LOWRY METHOD

After the incubation period, use the Lowry method to measure the protein concentration in each sample. This method involves adding 0.3ml of Folin-Ciocalteau reagents to the samples, followed by colour development and measure OD value of the standard and test solution at 660 nm and plot the standard graph. (TABLE 9)

The percentage inhibition for anti-inflammatory activity can be calculated using the following formula:

Percentage Inhibition = (1−Absorbance of Sample/Absorbance of Control) ×100

PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACT OF POMEGRANATE PEEL

The EPP extract (ethanol extract of *Pomegranate* peel) was subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites [Harbone et al., 1998].(TABLE 10) (PLATE 11)

1. Alkaloids: To 0.5 g of each extract, was added 5 ml of 2 N HCl and filtered. Dragndoff's reagent was added and formation of a red precipitate was used to indicate the presence of alkaloids.

2. Phenols: About 3 to 4 drops of ferric chloride solution was added to the extract. The presence of phenol was indicated by the formation of bluish black colouration.

3. Double bond: About 2ml of each plant extracts were mixed with a 2ml of potassium permanganate. The formation of brown colour indicates the presence of double bond.

4. Ninhydrin test : About 2 drops of Ninhydrin reagent was added with the 2ml of Plant extract. The positive indicates the appearance of a complex with a purple colour in the test tube.

5. Flavonoids: Sodium hydroxide (10%) was added to the plant extract. The formation of yellow colouration indicated the presence of flavonoids.

6. Phenolics and Tannins: A 10% Lead acetate solution was added to little quantity of the extract already dissolved in distilled water. The presence of tannin was indicated by formation of white precipitate.

7. Carbohydrate test : Molisch's Test: About 2ml of each extract were mixed with two drops of α-naphthol and shaken thoroughly in a test tube. Then drops of concentrated sulphuric acid was added slowly.The appearance of violet ring indicated the presence of carbohydrate.

GC - MS ANALYSIS OF ETHANOL EXTRACT OF POMEGRANATE PEEL

The 2 micro liters of ethanol extract of *Pomegranate* peel was injected into the GC-MS system on a 30-m glass capillary column with a film thickness of 0.25 mm using the following temperature program: initial oven temperature of 40°C for 4 min, increased to 25°C at 158°C/ min, and then held at 25°C for 10 min. GC-MS was run under computer control at 70 eV. Chemical ionization was performed using ammonia as the reagent gas at 95 eV. The solvent (dichloromethane) peak was seen at 2.4 min during the GC- MS analysis followed by compound peaks. Identification of unknown compounds was made by probability-based matching using the system such as, WILEY Registry™95, NIST05 and NIST05s. MS data library and comparing the spectrum obtained through GC-MS compounds present in the ethanol extract of *Pomegranate* peel sample wase identified. (TABLE 11) (FIGURE 2)

ANTICANCER ACTIVITY OF ETHANOL EXTRACT OF POMEGRANATE PEEL

The anticancer activity of the ethanol extract was evaluated using the MTT assay, a widely used colorimetric method for assessing cell viability and cytotoxicity (Mosmann, 1983). Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to attach overnight. The cells were then treated with various concentrations of pomegranate peel ethanol extract (10–500 µg/mL) for 24 or 48 h, while untreated cells served as the control. After incubation, 20 µL of MTT solution (5 mg/mL) was added to each well and further incubated for 4 h at 37 °C. The purple formazan crystals formed were dissolved by adding 100 µL of dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability was

expressed as a percentage relative to the control, and the IC_{50} values were calculated from dose–response curves. Apoptotic activity was assessed using acridine orange/ethidium bromide (AO/EB) dual staining and Annexin V-FITC/propidium iodide (PI) assays following standard protocols (Vermes et al., 1995; Ribble et al., 2005). Treated and untreated cells were stained and analyzed using a fluorescence microscope or flow cytometer to distinguish viable, early apoptotic, late apoptotic, and necrotic cell populations based on membrane integrity and phosphatidylserine externalization.

RESULTS AND DISCUSSION:

The contamination of food products with microorganisms is a global concern due to the associated health risks and food spoilage. Bacteria, along with molds and yeasts, are the principal agents responsible for foodborne illnesses and spoilage (Blackburn et al., 2006). Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, as well as Gram-positive bacteria like *Staphylococcus aureus* and *Bacillus cereus*, have been frequently implicated in foodborne diseases (Mostafa et al., 2018). The pomegranate, widely cultivated in India, is notable for its bioactive-rich peel, often discarded as waste (Malviya et al., 2014). With increasing demand for pomegranate-derived products, including juice and dietary supplements, scientific attention has turned toward the bioactive potential of its peel, which constitutes approximately 60% of the fruit's weight (Lansky & Newman, 2007). It contains antioxidants, phenols, flavonoids, and shows significant antibacterial and antifungal activity, justifying its selection for the current study. This study utilized ethanol for extracting the pomegranate peel's bioactive compounds. While methanol has shown higher efficiency in phenolic extraction (Isbilir et al., 2012), ethanol is also a polar solvent and safer for food-related applications. Literature has shown mixed results regarding extraction efficiency using different solvents (Wang et al., 2011; Orak et al., 2012; Abid et al., 2017). Isolation and identification of foodborne pathogens from mutton samples included *E. coli*, *S. aureus*, *S. typhi*, *Shigella flexneri*, and *Campylobacter jejuni*, based on morphological and biochemical characteristics, consistent with findings by Rajput et al. (2014) and Cruickshank et al. (1975). The results confirmed the presence of *E. coli* through tests like catalase, indole, methyl red, and citrate utilization. Antibiotic susceptibility testing revealed that isolates were resistant to commonly used antibiotics like chloramphenicol and ampicillin, underscoring the issue of antibiotic resistance among food pathogens (Slobodníková et al., 2016). The ethanol extract of pomegranate peel (EPP) exhibited strong antibacterial activity. At 400 µl/well, EPP produced the largest inhibition zones against *S. typhi* (29 mm) and *S. flexneri* (28 mm), and smaller zones against *C. jejuni* and *E. coli* at 100 µl/well (16 mm and 17 mm respectively), aligning with findings from Kumar et al. (2013). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were determined for EPP, with MIC recorded at 0.45 mg/ml, closely matching the range reported by Sirikhwan et al. (2016). These results demonstrate the potency of EPP against multiple foodborne pathogens. EPP also showed promising results in other bioactivities. Antioxidant activity was recorded at 83.67%, which may be attributed to its high phenolic content, including ellagitannins such as ellagic acid and punicalagin (Gigliobianco et al., 2022). Antidiabetic potential was indicated by α-amylase inhibition of 61.7% at 400 µl, consistent with its traditional use in managing blood glucose levels. The antibiofilm activity of EPP was assessed against *S. aureus* and *S. typhi*, showing excellent inhibition. Biofilm inhibition was evident with no ring formation on test tubes and low OD values in crystal violet assays. This is in line with the understanding that plant-derived phenolics disrupt quorum sensing and biofilm formation (Nazzaro et al., 2013; Roy et al., 2018). Anti-inflammatory activity was also demonstrated, with the highest inhibition (43.65%) observed at 800 µl of EPP, showing a concentration-dependent response. These findings support the multifunctional bioactivity of pomegranate peel extracts. Phytochemical screening confirmed the presence of alkaloids, double bonds, phenols, flavonoids, saponins, carbohydrates, and tannins, all contributing to the antimicrobial and antioxidant effects. GC-MS analysis revealed the presence of various bioactive compounds such as furfural, 2-furancarboxaldehyde, and (3-nitro-phenoxy)-acetic acid pyridin-2-ylmethylene-hydrazide, which are known for antimicrobial and therapeutic activities. EPP-coated meat samples exhibited extended shelf-life compared to controls. After 7 days of refrigeration, only *Listeria* spp. (10 cfu/sample) was found in treated meat, while control samples showed TNTC levels. Similarly, EPP-coated guava fruits resisted spoilage for up to 5 days, with minimal microbial load on the 7th day (15 cfu/sample), whereas control fruits showed heavy fungal contamination. The ethanol extract of pomegranate peel (PP) exhibited significant anticancer activity against human cancer cell lines in a concentration-dependent manner. Untreated control cells maintained normal morphology with intact membranes and adherence, whereas PP-treated cells (60 and 80 µg/mL for 24 h) showed pronounced apoptotic characteristics, including cell shrinkage, membrane blebbing, loss of adherence, and cellular distortion. These morphological alterations are consistent with apoptosis-mediated cytotoxicity and align with earlier reports on pomegranate-derived bioactives (Lansky & Newman, 2007). Apoptotic

induction was further confirmed by AO/EB dual staining. Control cells emitted uniform green fluorescence, indicating viable cells with intact nuclear morphology. In contrast, PP-treated cells displayed yellowish-green fluorescence at 60 µg/mL, indicative of early apoptosis, while cells treated with 80 µg/mL showed orange-red fluorescence with nuclear condensation and fragmentation, confirming late apoptosis. The progressive increase in apoptotic cell population with increasing concentration demonstrates a dose-dependent apoptotic response, similar to previous observations in pomegranate-treated cancer models (Seeram et al., 2005). The apoptosis-inducing potential of PP may be attributed to its high polyphenolic content, including punicalagin and ellagic acid, which are known to modulate mitochondrial apoptotic pathways, elevate intracellular reactive oxygen species, and inhibit pro-survival signaling pathways such as PI3K/Akt and NF-κB (Adhami et al., 2009; Syed et al., 2013). Collectively, these findings support the role of pomegranate peel ethanol extract as a potent natural anticancer agent capable of inducing programmed cell death in cancer cells.

TABLE 1 : THE BIOCHEMICAL CHARACTERISTICS OF FOOD PATHOGENIC BACTERIA

Organisms	Oxidase test	Catalase test	Indole test	Methyl red test	Voges Proskauer test	Citrate test	Gram staining
Salmonella typhi	-	+	-	+	-	+	-
Shigella flexneri	-	+	-	+	-	-	-
E.coli	-	+	+	+	-	-	-
S.aureus	-	+	-	+	+	-	+
Camphylobacter jejuni	+	+	-	+	-	+	-

TABLE 2: TO DETERMINE SUSCEPTIBILITY OF COMMERCIAL ANTIBIOTICS AGAINST FOOD PATHOGENS

Food Pathogens	Zone of inhibition (mm)				
	Streptomycin (10mcg)	Ampicillin (10mcg)	Chloramphenicol (30mcg)	Tetracyclin (30mcg)	Levofloxin (5m cg)
Staphylococcus aureus	18	Resistance	Resistance	24	8
Shigella flexneri	16	Resistance	Resistance	22	6
E. coli	15	Resistance	Resistance	22	5
Salmonella typhi	14	Resistance	Resistance	20	4
Camphylobacter jejuni	18	Resistance	Resistance	23	5

TABLE 3: ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF POMEGRANATE PEEL AGAINST FOOD PATHOGENS

Food pathogens	Concentration of ethanol extract of EPP (µl/well)			
	100	200	300	400
	Zone of Inhibition in mm			
Staphylococcus aureus	18	21	23	25
Shigella flexneri	20	22	25	28
Escherichia coli	17	20	22	24
Salmonella typhi	19	25	27	29

Camphylobacter jejuni	16	18	19	20

TABLE 4: MIC AND MBC VALUE OF ETHANOL EXTRACT OF POMEGRANATE PEEL AGAINST FOOD PATHOGENS

S.No	Food pathogens	MIC Concentration of ethanol extract of *Pomegranate* peel (µg/ml)					MBC Concentration of ethanol extract of *Pomegranate* peel (µg/ml)				
		OD value of Bacteria growth (620 nm)					Bacteria growth(cfu/ml)				
		100	200	300	400	500	100	200	300	400	500
1	*Staphylococcus aureus*	1.97	1.84	1.74	1.24	1.3	78	57	33	14	Nil
2	*Salmonella typhi*	1.84	1.74	1.66	1.25	1.1	87	63	49	24	Nil
3	*Shigella flexneri*	1.87	1.76	1.69	1.27	1.2	93	76	54	12	Nil
4	*Escherichia coli*	1.79	1.67	1.52	1.23	1.4	75	50	37	19	Nil
5	*Camphylobacter jejuni*	1.68	1.56	1.52	1.22	1.9	84	67	48	21	nil

TABLE 5: MIC AND MBC VALUE OF ETHANOL EXTRACT OF POMEGRANATE PEEL

S.No	Food pathogens	MIC value of ethanol extract of *Pomegranate* peel (µg/ml)	MBC value of ethanol extract of *Pomegranate* peel(µg/ml)
1	*Staphylococcus aureus*	400	500
2	*Salmonella typhi*	400	500
3	*Shigella flexneri*	400	500
4	*Escherichia coli*	400	500
5	*Campylobacter jejuni*	400	500

Table 6: ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT OF POMEGRANATE PEEL

Sample	Concentration µg/ml	OD @ 695 nm	% Antioxidant activity
Ascorbic acid	1000	1.96	100
EPP	400	0.32	83.67

Table 7: ANTI-BIOFILM ACTIVITY OF EPP AGAINST STAPHYLOCOCCUS AUREUS

Sample concentration	OD Value @ 620nm
Control	0.28
100µl	0.25
200µl	0.22
300µl	0.20
400µl	0.19

Table 8: ANTI-BIOFILM ACTIVITY TEST AGAINST SALMONELLA TYPHI

Sample concentration	OD Value @ 620nm
Control	0.29
100µl	0.22
200µl	0.20
300µl	0.18
400µl	0.16

FIGURE 1: INHIBITION PERCENTAGE OF BIO-FILM ACTIVITY OF ETHANOL EXTRACT OF POMEGRANATE PEEL

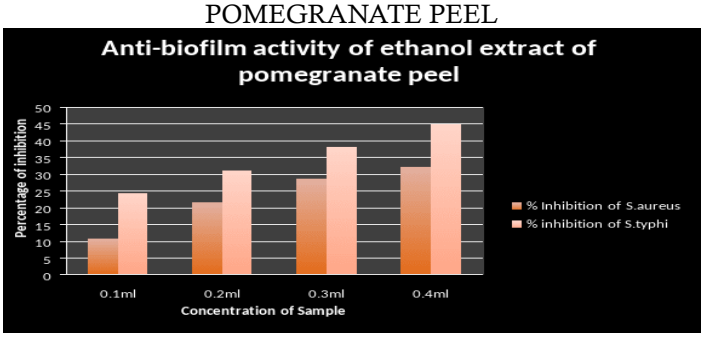


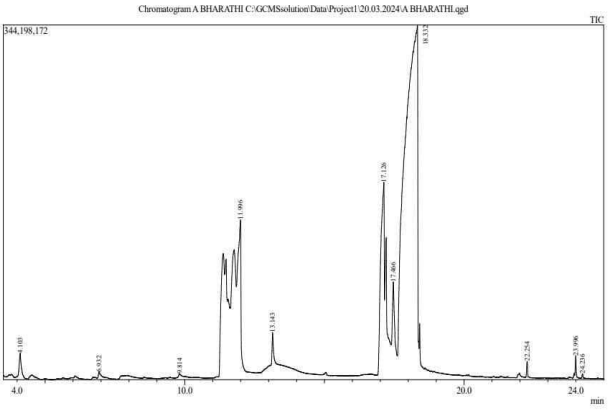
TABLE 9: ANTI-INFLAMMATORY TEST OF ETHANOL EXTRACT OF POMEGRANATE PEEL

Sample concentration	OD Value @ 620nm	% inhibition
Control	1.96	-
1st Tube (200µl)	1.23	37.56
2nd Tube (400µl)	1.19	39.59
3rd Tube (600µl)	1.14	42.13
4th Tube (800µl)	1.11	43.65

TABLE 10: PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT OF POMEGRANATE PEEL

Test	Presence or Absence of samples
Double bond test	PRESENCE
Tannins	PRESENCE
Flavonoids	PRESENCE
Alkaloids	PRESENCE
Carbohydrates	PRESENCE
Phenols	PRESENCE
Ninhydrin test	PRESENCE

FIGURE 2: GCMS ANALYSIS OF ETHANOL EXTRACTION OF POMEGRANATE



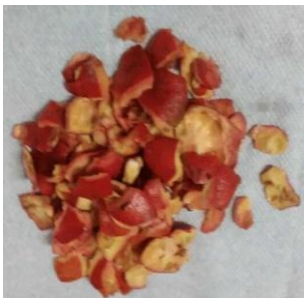
Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	4.103	115587239	0.69	22729525	2.84	Furfural
2	6.932	18257403	0.11	4960147	0.62	2-Furancarboxaldehyde, 5-methyl-
3	9.814	21168475	0.13	3149491	0.39	Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide
4	11.996	4562882242	27.38	1486975777	18.55	4-Hydroxy-2-nitro-m-ansaldehyde
5	13.143	111454056	0.67	31192223	3.89	Benzoic acid, 2-hydroxy-, ethyl ester
6	17.126	1953968535	11.72	173293025	21.62	Benzoic acid, 3-hydrazino-4-methyl-, ethyl ester
7	17.466	283572601	1.70	64154386	8.00	Diethyl 2-(p-tolyl)malonate
8	18.332	9515518466	57.10	314026179	39.18	(3-Nitro-phenoxy)-acetic acid pyridin-2-ylmethylene-hydrazide
9	22.254	35232148	0.21	15405845	1.92	Hexadecanoic acid, ethyl ester
10	23.996	39696167	0.24	19944453	2.49	Ethyl Oleate
11	24.236	8254531	0.05	4033212	0.50	Octadecanoic acid, ethyl ester
		16665591863	100.00	801586063	100.00	

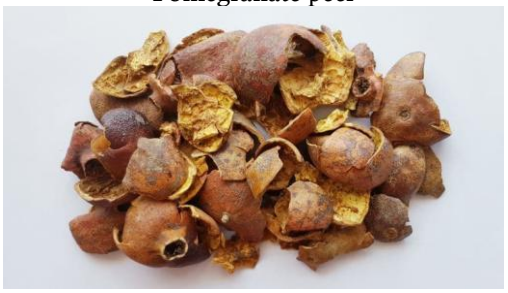
TABLE 11: GC MS ANALYSIS OF ETHANOL EXTRACT OF POMEGRANATE PEEL

IUPAC NAME	Molecular formula	Molecular weight	RT	Area	Area %	Activity	References
Furfural	C5H4O2	96.08 g/mol	4.103	11558723 9	0.69	Antimicrobial, Antioxidant, and Antitumor	Raman *et al.,* (2015). Yedi *et al.,* (2012) Lumina *et al.,* (2002) Wani *et al.,* (2016)
2-Furancarboxaldehyde,5-methyl	C6H6O2	110.11 g/mol	6.932	18257403	0.11	Anti- bacterial and antifungal	Li *et al.,* (2002)
Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide	C13H23NO	209.33 g/mol	9.814	21168475	0.13	Anti-bacterial and Anti fungal	Pradeep *et al.* (2022) Singh *et al.,* (2022)
4-Hydroxy-2-nitro-m-anisaldehyde	C8H7NO5	197.14 g/mol	11.996	45628824 2	27.38	antimicrobial activity	Kannappan *et al.,* (2023)
Benzoic acid, 2-hydroxy-, ethyl ester	C9H10O3	166.17 g/mol	13.143	11145405 6	0.67	antimicrobial activity	https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/benzoic-acid
Benzoic acid, 3-hydrazino-4-methyl-, ethyl ester	C10H14N2O2	198.23 g/mol	17.126	19539683 5	11.72	antimicrobial activity	https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/benzoic-acid
Diethyl 2-(p-tolyl)malonate	C15H18O4	262.30 g/mol	17.466	28357260 1	1.70	artificial flavourings. Pesticides preparation	Strittmatter *et al.,* (2007)
(3-Nitro-phenoxy)-acetic acid pyridin-2-ylmethylene-hydrazide	C15H12N4O5	324.28 g/mol	18.332	95155186 6	57.10	Antibacterial activity	Shaaban *et al.,*(2019)
Hexadecanoic acid, ethyl ester	C18H36O2	284.48 g/mol	22.254	35232148	0.21	Anti oxidant, Nematicide and Pesticide.	Sheela *et al.,* (2013)
Ethyl Oleate	C20H38O2	310.52 g/mol	23.996	39696167	0.24	Antimicrobial	Gabriel *et al.,* (2011)
Octadecanoic acid, ethyl ester	C20H40O2	C20H40O2	24.236	8254531	0.05	antibacterial agent, inflammatory processes	Zheng *et al.,* (2005);Das (2006)

PLATE 1 : PREPARATION OF POMEGRANATE PEEL POWDER



Pomegranate peel



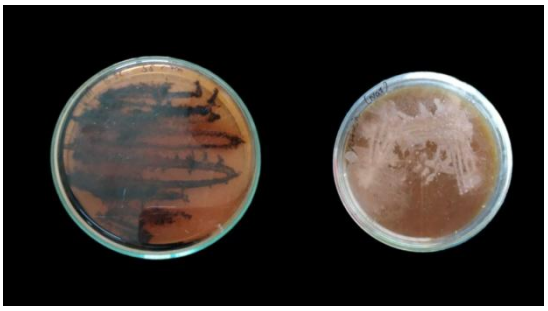
Dried Pomegranate peel

Powder of Pomegranate peel

PLATE 2 : ISOLATION FOOD PATHOGENIC BACTERIA FROM CONTAMINATED FOODS

EMB agar - *E.coli* Mannitol salt agar - *Staphylococcus aureus*

Camphylobacter isolation agar - *Camphylobacter jejuni*



SS agar - *Salmonella typhi* SS agar - *Shigella flexneri*

PLATE 3: BIOCHEMICAL TEST FOR FOOD PATHOGENIC BACTERIA

Biochemical test for *Salmonella typhi*

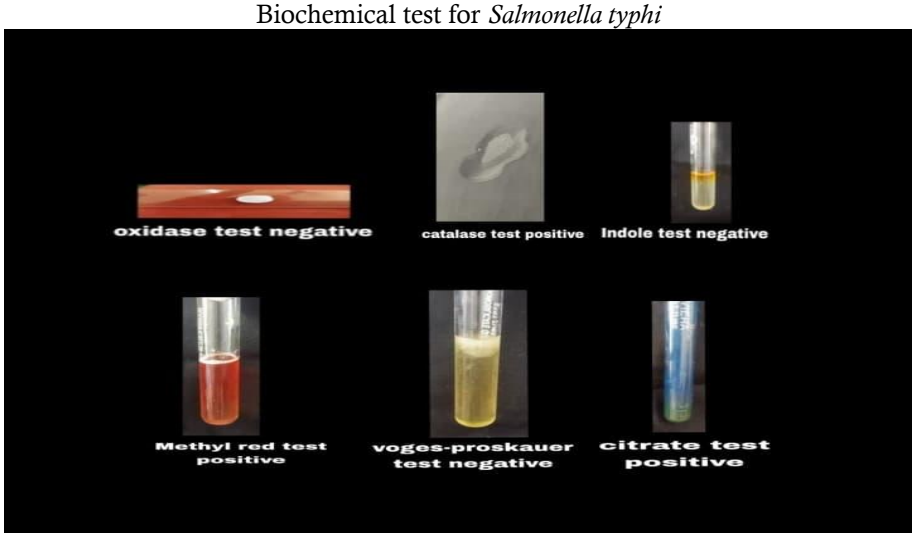


PLATE 4 : SUSCEPTIBILITY OF COMMERCIAL ANTIBIOTICS AGAINST FOOD PATHOGENS

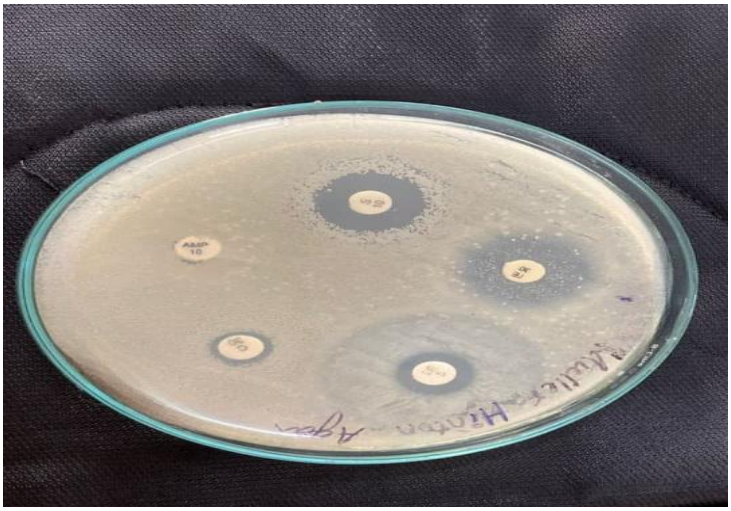
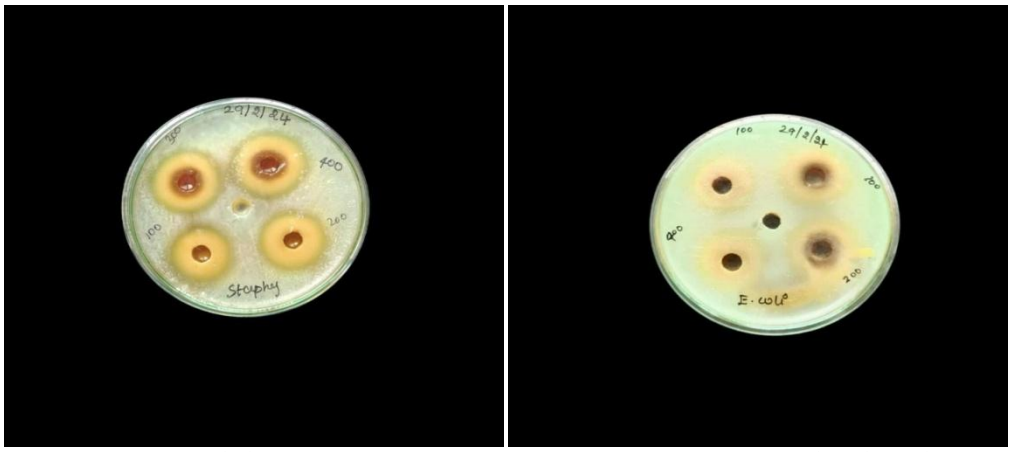


Plate 5: ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF POMEGRANATE PEEL AGAINST FOOD PATHOGENS

Camphylobacter jejuni *shigella fexneri*

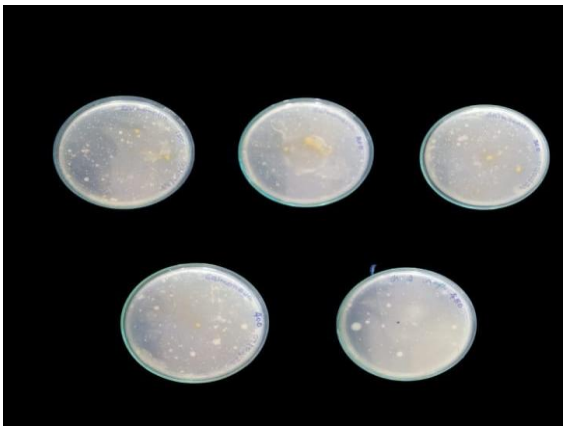
Salmonella typhi



Staphylococcus aureus *Escherichia coli*

PLATE 6: MBC OF ETHANOL EXTRACT OF POMEGRANATE PEEL

a) Salmonella typhi



b) *Staphylococcus aureus*

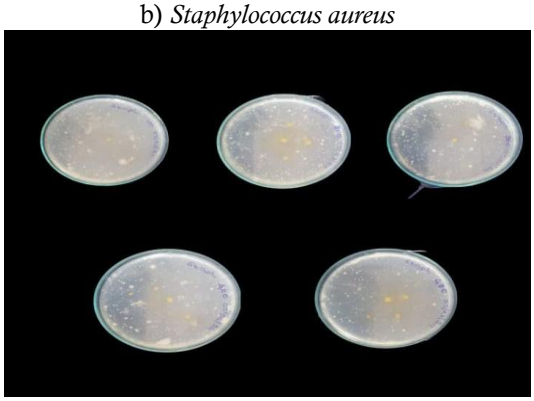


PLATE 6: ANTI-DIABETICS ACTIVITY FOR ETHANOL EXTRACT OF POMEGRANATE PEEL

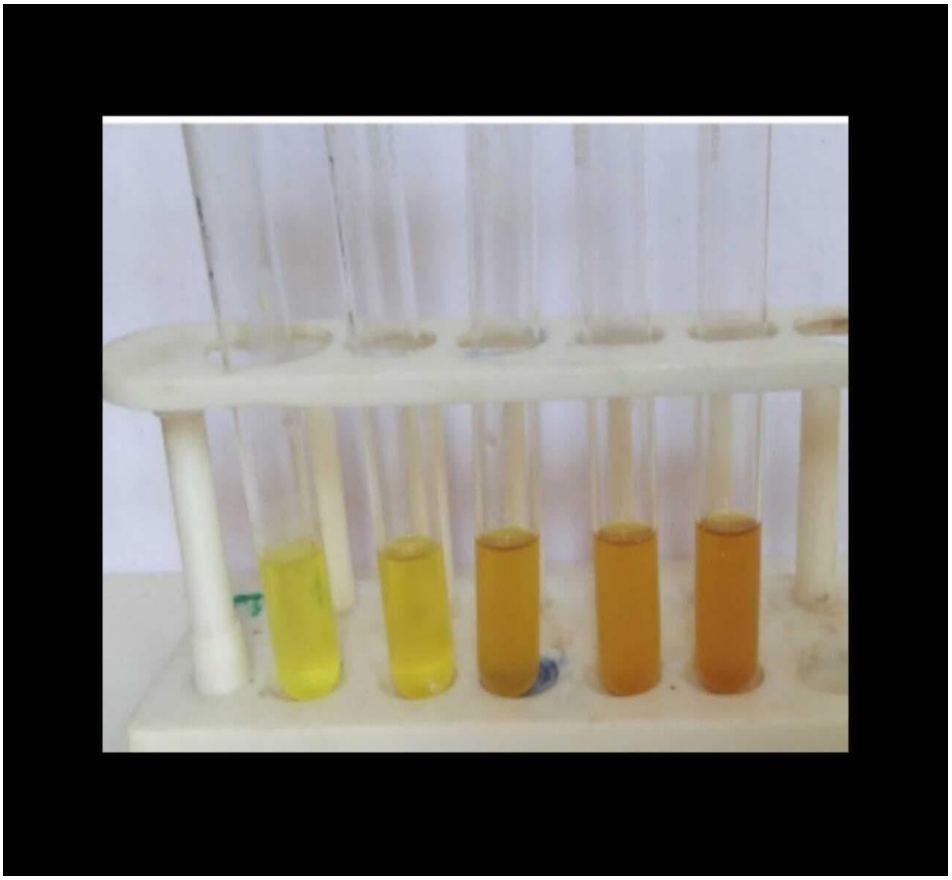


PLATE 7: ANTIOXIDANT ACTIVITY FOR ETHANOL EXTRACT OF POMEGRANATE PEEL

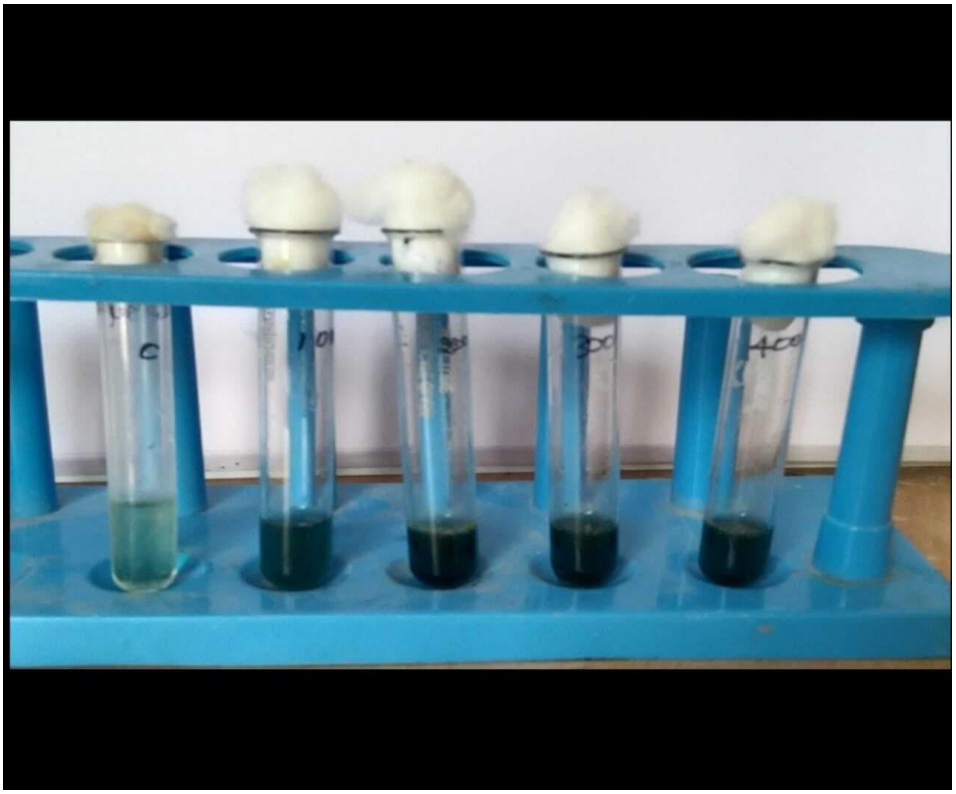
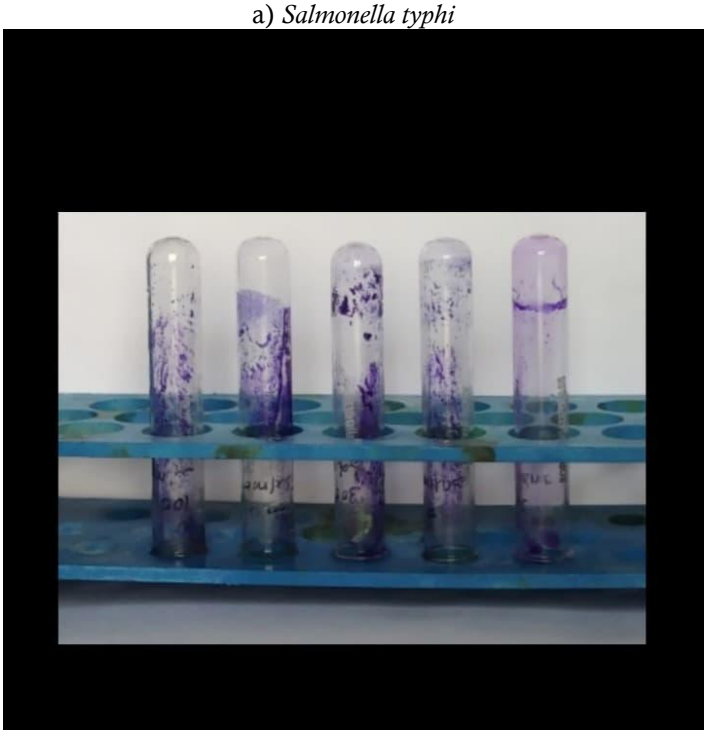


PLATE 8 : ANTI-BIOFILM ACTIVITY OF ETHANOL EXTRACTION OF POMEGRANATE PEEL

a) *Salmonella typhi*



b) *Salmonella typhi*

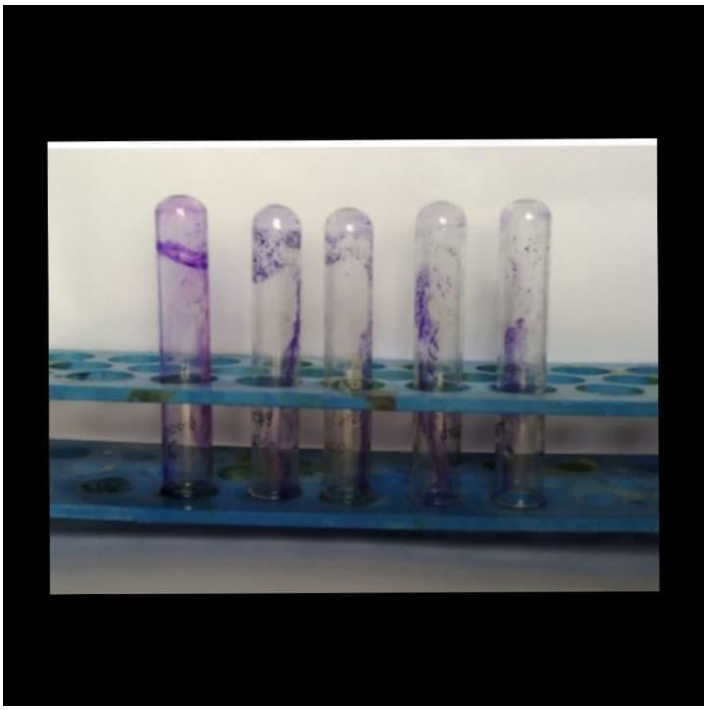
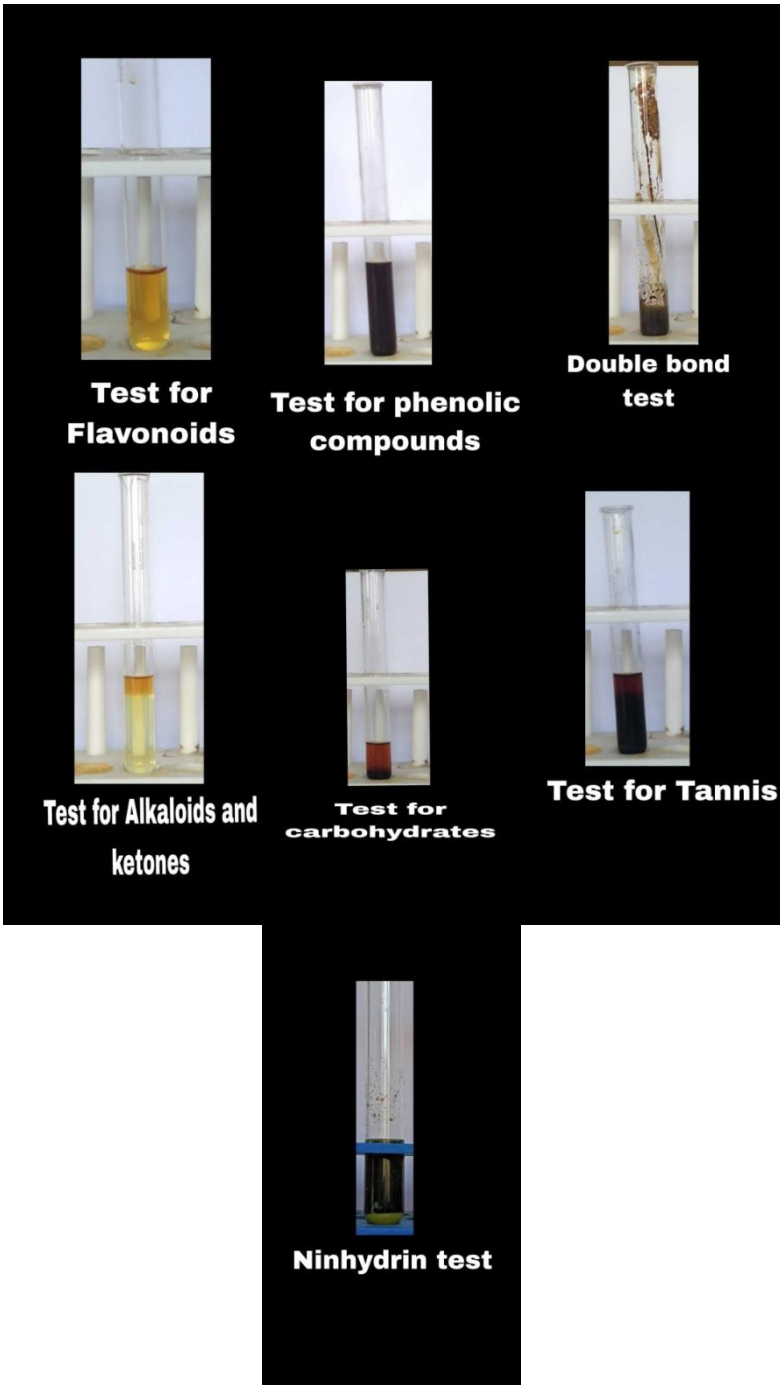


PLATE 9 : ANTI-INFLAMMATORY ACTIVITY FOR ETHANOL EXTRACT OF POMEGRANATE PEEL

PLATE 10: PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT OF POMEGRANATE PEEL



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