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Antibacterial Activity of Pomegranate Peel Against Multidrug Resistant Bacteria

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ABSTRACT

Bacterial resistance to antibiotics and their infiltration into cells are the main causes of antibiotic failure or difficulties. Since the second step would be used as a successful substitute therapy for getting rid of the multi-resistant bacteria, the initial step in our study rationale was intended as the aqueous peel of pomegranate extract. Effect of a clinical isolate made from pomegranate peel from Cairo's Al-Azhar University's El-Zahraa hospital college of Medicine (Girls). The antibacterial efficacy of pomegranate peel extractions against *Klebsiella pneumonia* and *E. coli* sample was examined using the well-diffusion method. Results: Two pathogenic bacteria were resistant to pomegranate peel extracts made with various solvents (D H2O, Fresh pp, ethanolic & methanolic). The powdered extract of pomegranate peel possesses potent antibacterial properties against *E. coli* and *Klebsiella pneumonia*.

INTRODUCTION

Some microbes display antimicrobial resistance to numerous antimicrobial medicines, called multiple drug resistance (MDR). Due to their antibiotic resistance, MDR bacteria are the biggest threat to public health. According to WHO (2018) and Magiorakos (2014), other MDR includes illnesses that are resistant to a number of antivirals, antifungal, and antiparasitic medications. Resistance may be influenced by a variety of biochemical and physiological factors. (Liu and Pop, 2009). The single main reason why there hasn't been much progress in the effective prevention and management of resistance development is that the mechanisms that lead to the establishment and spread of resistance in the specific situation of antimicrobial drugs are so complex. (Liu and Pop, 2009).

This level of complexity cannot be emphasised. There are distinct antimicrobial chemical types that can kill or inhibit microbes even at high dilutions Laxminarayan *et al.* (2013). According to Talaro & Talaro (2002) and Nester *et al.* (2004), antibiotics are chemicals that some microbes naturally make through their metabolic processes that can either inhibit or kill other microorganisms. The fruit of the pomegranate, Punica granatum L., is abundant in polyphenolic compounds, which have been demonstrated to possess exceptional antioxidant capabilities (Al-Musharfi NK *et al.*, 2015). Pomegranate peel (PP), an agricultural waste, accounts for over fifty percent of a pomegranate fruit's weight. Peels are rich suppliers of potassium, oxalic acid, and the vitamins A, B6, B9, and E. Peels from pomegranates have long been used to treat intestinal wounds and promote weight loss. According to a phytochemical analysis of PP extract, flavonoids and polyphenols are among the main phytoconstituents (Al-Rawahi A, *et al.*, 2014). Quercetin and gallic acid are two flavonoids and phenolics that considerably increase the concentration of pomegranate extracts [Orak H, Yagar H, Isbilir S (2012)]. People have been demonstrated to benefit greatly from the anti-inflammatory properties, angioprotective, and anticancer properties of the flavonoid quercetin (D'Andrea D 2015).

MATERIALS AND METHODS

1-Sample Collection:

In general surgery at Al-Azhar University Cairo's Faculty of Medicine (Girls), fifteen distinct human clinical samples including diabetic foot, abscesses, and wounds were gathered. On MacConkey and blood agar plates, transferred samples were cultured in the lab under aerobic conditions at 37 °C. On pure culture agar slants, the bacterial growth was separated from the plates and purified. Then subjected to Cultural, morphological, and biochemical identification.

2-Antimicrobial Susceptibility Test of Bacterial Isolates:

A disc diffusion method was used to test the susceptibility of antibiotics (Biemer, 1973), (Kirby et al., 1996). The isolates were exposed to the antibiotics Cefotaxime (CX 30 mg), Gentamicin (GN10 mg), and Norfloxacin (NX10 mg) to determine their susceptibility, Ceftazidime (CAZ 30 mg), Imipenem (IpM 10 mg), Amikacin (AK30 mg), Ofloxacin (OF5 mg), Cefadroxil (CFR 30 mg), Tigecycline (TGC 15 mg), Levofloxacin (Lev5 mg), Meropenem (MRP 10 mg), and Rifampicin (RIF)(5 mg) are some of the Cefoperazone (CPZ 15 g), Cefepime (CPM 30 g), Ertapenem (ETP 10 g), Tobramycin (TOB 10 mg), Fosfomycin (FO 200 mg), Cotrimoxazole (COT 25 g), Cefoperazone - Sulbactam (SCF 105 g), and Chloramphenicol (CL 10 mg)**.** 3–5 well-isolated colonies that resembled the test organisms in appearance were combined with 3–4 ml of sterile physiological saline using a sterile wire loop. To meet the 0.5 McFarland turbidity criterion, the suspension turbidity was modified. Prior to usage, the standard was blended immediately clean cotton swab was used to inoculate a Mueller Hinton agar plate. The swab was dipped in the suspension and the extra liquid was squeezed out. The plate was rotated by roughly 60 oC while the swab was streaked over the Mueller Hinton agar surface in three distinct directions in order to guarantee a flat distribution. The agar surface of the cultured plates was allowed to dry for three to five minutes. Using sterile forceps, the chosen antimicrobial discs were distributed across the plates. Following a night of incubation, the plates were incubated aerobically for 16–18 hours at 35 °C. The diameter of each inhibitory zone was determined in mm. Resistance, intermediate/moderate sensitivity, and sensitivity were deduced from the diameters. The Clinical and Laboratory Standardisation Institute (CLSI) has recommended that the conclusions were drawn (Wayne, 2019).

3. Prepare the Peel Extract:

Pomegranate fruits (Punica granatum) were purchased at a nearby store. After being repeatedly the fruits were dried

after being cleaned using water from the tap and then distilled water (DH2O). The peel was carefully taken off after washing. To avoid any surface contamination, at room temperature, the pomegranate peels were allowed to completely dry. Eventually, the peel was ground into a fine powder. The fine powder was soaked in ten grammes for a full day at room temperature. in 100 mL of (methanol, ethanol, and water). To obtain the aqueous extract, The filter paper from Whatman No. 1 was used to filter the final combination. The entire process was performed in sterile circumstances.

RESULTS

1-Sampling and Isolation of The Bacterial Isolates:

Fifteen different human clinical samples (diabetic feet, abscesses, and wounds) were collected from E-Zahraa Hospital, Faculty of Medicine (Girls) (Table 1), Al-Azhar University Cairo, the contaminated area (such as diabetic feet, an abscess, or wounds) was swabbed with a sterile swab to collect the specimens. These Swabs were kept in nutrient broth medium tubes till they were transported consecutively to the Al-Azhar University of Cairo, Faculty of Science (Boys) microbiology department.

Table 1: The collected specimens investigated for isolation of bacterial isolates:

No. of patient	Code of Surgical isolates	Gender	Age	Origin
1	H1	Female	51	Diabetic Foot
2	H2	Female	49	Abscess
3	H3	Female	54	Diabetic Foot
4	H4	Male	22	Wound
5	H5	Male	56	Abscess
6	H6	Male	35	Wound
7	H7	Female	54	Diabetic Foot
8	H8	Male	55	Diabetic Foot
9	H9	Female	40	Diabetic Foot
10	H10	Female	45	Abscess
11	H11	Female	18	Wound
12	H12	Female	27	Abscess
13	H13	Male	51	Wound
14	H14	Male	49	Abscess
15	H15	Male	45	Diabetic Foot

2. The 15 Isolates' Susceptibility to Antibiotics:

All isolates were screened against 23 different antibiotics using the disc diffusion method (Table 2). As shown in Table (2) and Figures (1&2) H5 isolate show 95% resistance to antibiotics and H14 shows 83 % resistance to antibiotics.

Table 2. Antibiotics susceptibility test for bacterial isolates.

Antibiotic Name	Conc. (mcg)	*H1*	H2	H3	*H4*	*H5*	*H6*	*H7*	*H8*	*H9*	*H10*	*H11*	*H12*	*H13*	*H* 14	H15
Cefotaxime (Cx)	30	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S
Chloramphenicol (Cl)	10	S	S	I	I	R	I	I	I	I	S	S	S	S	I	S
Piperacillin / Tazobactam (PIT)	100/10	S	S	S	S	R	S	S	S	S	S	S	S	*S*	*R*	S
Gentamicin (GEN)	10	I	I	S	S	R	S	S	S	I	I	I	I	I	R	I
Tobramycin (TOB)	10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cotrimoxazole (COT)	25	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S
Cefoxitin (CX)	1	I	I	R	R	R	S	S	S	I	I	I	I	I	R	I
TIGECYCLIN (TGC)	15	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S
Rifampicin (RIF)	5	S	S	I	I	R	I	I	I	I	S	S	S	S	R	S
Ceftazidme (CAZ)	30	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R
Cefoperazone (CPZ)	75	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S
Imipenem (IPM)	10	I	I	S	S	R	I	I	I	I	I	I	I	I	R	I
Cefatzidime / Avibactam (CZA)	50	S	S	S	R	R	S	S	R	S	S	S	S	S	S	S
Cefoperazone - Sulbactam (SCF)	75/30	S	S	S	S	R	R	R	R	S	S	S	S	S	R	S
Levofloxacin (LEV)	5	S	S	R	R	R	S	S	S	S	S	R	S	S	R	S
Amikacin (AK)	30	S	S	S	S	R	R	S	S		S	S	S	S	S	S
Ofloxacin (OF)	5	S	R	S	S	R	R	R	R	R	R	S	S	S	R	R
Norfloxacin (NX)	10	S	S	R	R	R	S	S	S	R	R	R	R	S	R	R
Meropenem (MRP)	10	S	S	S	S	R	R	R	R	R	S	S	S	S	R	S
Ertapenem (ETP)	10	S	S	S	S	R	I	I	I	I	S	S	S	S	R	S
Cotrimoxazole (COT)	25	S	R	S	S	R	S	S	S	S	S	S	S	S	R	S
Fosfomycin (FO)	200	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S
Ampicillin – Sulbactam (A/S)	10/10	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S

Denotes for Resistant (R), Intermediate (I) and Susceptible (S).

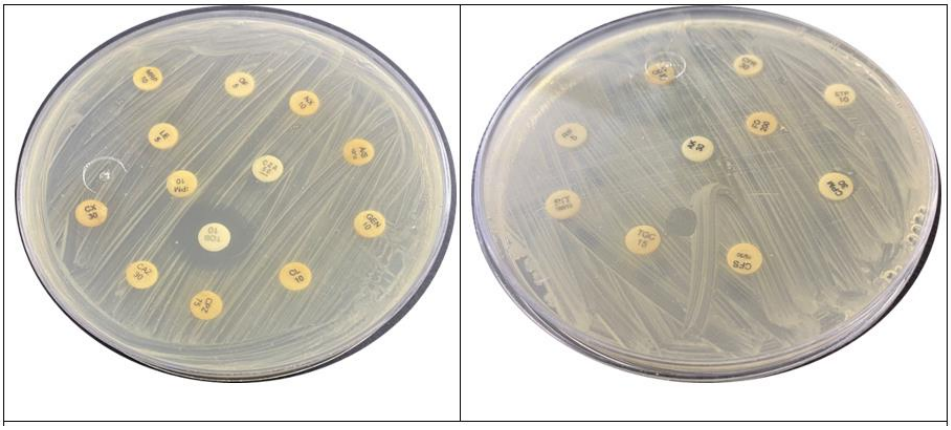

Fig. 1: The H5 isolates' susceptibility to antibiotics.

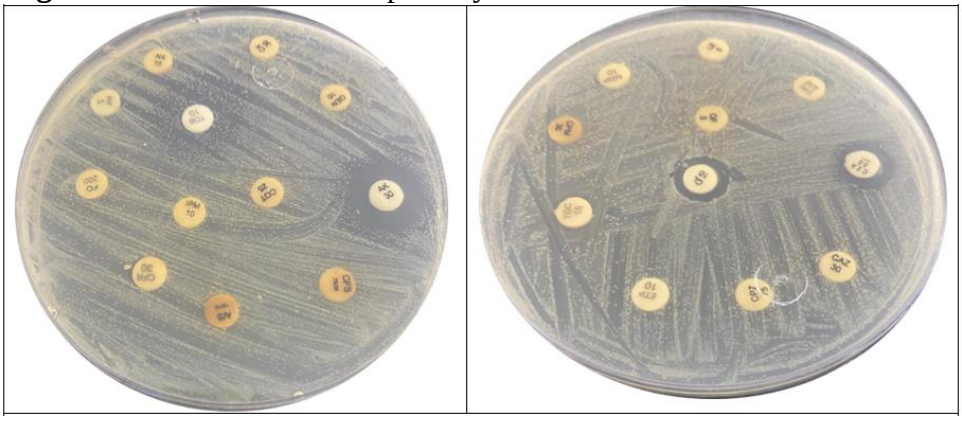

Fig. 2: The H14 isolates are susceptible to antibiotics.

3. Identification of the Most Effective Isolates by Physiological and Biochemical Means:

Cultural Characteristics:The cultural characteristics of two bacterial isolates were examined on a nutrient agar medium (Table 3). The isolates (H5) were rod-shaped, gram-negative, large, white, mucoid, and on MacConkey agar, large, pink to red, mucoid, lactose-fermenting colonies. The isolates (H14) on MacConkey agar had large, pink to red, mucoid lactose-fermenting colonies and were gram-negative, rod-shaped, and greyish-white with a smooth surface.

Morphological Characteristics: Morphological characteristics (cell shape, cell arrangement, gram stain reaction) Bacterial isolate (H5) were studied microscopically using oil immersion objective. rods arranged in pairs, gram-negative and non-motile. Isolate No (H14) were rods arranged in pairs, gram-negative and motile.

Table 3: Bacterial isolates from the collected samples on different media.

Specimen No.	Nutrient Agar	Blood Agar	MacConky Agar	Manitol Agar	Gram Stain	KOH Test
H5	+Ve	+Ve	+Ve	-Ve	-Ve	+Ve
H14	+Ve	+Ve	+Ve	-Ve	-Ve	+Ve

Biochemical Characteristics:

Isolate No. (H5) has the following characteristics: Motility was negative, catalase positive, coagulase negative, Indol negative, MR (Methylred) positive, carbohydrate fermentation positive, nitrate reduction positive, VP (Voges proskauer) negative, oxidase was negative, citrate positive, H2S production negative and urease positive (Table 4). Isolate No. (H14) had positive results for motility, catalase, Indol, MR (Methylred), carbohydrate fermentation, nitrate reduction, negative results for oxidase, citrate, VP (Voges proskauer) H2S production, and negative results for urease.

Table (4). Show Bacterial isolates and biochemical characterisation.

Characteristics	Bacterial Isolates	
	H 5	H 14
Catalase	(+)	(+)
Motility Test	(-)	(+)
Coagulase	(-)	(-)
Indol	(-)	(+)
MR(Methylred)	(+)	(+)
VP(Voges proskauer)	(-)	(-)
Oxidase	(-)	(-)
Urease	(+)	(-)
Citrate	(+)	(-)
Carbohydrate Fermentation Test	(+)	(+)
Nitrate Reduction Test	(+)	(+)
H2S Production Test	(-)	(-)

(+) Positive result – (-) Negative result.

4. Aqueous Plant Extracts' Antibacterial Action Against Pathogenic Microorganisms:

Antimicrobial activity of different Solvents (Figs. 3&4) (pp powder used DH2O as a solvent, fresh pp, 70% Methanol and 70% ethanol) of *Pomogrante peel* on two bacterial species.

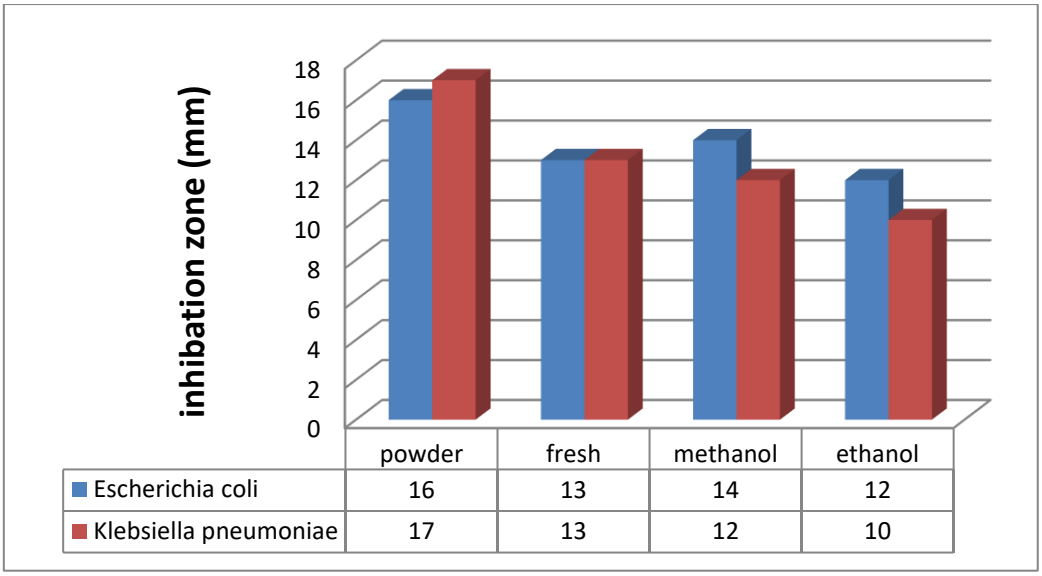

Fig. 3: Pomegranate peel extracts' antibacterial properties when combined with various solvents.

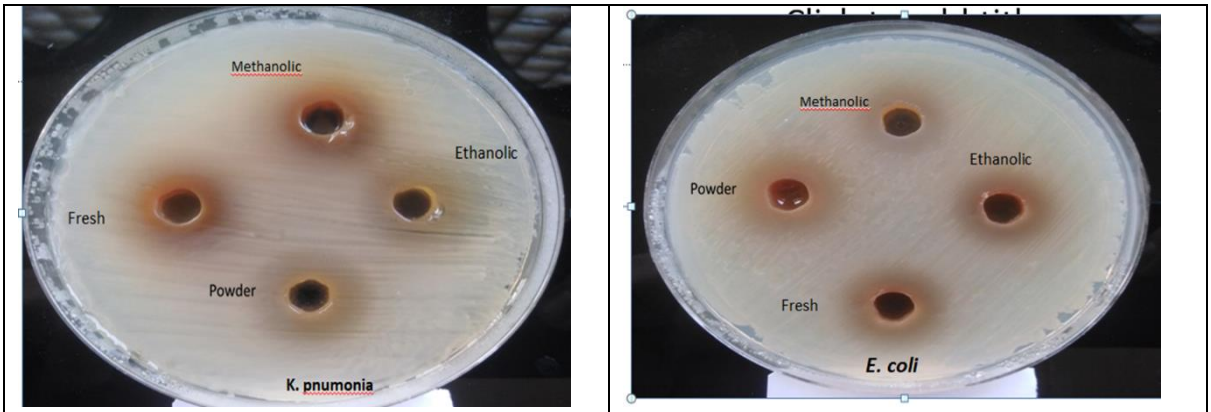

Fig.4: Shows that *Punica granatum* extracts have antibacterial activity against *Klebsiella pneumonia* and *E. coli*.

5-Automated Identification by Using the Biomerieux Vitek 2 System:

The VITEK2 system version: 07.01 apparatus was used to identify the two bacterial isolates using conventional biochemical techniques, as exhibited in Table(5), H5, H 14. According to morphological, cultural, and conventional biochemical characteristics and on the basis of confirmation of identification by the VITEK2 system isolates H 5 and H14 were identified as Klebsiella pneumonia H 5, and *Escherichia coli* H 14, respectively.

Table 5. Vitek 2 System for *Klebsiella pneumoniae* (H5) and *Escherichia coli* (H14)

Character (Test)	**abbreviation**	***Klebsiella pneumoniae* (H5)**	***Escherichia coli* (H14)**
Gram stain	GS	- ve	- ve
Ala-Phe-Pro-Arylamidase	APPA	- ve	- ve
Adonitol	ADO	+ve	+ ve
L-Pyrrolydonyl-Arylamidase	Pyra	+ ve	- ve
L-Arabitol	IARL	- ve	- ve
D-cellobiose	dCEL	+ve	-ve
Beta-galactosidase	BGAL	+ ve	+ve
H2S production	H2S	- ve	- ve
Beta-N-acetyleglucosaminidase	BNAG	- ve	- ve
Glutamyle aryleamidase pNA	AGLTp	-ve	- ve
D-glucose	dGLU	+ ve	+ ve
Gamma glutamyle transferase	GGT	+ ve	- ve
Fermentation/glucose	OFF	+ ve	+ ve
Beta-glucosidase	BGLU	+ ve	- ve
D-maltose	dMAL	+ ve	+ ve
D-manitol	dMAN	+ve	+ve
D-mannose	dMNE	+ ve	+ ve
Beta-xylosidase	BXYL	+ve	-ve
Beta-alanine aryleamidase pNA	BALap	- ve	- ve
L-proline arylamidase	ProA	- ve	- ve
Lipase	LIP	- ve	- ve
Palatinose	PLE	+ve	-ve
Tyrosine arylamidase	TyrA	+ ve	+ ve
Urease	URE	+ve	-ve
D-sorbitol Salicin	dSOR	+ve	+ve
Saccharose/Sucrose	SAC	+ve	-ve
D-Tagatose	dTAG	- ve	- ve
D-trehalose	dTRE	+ve	+ve
Citrate(Sodium)	CIT	+ ve	- ve
Malonat	MNT	+ ve	- ve
5-Keto-Gluconate	5KG	- ve	- ve
L-lactate alkalinisation	ILATK	+ ve	- ve
Alpha glucosidase	AGLU	- ve	- ve
Succinate alkalinisation	SUCT	-ve	+ve
Beta N-acetyle galactoseaminidase	NAGA	- ve	- ve
Alpha-galactosidase	AGAL	+ ve	+ ve
Phosphatase;	PHOS	+ve	-ve
Glycine aryleamidase;	GLYA	-ve	-ve
Decarboxylase base;	ODEC	-ve	-ve
Ornithine decarboxylase;	ODC	- ve	+ ve
Lysine decarboxylase;	LDC	+ve	+ve
L-histidine assimilation	IHISa	- ve	- ve
Coumarate	CMT	- ve	+ ve
Beta-glucoronidase;	BGUR	- ve	+ ve
O/129 Resistance;	O129R	+ ve	+ ve
Glu-Gly-Arg-Aryleamidase;	GGAA	- ve	- ve
L-malate assimilation;	IMLTa	- ve	- ve
ELLMAN	ELLM	- ve	- ve
L-Lactate assimilation	ILATa	- ve	- ve

DISCUSSION

Due to the rapid rise in antibiotic resistance in microorganisms, antimicrobial herbal products have been added to the researchers' special interests (Essawi et al., 2000). Years of frequently inappropriate use of antimicrobial medicines have led to the current issue of widespread antibiotic resistance, which is particularly challenging to treat multidrug-resistant organisms (Lerner et al., 1998; Hellinger et al., 2000). Despite the fact that resistance mechanisms undoubtedly existed before antimicrobial drugs were clinically used, their widespread use has tended to favour bacteria that express resistance genes or naturally resistant species (Livermore et al., 2000). The cost of medical care, rising sickness and mortality rates, and all are connected difficulties (Hellinger et al., 2000). Although understanding the mechanisms of resistance itself may teach us a lot, the present focus on identifying novel drug targets is driven by the need for new antimicrobials to tackle multidrug-resistant microorganisms. (Coleman et al., 1994; Renau et al., 1998; Wright et al., 2000). In order to treat bacterial infections, there is increased interest in the creation of innovative antimicrobial medications. In the present study, twenty-three antibiotics used to treat the most pathogenic infection were selected to study their antibacterial activity. *Klebsiella pneumonia and E. coli*, show 95% resistance to antibiotics and *Klebsiella pneumonia*, *E. coli* show 83% resistance to antibiotics. Stefanovic et al. (2011) recognised that restoring antibiotic effectiveness One of the most efficient ways to prevent bacterial resistance is by the combined effect of antimicrobial substances from both synthetic and natural agents. The major goals of the current investigation were to assess how well plant extracts could limit the development of harmful bacteria both with and without the use of antibiotics as well as how well they might increase the effectiveness of antibiotics. Plant active components that limit bacterial growth may also have antibacterial properties, which are reflected in the antibacterial activity of plant extracts against pathogenic bacteria. In our testing, the well-diffusion method was used to assess how well plant extracts worked against the bulk of the harmful bacteria that were looked at. Pharmaceutical formulations for the treatment of respiratory issues are made using extracts from the Punica granatum peel. According to Supayang PV et al. (2005) and Vasconcelos et al. (2003), the tannin-rich ellagitannins and phenolic acids of Punica granatum have anti-fungal, antibacterial, and antiprotozoal properties. The Punica granatum powder aqueous extract's zone of inhibition against *Klebsiella pneumoniae* was at least 17 mm, which was higher than the zones for the methanolic and ethanolic extracts. For *E. coli*, the zones were at least 16 mm, which was also higher than the zones for the methanolic and ethanolic extracts.

Conclusion

In this study, an effort was made to comprehend the antibacterial properties of Punica granatum peels, which are frequently thrown away as trash. Punica granatum peels contain bioactive compounds like polyphenols, tannins, flavonoids, and anthocyanins (Cyanidins, delphinidins), according to past studies *Punica granatum peel* extracts exhibit antibacterial efficacy against *Klebsiella pneumonia* and *E. coli*, two bacterial pathogens.

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