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Punica Granatum Bioactive Activity Against Gram Negative Isolates From Post-Surgical Infections In Erbil Province

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Abstract

Background: Post-surgery wound infections complication becoming more common and global trend due to multi drug resistant bacteria. The problem is causative agent changing their strategy continuously toward antibiotic, so the antibiotic cannot manage the treatment. Therefore, we tried for alternative treatment or using substance has antibacterial activity. Subsequently we used extracts from different part of Pomegranate. However, there hasn't been much published study employing using Pomegranate extract for surgical wound infections.
Study Design: A prospective study was conducted on 100 post-surgical patients at Mihrabani Hospital Private in Erbil Province, Kurdistan Region, Iraq, from September 2024 to April 2025. Swabs were collected from these patients. Out of the 100 samples, 80 (80%) yielded positive cultures, while 20 (20%) showed negative results. Gram-positive bacteria accounted for 30 (30%) of the positive culture isolates, which were not included in the study. The remaining 50 percent consisted of gram-negative bacteria. Among the patients, there were 30 females (60%) and 20 males (40%), with ages ranging from 20 to 80 years. Researchers employed various solvents at multiple concentrations to evaluate their effectiveness against the isolated bacteria.
Results: percent study investigates 50-gram negative bacteria isolated from post-surgical infections using VITEK 2 system. Other gram-positive bacterial isolates excluded from the study. Cassette within 26 antibiotics assessed against gram negative bacteria. The study found most frequent isolates coursing infection was Escherichia coli (E. coli) and Pseudomonas aeruginosa. Various solvent used for extraction of (PP) pomegranates peel, (PC) pomegranates core, (PPC) peel and core pomegranate tested against isolated bacteria. Punica granatum contains ellagitannins, gallic acid, and phenolic acids that are rich in tannins and exhibit antibacterial properties. This study demonstrated the sizes of the inhibitory zones for different types of bacterial infections caused by each extract. We evaluated the effectiveness of these compounds in killing bacteria by using methanol, ethanol, and distilled water (DW) extracts at concentrations of 100%, 75%, 50%, and 25%.
Conclusion: In the present study many etiological agents have been isolated causing surgical infection with their susceptibility pattern. P. granatum extracts used with various solvent. We found most effective solvent was methanol followed by ethanol. While, less effective solvent was water.

Keyword: Post-surgery wound infections - (PP) pomegranates peel, (PC) pomegranates core, (PPC) peel and core pomegranate.

1- Introduction

Postoperative wound infections are a great problem in surgical profession causes countless complications, counting additional morbidity and mortality. Various hospitals raise different kinds of postoperative wound

infections (1, 2). The annual occurrence of antimicrobial resistance (AMR) is a major cause of hospitalizations and deaths, which is a major concern in global health, 10 million people would die each year (13, 14). Within the absence of consistent diagnostic criteria makes it difficult to track the global incidence of surgical site infection. A further challenge in managing and treating post-operative wound infections occur with increasing prevalence of bacteria with high levels of resistance to antibiotics (3).

As a potential alternative to antibiotics, plant-based medicines are already receiving more scientific attention. There has been a surge in research into the potential antibacterial properties of plants, fruits, and vegetables. An increasing number of studies are looking at fruit peels as a potential source of antibacterial and antioxidant bioactive compounds (15, 16). These natural substances are rich in bioactive secondary metabolites, which have an intriguing antibacterial effect. The pomegranate, scientifically known as Punica granatum L is a type of fleshy berry. Its exocarp, or pomegranate skin, makes up around half of it (4). Pomegranate extracts from Different parts have diverse mod of action against bacteria. Several studies have linked the high flavonoid and tannin content of PPE to its superior antibacterial effect compared to other components (9). The physiological significance of pomegranate peel can be attributed to its abundance of polyphenols and other compounds, including quercetin, gallic acid, ellagic acid, ellagitannins, and nitrate. Pomegranates contain anthocyanins such as cyanidin, delphinidin, and pelargonidin in addition to other tannins like punicalin, pedunculagin, and punicalin. The antibacterial action and antioxidant potential are just a few of its many health advantages (5-8).

Scientific studies have shown that pomegranate byproducts (9), especially pomegranate peel extract (PPE), have anti-inflammatory, anti-cancer, and anti-ulcer properties (9). In addition of promoting healing process. Study have been shown that Scars may heal more quickly after using pomegranate skin care products. Wound healing is facilitated by an enhancing the production of collagen and proteins, as well as an acceleration of the contraction rate and tensile strength. The extract demonstrated a notable antibacterial effect against wound pathogens, including strains of Pseudomonas aeruginosa, S. aureus, E. coli, and Klebsiella pneumonia (10).

Pomegranate peel extracts, especially those prepared with ethanol or methanol, have a high total phenolic and flavonoid content and exhibit excellent antioxidant capabilities (11, 12). Pomegranate seed and peel extracts have demonstrated significant antimicrobial activity against a wide variety of bacteria and human pathogens, including Bacillus subtilis, E. coli, Helicobacter pylori, Shigella spp, Klebsiella pneumoniae, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhi, , S. aureus, Candida albicans, Clostridium spp, Vibrio cholerae, Yersinia enterocolitica, and other microorganisms (17). Pomegranate peel is considered an agro-waste; however, it may really include antioxidants, phenols, flavonoids, and even antifungal and antibacterial properties. The antioxidant activity of pomegranate peel is higher than that of the fruit's blossoms, leaves, and seeds, according to previous studies (18). The study aims to detect microorganisms associated with post-surgical process area infections and their susceptibility pattern against antibiotic using Vitek compact system. in addition, using pomegranate extract from various part of pomegranates, each extract with 4 concentrations testing their antimicrobial activity against gram-negative isolates from post-surgical wound.

2- Methodology

2.1- Sample collection

Specimens were collected from 100 post-surgical patient admitted to General Surgery Department at Mihrabani Hospital, Erbil, Iraq from September 2024 to April 2025. (60%) of the patient was females and 20 males (40%) within ages spanning from 20 to 80 years. The area surrounding the surgical wound was cleaned with 70% ethyl alcohol, samples within inflamed and discharging pus of wound obtained using disposable swabs by rubbing sterile swabs in the inflamed or discharging area.

2.2- Bacterial culture, Isolation and identification

Cultures were performed on blood and MacConkey agar using streak plate method, incubated aerobically at 37ºC for 24 hours. The isolated bacteria were subjects to VITEK 2 system for identification and susceptibility of antibiotic. The system is employed in various clinical settings to provide rapid results, which are crucial for effective patient management and treatment decisions (19).

2.3- Extract Process of Pomegranate Peel

One of the most widely used methods for pomegranate extraction is the methanol, ethanol & water extraction methods. The Pomegranate were separated and washed with tap water, pomegranates separated in to three group's includes peel (PP), pomegranates core (PC), pomegranates peel and the last group core and peel (PPC), all groups subjected to drying in oven at 50 °C up to dryness. The dried peels were ground with an electric blender to coarse powder of approximately 1 mm size and stored in an incubator at 40 °C for 24h (9). To prepare antimicrobial activities, 10g of powder sample is extracted with 250ml of 80% methanol, 80% ethanol at (∼37°C) temperature for 24h; the powdered sample (10g) is extracted with 100ml of distilled water (DW) at (∼37°C) temperature for 24h all incubated with agitation. final extract is then filtered with Whitman paper (7). The extract was used for the analysis of antioxidant and antimicrobial activities.

2.4- Extract Dilution in (Ethanol, Methanol, and Deionized Water)

Selection of solvent is an important step for obtaining pomegranates extract (PE), in another ward, Extracts with acceptable yields and strong antimicrobial and antioxidant activity. The yields of extract from numerous concentrations were prepared from each pomegranate extract {(PP), (PC), (PPC)} pomegranate. 75 % ethanol: 25% PE, 50% ethanol: 50 % PE, 25 % ethanol: 75 % PE, absolute PE. 75 % methanol: 25% PE, 50% methanol: 50 % PE, 25 % methanol: 75 % PE, absolute PE. 75 % DW: 25% PE, 50% DW: 50 % PE, 25 % DW: 75 % PE, absolute PE.

2.5- Assessment of Antibacterial Activity

The antibacterial efficacy of pomegranate peel water extraction (PPWE), methanol (PPME), and ethanol extracts (PPEE) was evaluated in this study using the agar well diffusion method for nine bacterial strains.

The investigation conducted on pomegranate core water (PCWE), methanol (PCME), and ethanol extracts (PCEE), which included pomegranate peel core extracts (PPCEE and PPCME). This comprehensive evaluation of an array of extracts for antibacterial activity was demonstrated. The agar cultures of Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Achromobacter denitrificans, Serratia marcescens, Enterobacter Kobi, Stenotrophomonas maltophilia and Proteus.

The inhibitory effects of pomegranate extract were assessed using Muller Hinton agar. Flasks containing the agar medium were sterilized in an autoclave at 121°C for 15 minutes. Bacterial cultures were grown at 37 °C for 24 hours through inoculation onto Muller Hinton agar. Petri dishes with 15 ml of agar were prepared and inoculated with 100 µl of bacterial culture. Wells measuring 9.0 mm were made in the agar and filled with 100 µl of the test substance, while control solutions (deionized water, methanol, and ethanol) were applied as well. The inoculated plates were incubated for 24 hours at 37 °C. After incubation, inhibition zones appeared, and their diameters were measured from the outer edge to the well's periphery.

2.6- Minimum Inhibitory Concentration

The MIC test is a laboratory technique that establishes the minimum concentration of an antimicrobial agent required to impede the visible growth of a microorganism (24). The determination of such MIC is crucial in the testing of antibiotics, antifungals, and other antimicrobial agents to ascertain the efficacy of a compound against the target microorganism. The current study employed the MIC ratio test to determine the effectiveness of pomegranate extraction (PE) against gram-negative, multi-resistant bacteria, such as E. coli. The objective of the MIC test is to ascertain the lowest concentration of an antimicrobial agent, such as PE, that prevents the visible growth of a microorganism.

2.7- Dilution Method Determines the MIC

The broth dilution method determines the MIC of an antimicrobial agent by serially diluting it in a liquid medium and adding a standardized bacterial suspension (24). Serial Dilution Method: MIC is determined using two-fold dilutions of the antimicrobial agent in a growth medium, with the lowest concentration that inhibits visible growth being recorded as the MIC (25, 26). Furthermore, the agar dilution method determines MIC by evaluating microbial growth on agar plates with serial dilutions of an antimicrobial agent (24).

Inhibition as a Ratio: Formula: Inhibition= OD (Optical Density) of Sample / OD of Positive Control (27). Represents the fraction of growth relative to the control. Useful for normalization and dose-response analysis (28). OD measures how cloudy the bacterial solution is, which reflects bacterial growth. Positive Control has full bacterial growth with no treatment. Interpreting the inhibition ratio of MIC show in (Table 1).

Table 1: Interpreting the inhibition ratio of MIC on agar dilution

Ratio Value	Meaning
> 1	Sample has more bacterial growth than the control (possibly stimulating growth)
= 1	No effect compared to control
< 1	Inhibition of growth; the smaller value, stronger inhibition
≈ 0	Complete inhibition (no bacterial growth)

3- Result

3.1- Frequency of type growth cultures isolated in post Patients

Among 100 samples examined, 80 (80%) produced positive culture, whereas 20(20%) produced negative cultures. among the positive culture 30 (30%) isolates were gram-positive bacteria and excluded from the study, while only 50(50%) were gram-negative bacteria as shown in table 2.

Table 2: Frequency of type growth cultures isolated of post-surgical patients

Type of growth cultures	No. of isolates	Percentage
Positive culture (include gram positive and negative)	80	80%
Gram positive(excluded)	30	30%
Gram negative	50	50%
Negative culture	20	20 %
Total	**100**	**100%**

3.2- Identity of isolated Gram-negative bacteria

The identity, number and percentages of the most common gram-negative organism isolated from the post-surgical patients are shown in table 3. The results showed that the highest percent of the isolates belonged to the genus Escherichia (30%) of the total isolate, followed closely by Pseudomonas aeruginosa 14 (28%) of the isolates. E. coli and P. aeruginosa standing out as the most prevalent, suggesting their crucial involvement in nosocomial infections. Additional species identified included Klebsiella pneumonia 6 (12%), along with a variety of less common pathogens. As well as, the lowest rate 1 (2%) of the total isolates were Serratia marcescens, Achromobacter denitrificans, Enterobacter kobei, Stenotrophomonas maltophilia, and Proteus mirabilis.

Table 3: Prevalence of clinical isolation bacterial species form post-surgical process.

Bacterial Spp.	Number of Cases	%
E. coli	15	30%
Pseudomonas aeruginosa	14	28%
Acinobacter baumanii	10	20%
K. pneumoniae	6	12%
Serratia marcescens	1	2%
Acromobacter denitrificans	1	2%
Entrobacter kobi	1	2%
Stenotrophomonas maltophilia	1	2%
Porteous mirabils	1	2%

3.3- Antimicrobial susceptibility patterns against gram negative bacterial Isolate from post-surgical patients

susceptibility tests done for isolates by vitić system 2. cassette within 26 antibiotics assessed against gram negative bacteria. Notably, Pseudomonas aeruginosa was particularly showed resistant against Ceftriaxone (73%), Cefepime (68%), and Ampicillin/Sulbactam (65%), the sensitivity for these agents being remarkably low, ranging from 15% to 25%. In contrast, Tigecycline displayed relatively better efficacy, with a sensitivity rate of 62% in Pseudomonas aeruginosa, indicating its potential as a alternative therapeutic. On the other hand, E. coli shows resistance rates for Ceftriaxone and Trimethoprim/Sulfamethoxazole stood at 67% and 47%, respectively, while Amikacin and Imipenem exhibited higher sensitivity levels of 63% and 58%, respectively. Ciprofloxacin similarly exhibited poor performance, surpassingly with resistance rates 60% in both E. coli and Pseudomonas aeruginosa, which may reflect patterns of overuse or emerging resistance mechanisms. more concerning resistance profile noted in Acinetobacter baumannii, that resistance levels reached 100% for the majority of antibiotics evaluated. Agents such as Amikacin, Piperacillin/Tazobactam, and Cefepime displayed minimal or no sensitivity, whereas Colistin and Tigecycline remained the only therapeutic options with partial efficacy, with sensitivity rates ranging from 55% to 70%. Klebsiella pneumoniae exhibited intermediate resistance levels across most antibiotics analyzed, with Meropenem and Amikacin demonstrating moderate sensitivity, approximately between 45% and 50%. Overall, the highest resistance levels were evident within the β-lactam and cephalosporin classes of antibiotics, while carbapenems and last-resort agents, including Colistin, maintained comparatively improved sensitivity against organisms characterized by multidrug resistance.

4.4- Minimum Inhibitory Concentration in Broth Dilution

The minimum inhibitory concentration (MIC) was assessed for three types of pomegranate peel extracts: methanol (PM), ethanol (PE), and water (PW), by evaluating their effectiveness in suppressing bacterial growth across various dilutions. The MIC is defined as the lowest concentration at which no visible signs of bacterial growth occur. The methanol and ethanol extracts exhibited bacterial growth at all tested concentrations, suggesting that their MIC values exceed the highest dilution tested, meaning no MIC was identified within the evaluated range. In contrast, the water extract showed significant antibacterial properties, with no bacterial growth detected at the 128×dilution level. This finding indicates that the MIC

for the PW from pomegranate peel is established at the 128×dilutions, positioning it as the most potent extract among the three in terms of inhibiting bacterial growth under the experimental conditions applied listed in the (Table 3).

Table 3: Pomegranate peel extracts' MIC ratios with methanol, ethanol, and water show efficacy in inhibiting E. coli

Con	SD	PM	Result	PE	Result	PW	Result
1	1	1.843	G	1.471	G	1.194	G
2	2	1.744	G	1.410	G	1.120	G
3	4	1.671	G	1.166	G	1.077	G
4	8	1.285	G	1.112	G	1.071	G
5	16	1.144	G	1.075	G	1.068	G
6	32	1.055	G	1.039	G	1.053	G
7	64	1.040	G	1.033	G	1.044	G
8	128	1.027	G	1.028	G	0.956	NG

G; growth, NG; no growth bacteria, SD; serial dilution, Con; concentration, PM; Pomegranates Peel Methanol, PE; Pomegranates Peel Ethanol, PW; Pomegranates Peel Water

These findings reinforce the superior efficacy of water-based core extracts, particularly pell water extract PW, which achieved inhibition at the lowest concentration tested among all core-derived samples. The results suggest that polar, water-soluble phytochemicals present in the core are likely responsible for the observed antimicrobial activity, and further phytochemical analysis is warranted to isolate and characterize the active compounds (Table 4).

Table 4: MIC ratio of pomegranate core extracts with methanol, ethanol, and water their effective to suppressing E. coli.

OD of serial dilution and MIC according Positive control=serial dilution/positive control. If the OD of the extract is greater than 1.0, it indicates growth, and if it is less than 1, it indicates no significant growth							
TN	SD	CM	Result	CE	Result	CW	Result
1	1	1.507	G	1.294	G	1.035	G
2	2	1.317	G	1.184	G	0.993	NG
3	4	1.118	G	1.109	G	0.952	NG
4	8	0.990	NG	1.042	G	0.940	NG
5	16	0.905	NG	1.038	G	0.933	NG
6	32	0.902	NG	1.010	G	0.928	NG
7	64	0.885	NG	0.956	NG	0.909	NG
8	128	0.811	NG	0.923	NG	0.645	NG

G; growth, NG; no growth bacteria, SD; serial dilution, Con; concentration, CM: Pomegranates Core Methanol, CE: Pomegranates Core Ethanol, CW: Pomegranates Core Water.

The findings emphasize a noticeable pattern seen among various types of extracts. Specifically, extracts that are water-based, especially those obtained from the peel and core of pomegranates, demonstrate significantly improved antimicrobial qualities. This insight indicates that the water-soluble

bioactive compounds play a vital role in the antimicrobial effectiveness of pomegranates. This is likely attributed to their enhanced extraction efficiency when using water compared to the use of alcoholic solvents (table 5).

Table 5: MIC ratio of pomegranate peel: core extracts with methanol, ethanol, and water their effective to suppressing E. coli.

OD of serial dilution and MIC according Positive control=serial dilution/positive control If the OD of the extract is greater than 1.0, it indicates growth, and if it is less than 1, it indicates no significant growth							
Con	SD	PCM	Result	PCE	Result	PCW	Result
1	1	1.599	G	1.718	G	1.044	G
2	2	1.518	G	1.359	G	1.024	G
3	4	1.438	G	1.039	G	0.967	NG
4	8	1.066	G	0.987	NG	0.958	NG
5	16	0.956	NG	0.908	NG	0.956	NG
6	32	0.907	NG	0.901	NG	0.929	NG
7	64	0.897	NG	0.869	NG	0.907	NG
8	128	0.614	NG	0.712	NG	0.835	NG

G; growth, NG; no growth bacteria, SD; serial dilution, Con; concentration, PCM: Pomegranates Peel & Core Methanol, PCE: Pomegranates Peel & Core Ethanol, PCW: Pomegranates Peel & Core Water.

In a comparative analysis, extracts obtained from the core pomegranate exhibited more significant inhibitory effects than those derived from other parts, particularly when the peel and core were combined. The water extract from the pomegranate core (PCW) exhibited remarkable potency, achieving total inhibition at a concentration of 4 µg/mL (OD = 0.967). In contrast, the methanol extract from the core (CM) and the ethanol extract combining both peel and core (PCE) revealed moderate levels of activity, each presenting a MIC of 8 µg/mL. The methanol extract from the peel and core (PCM) demonstrated a somewhat lesser effect with a MIC of 16 µg/mL (OD = 0.956). These results highlight the superior antimicrobial characteristics of aqueous extracts, particularly those sourced from the pomegranate core, when compared to extracts utilizing methanol or ethanol solvents. The combined summary result of Effectiveness, the most effective: PW at 2 µg/mL (lowest MIC value). PCW (Water Extract) is the second most effective at 4 µg/mL**.** CM and PCE show moderate activity at 8 µg/mL**.** PCM and PE are the least effective, with PCM showing the highest MIC at 16 µg/mL and PE at 64 µg/mL

3.5- Antibacterial activity of various extracts

The tannin - rich ellagitannins, gallic acid and phenolic acids of Punica granatum have antibacterial activity (7) . In the current study inhibition zone presented of each extract from 3 pomegranate composite (C, PC, P) separately. Using methanol, ethanol, and distilled water (DW) extracts at 100%, 75%, 50%, and 25% concentrations. In order to examine these extracts, nine distinct strains of Gram-negative bacteria were utilized (Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Serratia marcescens, Pseudomonas aeruginosa, Achromobacter denitrificans, Enterobacter kobei, Stenotrophomonas maltophilia and Proteus mirabilis).

One could evaluate the antibacterial potency by measuring the inhibitory zone sizes in millimeters. Panel A, the methanol extract, had the most antibacterial characteristics of 3 pomegranate composite (C, PC, P.) overall and was the most effective of the extracts tested. The most potent inhibitory zones were observed against Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Escherichia coli, particularly at doses of 75% and 100%. The pattern of decreasing antibacterial activity as

a function of concentration was readily apparent. In contrast, E. kobei, S. maltophilia, and P. mirabilis, which were the least susceptible strains, exhibited smaller inhibition zones.

There was a moderate antibacterial effect from the ethanol extract in Panel B. Although it was not as effective as the methanol extract at lower concentrations, it still managed to inhibit large areas of Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii, E. kobei, S. maltophilia, and P. mirabilis were the strains least affected.

The distilled water extract (Panel C) demonstrated the least effective bactericidal action compared to all of the others. Particularly against Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii, just a slight suppression was observed even at the highest dose.

Water may not be the best solvent for extracting or stabilizing the active antibacterial components in compound C, according to this result as shown in figures (1,2,3) here the explanation of each extract with various concentration of each extract

1. The antibacterial activity of Pomegranate core (PC) compound is sensitive to the solvent and concentration used; the most effective extractants are methanol and ethanol, respectively; distilled water has the lowest efficacy. E. coli, A. baumannii, P. aeruginosa, and K. pneumoniae had the highest levels of sensitivity among all Gram-negative bacteria tested, E. kobei, S. maltophilia, and P. mirabilis exhibited the highest levels of resistance. Additionally, Serratia marcescens and Achromobacter denitrificans produced only moderate reactions across all solvents tested.

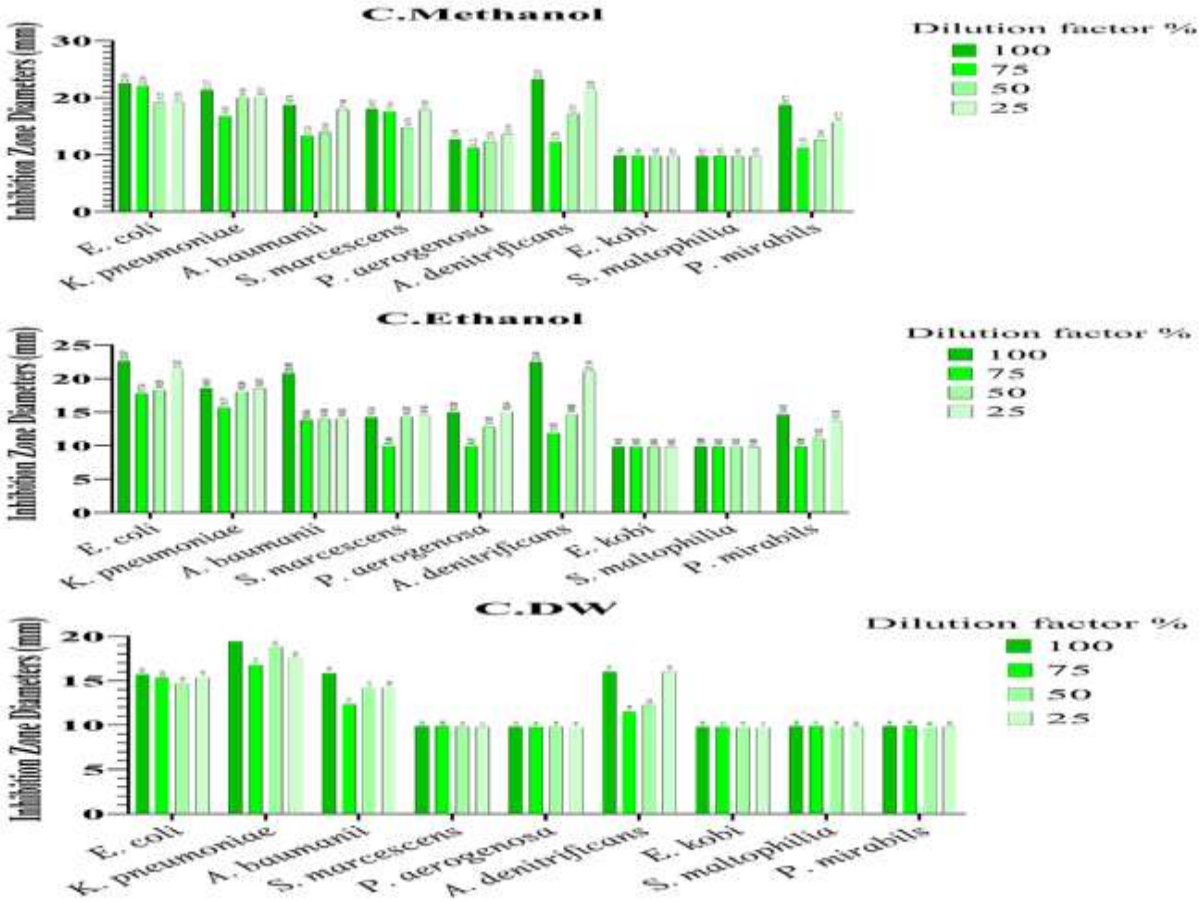

Figure 1: Pomegranate core (PC) methanol extract has unique antibacterial activity at different concentrations. The most effective extractants against Pomegranate core are methanol and ethanol, respectively; distilled water has the lowest efficacy, compound Core 's antibacterial property was sensitive to the solvent and concentration utilized.

2- Several PC extracts have antibacterial properties, with its most striking antibacterial properties, the methanol extract stands out among the investigated solvents in (A). This extract, particularly at higher concentrations, creates wide-ranging inhibitory zones (typically 20 mm) against various bacterial species. The ethanol extract demonstrates a moderate level of antibacterial action, as we move on to (B). The fact that it continues to exhibit efficacy against numerous infections, despite having somewhat smaller inhibition zones than the methanol extract, clearly demonstrates a dilution-dependent response. (C) displays the PC distilled water extract, which has the lowest antibacterial effect and a significant contrast. The inhibitory zones for this extract are typically smaller, and its efficacy is restricted, especially with species such as Pseudomonas aeruginosa, multidrug-resistant bacteria, and Proteus mirabilis. The results demonstrate that the powerful antibacterial components in the PC extract can be more easily extracted using organic solvents, with methanol proving to be particularly effective.

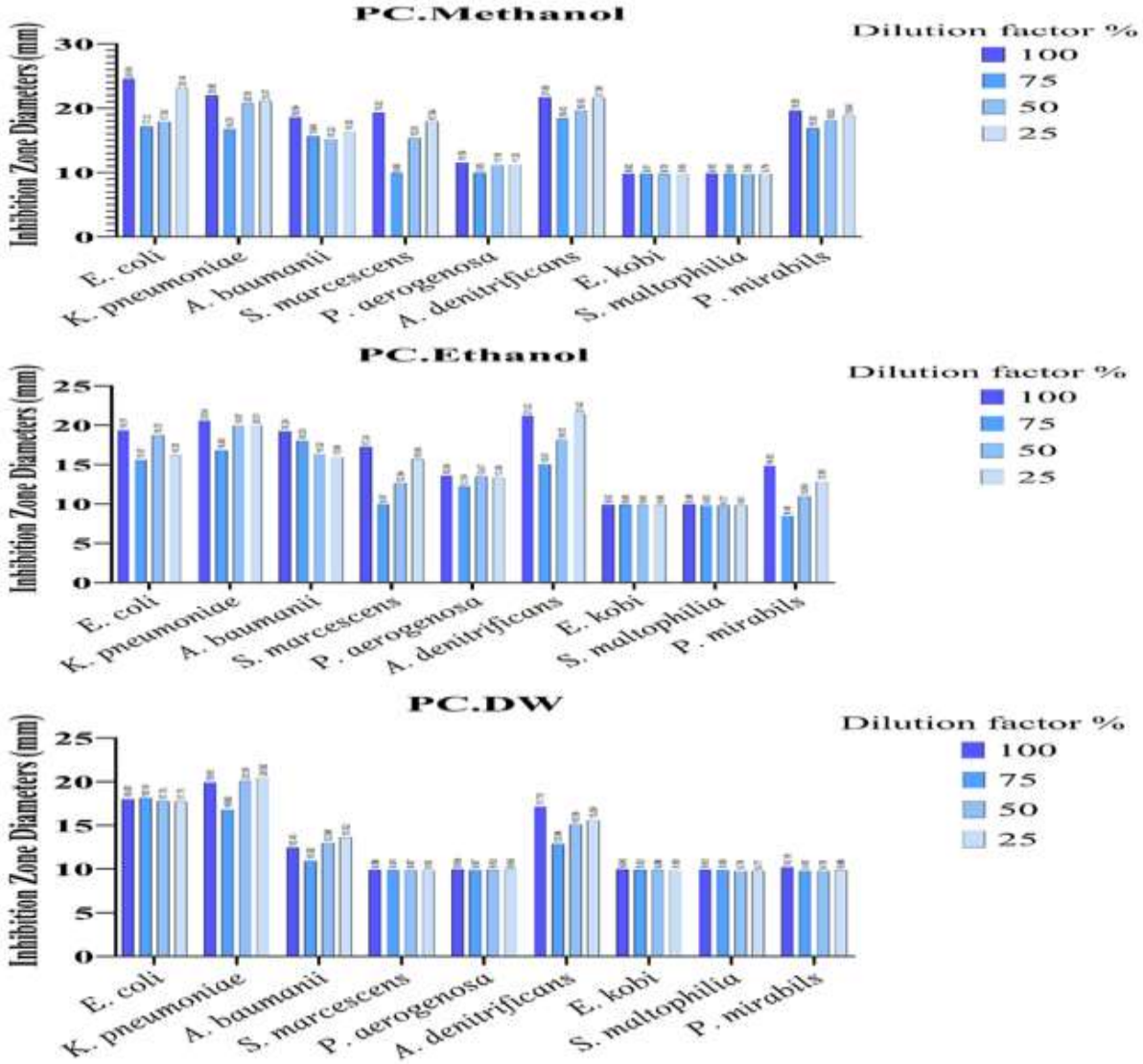

Figure (2) pell and core methanol concentration dependent extract stands out for having the most pronounced antibacterial qualities. Methanol extract exhibits the most notable antibacterial qualities. This extract produces broad-ranging inhibitory zones (usually 20 mm) against a variety of bacterial species, especially at higher concentrations.

3- The result show that pell in three solvents evaluated, the P. methanol extract (A) exhibited the most remarkable antibacterial properties. Across all bacterial strains tested, the inhibitory zones ranged from 15 to 23 mm, indicating that the effects were concentration dependent. The P. ethanol extract (B) demonstrated strong antibacterial activity; nevertheless, its inhibition zones were noticeably smaller than the methanol extract, particularly at higher dilutions. On the other hand, the antibacterial activity of the P. distilled water extract was the weakest. It had inhibition zones that did not surpass 15 mm against several bacterial species, including P. aeruginosa and S. maltophilia. These results provide further evidence that methanol is the best solvent for removing antimicrobial components from the P. extract, as demonstrated in Figure 3.

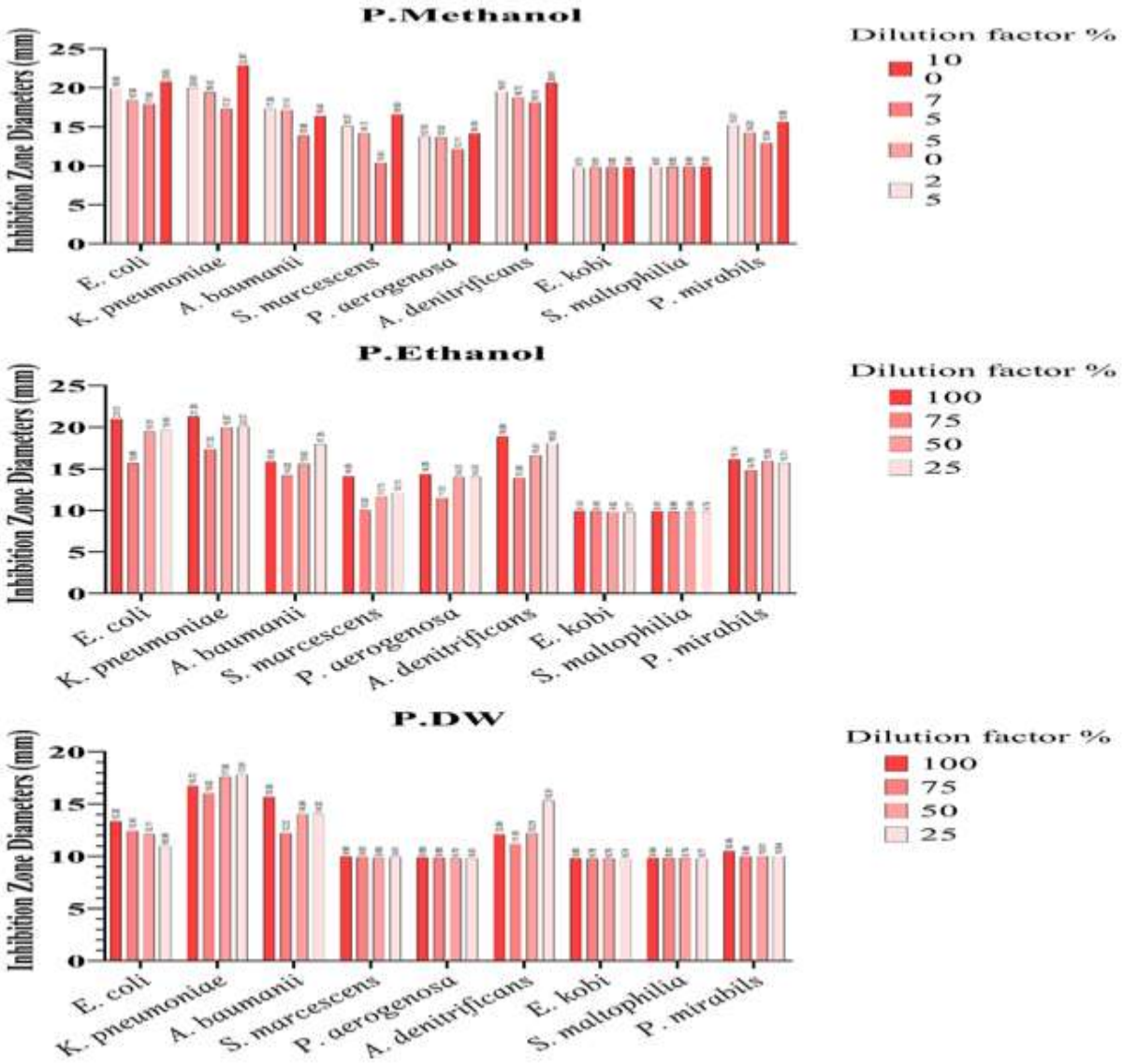

Figure 3: (Pell). P-methanol extract exhibited the most remarkable antibacterial properties. Across all bacterial strains tested. P. methanol extract (A) had the most notable antibacterial qualities among the three solvents tested. The inhibitory zones varied from 15 to 23 mm for every bacterial strain examined, suggesting that the effects were concentration dependent. Although the P. ethanol extract (B) showed robust antibacterial activity, especially at higher dilutions, its inhibition zones were noticeably smaller than those of the methanol extract. However, the P. distilled water extract had the least effective antibacterial activity. Its inhibition zones against a number of bacterial species were less than 15 mm, including

4- Discussion

The findings of this study highlight the high frequency of Gram-negative bacteria in clinical isolates obtained post-surgery. The most frequently found species were Escherichia coli (30%) and Pseudomonas aeruginosa (28%), followed by Acinetobacter baumannii (20%) and Klebsiella pneumoniae (12%). Because of their resilience and quick development of resistance mechanisms, these microbes are generally recognized as important agents in nosocomial infections, especially in post-operative settings**.** These microorganisms are widely acknowledged as significant agents in nosocomial infections, particularly within post-operative settings, attributed to their robustness and rapid acquisition of resistance mechanisms (29, 30) .The testing of antibiotic susceptibility revealed alarmingly elevated resistance levels, especially among K. pneumoniae, E. coli, and A. baumannii. The resistance of K. pneumoniae was most pronounced against β-lactams, including ceftriaxone (73%) and cefepime (68%), while E. coli exhibited similar resistance rates to ceftriaxone (67%) and trimethoprim/sulfamethoxazole (47%). Such findings are consistent with global patterns indicating increasing resistance among Enterobacteriaceae, likely fueled by the overutilization and improper application of antibiotics (31, 32). Notably, Acinetobacter baumannii presented nearly universal resistance across the tested antibiotic spectrum, with only colistin and tigecycline revealing moderate sensitivity (55–70%). This aligns with earlier research identifying A. baumannii as a highly multidrug-resistant pathogen and a predominant cause of ventilator-associated and surgical site infections in critical care environments (33-35). The intermediate resistance observed in P. aeruginosa further accentuates the difficulties in effectively managing infections resulting from opportunistic Gram-negative bacilli, which frequently demonstrate both intrinsic and acquired resistance to numerous drug categories (36, 37). Given the current resistance crisis, there is an increasing interest in exploring alternative antimicrobial strategies, particularly those derived from natural sources.

This study examined the antibacterial efficacy of Punica granatum (pomegranate) peel and core extracts employing methanol, ethanol, and water as solvents. Among these, the methanol extracts displayed the most substantial inhibition zones (>20 mm), particularly against E. coli, P. aeruginosa, and A. baumannii, indicating the presence of potent bioactive compounds that can be extracted using organic solvents (37-39). Prior studies have similarly indicated that pomegranate extracts are abundant in tannins, flavonoids, and phenolic acids, contributing to their broad-spectrum antimicrobial properties (40). Notably, the MIC assessments demonstrated that the water-based extract from the pomegranate core (PCW) exhibited the most effective antibacterial activity, with growth inhibition observed at concentrations as low as 4 µg/mL, closely followed by the water extract from the pomegranate peel (PW) at 2 µg/mL. This finding challenges the prevailing assumption that organic solvents yield more bioavailable antimicrobial agents and suggests that water-soluble phytochemicals, such as ellagitannins, might play a crucial role in antimicrobial effectiveness (41). The peels of Punica granatum, commonly known as pomegranate, exhibit noteworthy antibacterial characteristics, hinting at the existence of metabolic toxins or a range of antimicrobial compounds that can target both gram-positive and gram-negative bacteria [28]. Remarkably, the extracts derived from these peels demonstrated the most potent antibacterial action against a variety of pathogens, including Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa, Acromobacter denitrificans, and Klebsiella pneumoniae. Additionally, Punica granatum is recognized for its substantial tannin content, which constitutes about 25% of its composition. This antibacterial proficiency likely points towards the presence of secondary metabolites within the plant. Prior investigations have also delved into

the antibacterial properties of pomegranate peels, revealing a spectrum of outcomes across different studies [4].

These results imply that aqueous extracts from P. granatum—particularly those derived from the core—could be viable alternatives or complements to traditional antibiotics in the battle against multidrug-resistant pathogens. This holds particular importance in resource-limited settings where synthetic antibiotics may be scarce, and the risk of resistance proliferation is significant [12].

5- Conclusion and Recommendations

The research advocates the integration of ethnobotanical knowledge into contemporary antimicrobial studies and encourages further phytochemical investigation of P. granatum extracts to isolate and amplify bioactive components. Additionally, it emphasizes the urgent need for ongoing surveillance of antibiotic resistance in clinical pathogens as well as the development of novel therapeutic strategies.

6- Ethical Approval

This study was submitted to the Ethics and Scientific Committees of the Erbil Technical Health and Medical College/ Erbil Polytechnic university (1766) on 9-10-2024.

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