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The inhibitory effect of *Punica granatum* on *Escherichia coli* and *Klebsiella pneumonia* Extended spectrum β-lactamase strains

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Abstract

Each year, millions of people worldwide suffer from urinary tract infections (UTIs), which are the second most common type of infection in the human body. An infection of the urinary tract (UTI) affects the urinary bladder, kidneys, and\ or urethra. In order to eliminate the urine from the body, it passes through these organs. However, most UTIs are caused by the uropathogenic *Escherichia coli* and *Klebsiella pneumonia*, making treatment more difficult. Recurrent UTIs can be effectively treated with long-term antibiotics; however, they can have several adverse side effects, and sometimes they may generate antibiotic-resistant strains. Due to these downsides, alternative remedies based on plant extracts are increasingly being considered for the prevention and treatment of urinary tract infections, particularly in the context of a synergistic antibiotic strategy. There are many medicinal benefits of the pomegranate (*Punica granatum*) plant that makes it to be known as a wonder fruit. Pomegranates are the predominant species that belong to the family *Lythraceae*. Due to its extensive range of bioactive compounds, the diverse parts of this *P. granatum* plant exhibit significant pharmacological activities. The bioactive compounds of this plant have been shown to possess several antioxidants; anti-inflammatory, antimicrobial, anti-diabetic, anti-atherosclerotic, and many other biological effects. Consequently, the purpose of this review was to highlight the inhibitory potential of *P. granatum* extracts on *E. coli* and *K. pneumonia* pathogens, to be used in the effective management of UTIs.

Keywords: Urinary tract infections, ESPL, Antimicrobial activity, *Enterobacteriaceae*

1. Introduction

Urinary tract infection (UTI) is the second most common bacterial infection worldwide. About 150 million urinary tract infections are diagnosed annually (Öztürk and Murt, 2020). The costs of treatment of the UTI are high, with previous studies from France and the US reporting €58 million and $2.47 billion; respectively, for treatments of the UTIs (Foxman *et al*., 2000; François *et al*., 2016). UTIs occur when the

bacterial pathogen enters into different parts of the urinary tract system, including the urethra; bladder, ureter, and kidneys, and its load may reach more than 10^5 cells\ ml in urine (Osungunna and Adeyemi, 2016). The prevalence of UTIs is influenced by the age; sex, comorbidities, and functional abnormalities in the lower urinary tract (Byron, 2018). Due to the anatomical differences, the women are more prone to the UTIs than the males (Geerlings, 2017). The incidence of UTI peaks in individuals that are in their early 20s age and after the age of 85 (Foxman, 2014). Generally, it has been predicted that only half of all women will get at least one UTI throughout their lives (Nicolle, 2001). According to a recent Saudi Arabian study, the frequency of UTI among children is almost 25.8 % (Alrasheedy *et al*., 2021). Another study conducted by Alanazi, (2018) has reported an overall prevalence of UTI by 9.9 %, with the distribution of the following age groups: pediatric (18.6 %), adult (59.2 %), and elderly (22.2%) patients. UTIs can be defined based on their sites of infection. According to González de Llano et al., (2020) study, when the upper part of the urinary tract becomes affected, it is termed pyelonephritis and kidney infections (ureters and parenchyma of the kidney); however, when they affect the bladder or the urethra, the condition is known as cystitis or urethritis (lower tract infection). The uncomplicated UTIs normally affect the healthy people who do not have any urinary tract abnormalities. On the contrary, the complicated UTIs are normally linked to factors that weaken the host's ability to protect their urinary tract, including urinary retention or obstruction generated by the neurological conditions; renal failure or transplantation, and pregnancy, in addition to the presence of foreign objects such as renal calculus or indwelling catheters (Spaulding and Hultgren, 2016). The age; infection stage, host reaction, and the bacterial species causing the UTI, all affect the clinical symptoms of this infection. Recently, Tsetsou *et al*., (2022) reported that young newborns frequently exhibit vague symptoms, such as fever; agitation, nausea, lethargy, and\ or poor feeding. Greater frequency and more overt signs; including discomfort upon urination, are prevalent as the children get older and in the adults as well. Nevertheless, fever and flank pain are also connected to the upper UTI (Tan *et al*., 2021). The effectiveness of UTI symptoms and signs in children has been recently assessed through a meta-analysis. Infants are more likely to develop UTIs if they have a history of previous UTIs; have a fever that lasted for more than 24 h, have suprapubic pain, and\ or have not been circumcised yet. However, for the older kids, these symptoms include fever; lumbago, newly developed incontinence, and dysuria. Additionally, the postmenopausal women have more severe clinical manifestations, such as urine incontinence and more generalized nonspecific symptoms (Zhu *et al*., 2020). Bacteria from the surrounding vagina and perineum cause UTIs when they enter the urinary tract (Chanderraj, 2021). In these regions that are often highly populated with bacteria; the urethral opening is located, thus making the urinary system more susceptible to the bacterial infection (Kaur and Kaur, 2021). The objective of the current study was to present an overview on the phytochemical and pharmacological effects of *P. granatum* extracts on *E. coli* and *K. pneumonia* extended spectrum β-lactamase strains.

2. The *Enterobacteriaceae*

The appearance of ESBLs in the bacterial species such as *Klebsiella* sp. and *E. coli* indicates that β-lactamase-producing genes, including the TEM-type and SHV-type genes, which are encoded on a plasmid (R plasmid), have broadened the range of target drugs through a genetic mutation. Since the ESBL genes are being plasmid-derived resistance genes, they can be easily transmitted between bacteria of the same genera, i.e., the *Enterobacteriaceae* and other genera as well (Vachvanichsanong *et al*., 2021). It is well-known that the *Enterobacteriaceae* bacteria can cause diseases associated with high mortality and morbidity rates, such as urinary tract infections; however their treatment is challenging since resistance to antibiotics is increasing (Alotibi *et al*., 2022). Therefore, more than 95 % of the uncomplicated UTIs are caused by a single bacterial pathogen. According to previous

studies reported by Al-Sarraj, (2021); Vachvanichsanong *et al*., (2021), *E. coli* and *K. pneumoniae* are the two most prevalent bacteria that cause UTIs.

2.1. *E. coli*

Escherichia coli lives in the intestines of vertebrates, where it has its natural habitat. However, E. coli is also widely found in the soil and water (Berthe *et al*., 2013). Therefore, when existing in the aquatic habitats, E. coli is taken frequently as an indicator of fecal contamination (Petersen and Hubbart, 2020). In addition, the bacterial surface is covered with fimbriae, which are crucial for colonizing the hosts and for adhesion to the human cells (Gomes *et al*., 2021).

Recently, Wang *et al*., (2022) revealed that *E. coli* produces a wide range of enzymes that are crucial for its pathogenicity; interactions with the local microbiome, access to the different resources, and survival under the varied conditions. Like many other bacterial species, *E. coli* is an opportunistic pathogen (Denamur *et al*., 2021). This indicates that *E. coli* causes no harm under the normal conditions, because it is a standard component of the microbial flora of humans. However, *E. coli* can potentially cause fatal infections when it spreads from the intestinal mucosa to the other organs, including the urinary tract; the gall bladder, and\ or the bloodstream (Khalid and Andreoli, 2019).

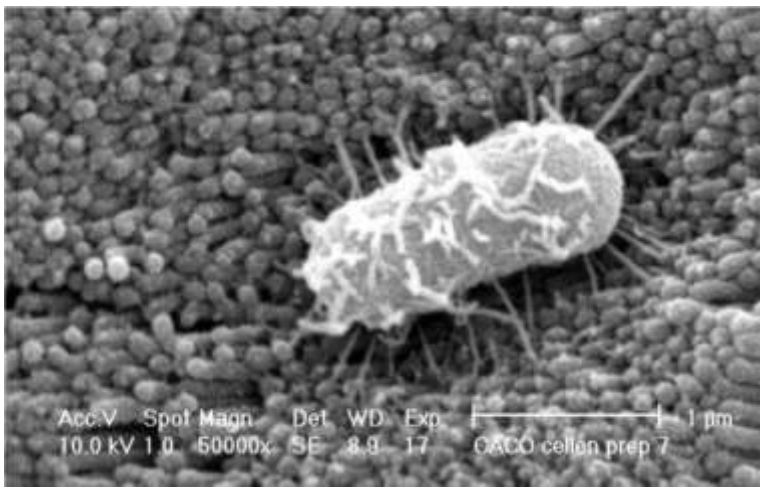

Fig. 1: A single cell of the *E. coli* adhering to 19-day-old Caco-2 cells, which is observed under a scanning electron microscopy (SEM), adopted by Starčič *et al*., (2002)

The *E. coli* genome encodes up to 5000 genes. However, approximately half of these genes only constitute the core genome shared by all the *E. coli* strains. In contrast, the remaining genes comprise the highly variable accessory genome, which generates a wide genomic diversity within the species (Nowicki *et al*., 2021). For example, the accessory genome can encode for certain pathogenicity factors and other properties, which enhance the bacterial survival in the particular ecological niches. These virulence factors include adhesins; secretion systems, toxins, iron uptake systems, and capsule synthesis (Desvaux *et al*., 2020). The extra-intestinal pathogenic *E. coli* (ExPEC) is an important human pathogen and is the most common causal agent of the urinary tract infection (Guglietta, 2017). In addition, the ExPEC is the most

common Gram-negative bacteria that is involved in the bloodstream infections (BSIs) (Pitout, 2012; Flores-Mireles *et al*., 2015). Furthermore, ExPEC has the potential to infect several human organs, which range from the biliary system to the central nervous system (CNS). In the gut, the ExPEC can act as harmless commensals until leaving the gastrointestinal tract, where it may cause infections in the different regions of the human body. ExPEC strains that tend to cause UTIs are designated as Uropathogenic *E. coli* (UPEC). Adhesion on the tip of the type 1 fimbriae; *FimH*, is an important virulence factor of the UPEC. These fimbriae allow the bacteria to adhere and invade the urinary bladder epithelial cells, which facilitate their infection (Govindarajan and Kandaswamy, 2022).

2.2. *K. pneumonia*

According to the first description of the *K. pneumonia* bacterium (Fig. 2) in 1882, it has been detected in the lungs of pneumonia patients who have died (Fliss *et al*., 2022). *K. pneumonia* belongs to the *Enterobacteriaceae* family, and is a Gram-negative bacterium; rod-shaped bacillus, lactose-fermenting, and has a prominent capsule (Hussein and Hamed, 2017). *K. pneumoniae* can colonise the healthy individuals' intestinal tracts; skin, nasopharynx, and oropharynx. Nevertheless, it has been reported that *K. pneumoniae* have the potential to cause several diseases in the hospitalized patients, including pneumonia; wounds, soft tissue infections, and urinary tract infections (Reyes *et al*., 2019).

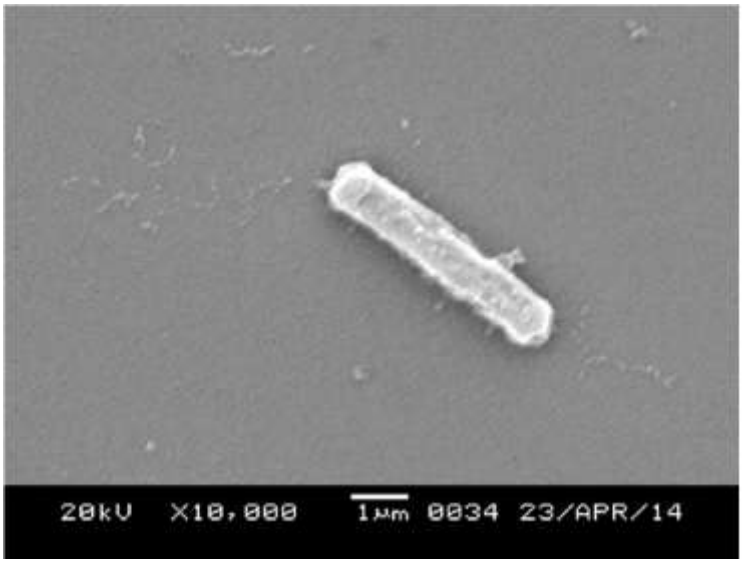

Fig. 2: Scanning electron micrograph of *K. pneumoniae* (Nath and Joshi, 2015)

Infections caused by this bacterium have become a serious public health concern, due to the advent of carbapenem-resistant strains (Wyres and Holt, 2018; Shrivastava *et al*., 2018). As a result, patients with immunocompromised status and those with severe illnesses can develop life-threatening nosocomial infections, such as urinary tract infections; pneumonia, and bloodstream infections (Podschun and Ullmann, 1998). *K. pneumoniae* are widely recognized as reservoirs of the plasmids that carry the antimicrobial resistance genes. Many of these plasmids can be acquired, transferred, and spread within and outside

their populations (Hawkey *et al*., 2022). *K. pneumoniae* strains frequently spread in the hospitals and are the key causes of the hospital and community-acquired infections worldwide (Aljanaby and Alhasnawi, 2017; MOTAWEQ, 2022). Because of the two key factors listed below, nosocomial infections produced by *K. pneumoniae* have a tendency to be chronic. *In vivo* biofilms produced by *K. pneumoniae* shield this bacterial pathogen from the host immunological responses, as well as from the drugs used to treat them (Jagnow and Clegg, 2003). Moreover, nosocomial isolates of *K. pneumoniae* frequently exhibit multidrug-resistance phenotypes, which are induced by the presence of extended-spectrum β-lactamases or carbapenemases, making these isolates challenging for selecting the effective antibiotics for their treatment and\ or therapy (PETERSON, 2004; Munoz-Price *et al*., 2013).

3. The Extended spectrum β-lactamase (ESBL) bacteria

Antibiotics with a β-lactam ring in their molecular structure are known as β-lactams. They work through binding to the bacterial cell wall's "penicillin-binding proteins" (PBPs). PBPs are bacterial enzymes that play an essential role in the cell wall synthesis (Jubeh *et al*., 2020). Their primary purpose involves synthesizing a cross-linked peptidoglycan, which forms a fishnet-like polymer that maintains the shape of the bacterial cells and protects them from the internal osmotic pressure (Letourneau, 2019). The PBPs are known to be inactivated by covalent binding of all the β-lactams; however the binding profiles of the different PBPs for β-lactams vary substantially. According to Jiao *et al*., (2019), in order to achieve synergistic bacterial killing and reduce the antibiotic resistance; two β-lactams can be combined, which will allows for the inactivation of several PBPs. A covalent acyl-enzyme ester link is created when a β-lactam binds to a PBP, leading to inhibition of the transpeptidases; through the acylation of an active site serine in these enzymes, which are essential for growth of the replicating bacteria (Waxman and Strominger, 1983). β-Lactams are among the safest and most widely prescribed antibiotics (Bush, 2017; Klein *et al*., 2018). The β-lactam ring is fused to various molecular ring structures in the β-lactam antibiotics, and there are four main groups for the therapeutic uses, including penicillins; cephalosporins, and carbapenems that belong to three categories and have a bicyclic structure, in addition to a fourth group that has a monocyclic structure (*i.e*., monobactams). In one case, the four-membered 2-azetidinone ring that is also known as the β-lactam ring, either complete the five-membered pyrroline (carbapenems), or it becomes integrated into a thiazolidine ring (found in penicillin), or it is integrated into a six-membered dihydrothiazine ring (found in cephalosporins) (Klein *et al*., 2018). The natural products found in each group were subjected to extensive modification programs, resulting in a variety of semi-synthetic derivatives (Bush and Bradford, 2016). The development of β-lactamase enzymes, which break down the β-lactam compounds, is a common mechanism by which the Gram-negative bacteria; including the *Enterobacteriaceae*, resist the β-lactams (Bush, 2018). According to the amino acid sequences, these β-lactamase enzymes are classified into four classes (A, B, C, and D) (Tsivkovski *et al*., 2020). The β-lactamases that belong to classes A, C, and D, belong to the family that inhibits the β-lactamase activity through covalently binding to the serine amino acid that resides in the active site of the enzyme. On the contrary, the class B β-lactamases commonly referred to as Metallo-β-lactamases (MBLs); utilize the bound Zn^{2+} ions to mediate hydrolysis of the β-lactam antibiotics (Tooke *et al*., 2019). Another classification that is currently used for the β-lactamases is called the Bush-Jacoby-Medeiros functional classification scheme, which categorizes the β-lactamases into groups 1-3, based on their ability to hydrolyze the specific β-lactam classes and on the their vulnerability to the inhibitors (Boyd *et al*., 2020). According to the molecular structural classification, Group 1 contains cephalosporinases that belong to class C, and their genes are originally chromosomal. In Group 2, the β-lactamases enzymes are harbored by the plasmids, making these plasmids easy to spread between the bacteria, ultimately resulting in rapid

resistance to these β-lactamases enzymes. Meanwhile, β-lactamases that belong to group 2 are inhibited by the tazobactam, clavulanic acid, and sulbactam. According to the molecular structural classification, group 3 involves the metallo-β-lactamases (MBLs) and belongs to class B (Ghafourian *et al*., 2015). Finally, β-lactamases in Group 4 include the penicillinases that cannot be inhibited by the clavulanic acid (Ghafourian *et al*., 2015). Mutations in the *TEM-1*, *TEM-2*, or *SHV-1* genes usually result in modifications of the amino acid structure that surrounds the active site of these β-lactamases. In this way, the enzymes can hydrolyze an expanded number of β-lactam antibiotics. Several previous reports have documented an increase in the number of ESBLs that are not related to TEMs or SHVs lineage (Paterson and Bonomo, 2005). The most common β-lactamase is found in *TEM-1*. However, more than 90 % of the *E. coli* ampicillin resistance is thought to be caused by the presence of *TEM-1* gene (Livermore, 1995). The *E. coli*-causing UTIs have steadily become harder to treat due to the development of ESBLs. Moreover, Al-sarraj, (2021) reported that the ESBL-producing bacteria may also acquire resistance to additional antibiotics, including the tetracyclines, trimethoprim\sulfamethox azole, quinolones, and aminoglycosides.

4. Treatments of the ESBL bacteria

The ESBL-producing bacterial strains are recognized as a worldwide challenge in treatment of both the hospital and community- acquired infections. Despite the *in vitro* activity of the other β-lactam antibiotics, carbapenems remain the first-line of therapy against the ESBL infections (Harris *et al*., 2018). Nevertheless, the enormous global increase in the ESBL incidence has resulted in major carbapenem misuse, which has prompted the development of novel β-lactamase enzymes that are capable of hydrolyzing the carbapenems (Montravers and Bassetti, 2018). Thus, there is an urgent need to adopt new approaches to minimize the use of carbapenems. A previous study conducted by Davies and Davies, (2010) revealed that the discovery and use of antibiotics to treat the human diseases and save their lives have significantly decreased the public health risks that have stemmed from bacterial infections, and also have been considered among the most significant advances in medicine in the 20th century. The most frequent classification of the antibiotics is based on their several criteria, including the bacterial spectrum (*i.e*., broad or narrow); administration route (*i.e*., injectable, oral, and\ or topical) and molecular structures (Etebu and Arikekpar, 2016). Antibiotics of the same chemical structure have been recorded to have similar efficacy; toxicity, and allergenic actions. Therefore, based on their physical-chemical or molecular structures, the antibiotics can be categorized into various groups, including β-lactams; macrolides, tetracyclines, quinolones, and sulfonamides (Oberoi *et al*., 2019). Furthermore, the antibiotics can be divided into four principal groups depending on how their active components affect the bacterial cell (Lade and Kim, 2021), including disruption of the bacterial cell wall synthesis (e.g. β-lactam antibiotics and glycopeptides); interference with DNA-synthesis (e.g. fluoroquinolones), inhibition of protein synthesis (e.g. tetracyclines and aminoglycosides), and inhibition of folic acid synthesis (e.g. trimethoprim).

The basis of resistance to all antibiotics is the genetic makeup of the bacteria. Levy and Marshall, (2004); Davies and Davies, (2010) highlighted that a bacterium may acquire antibiotic resistance through a gene mutation that alters the pre-existing DNA of the cell and changes its gene expression; however, this mutation does not add new genes or transfer new genes between the bacteria through the process known as "Horizontal gene transfer". Generally, the horizontal gene transfer takes place via three different mechanisms; mainly transformation, conjugation, and transduction. The transduction involves DNA transfer that is mediated by bacteriophages, which transfer the genetic materials between a donor and a recipient bacterium. Transformation is a form of genetic recombination in which free DNA fragments from a dead bacterium enter into a recipient bacterium, and these fragments are then incorporated into the bacterial

chromosome (Thomas and Nielsen, 2005; Norman *et al*., 2009). However, in the conjugation, the mobile genetic element (MGE) mobilizes and reorganizes the genes within a given genome and\or between the bacterial cells (De Boever *et al*., 2007). Several previous studies conducted by Rijavec *et al*., (2006); Partridge *et al*., (2018) reported that the MGEs that allow for mobility within a chromosome or across the bacteria are often classified into plasmids; integrons, and transposons. The phenotype of bacterial resistance is determined by its gene content and the way it is expressed.

Several previous studies reported by Nikaido, (2009); Blair *et al*., (2015) that the resulting biochemical mechanisms of antibiotic resistance have been frequently split into several groups; mainly

1-Modifying the enzymes or inactivating the antimicrobial agents:

Bacterial inactivation of the antibiotic molecule is made possible through synthesizing enzymes, which may modify or destroy the antibiotic, e.g. β-lactamase that is produced by *E. coli* hydrolyzes the β-lactam antibiotics, while *K. pneumoniae* destroy the ampicillin by similar enzymes.

2-Decreased permeability and efflux pumps:

The pumps are found in the cytoplasmic membrane, and have the potency to remove the harmful substances from the bacterial cell, including the antibiotics. This method of antibiotic resistance impacts wide range of antimicrobial classes, along with additional hazardous substances that the bacteria may come in contact with (e.g. *E. coli* with altered porins or efflux pumps reduces the concentration of the tetracyclines).

3-Alterations of the target sites of the antimicrobial agents:

One of the most prevalent methods by which the bacteria develop resistance to antibiotics is through the impairment of the antimicrobial molecule's ability to bind to its intended target. The target modifications may take the form of an enzymatic change to the target site; a point mutation in the gene that codes for the target site, and\ or a replacement or bypass of the original target [e.g. Several β-lactamase drugs do not bind to *Staphylococcus aureus* due to its modified binding site, and is named as methicillin resistant *Staphylococcus aureus* (MRSA)].

The growing resistance of bacteria to the antibiotics has become a serious problem, due to the biological processes of natural selection and bacterial adaptation towards the exposure to antibiotics, as a result of the indiscriminate use of the antibiotics in humans and animals (Fymat, 2017). The rapid spread of bacterial resistance while few new antibiotics are developing has led to the development of stewardship and treatment guidelines, so as to better manage the dwindling antibiotic resources. According to Karpiński, (2019), every year there are about 700,000 deaths that are possibly caused by microorganisms. However, different cases have been reported to be related to antibiotic resistance, including penicillin-non-susceptible *Streptococcus pneumoniae* (PNSSP); methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum β-lactamase- (ESBL) producing *Enterobacteriaceae*, vancomycin-resistant enterococci (VRE), and Candida sp. that is resistant to imidazoles (de Oliveira *et al*., 2015). This highlights the growing need for new antibacterial compounds that are active against the pathogenic bacteria (Jubeh *et al*., 2020). In spite of the extensive research works that have been performed to find alternative drugs; however, the natural products with their diverse chemical structures have been considered as important sources of the novel bioactive agents that have useful biological activities (Zerrifi *et al*., 2018). Almost all forms of living cells, including the animals, plants, and microorganisms, can produce natural bio-products (Abdel-Razek *et al*., 2020).

5. Treatments of the ESBL bacteria using herbals medicine

The cultivation of plants can be traced back to thousands of years when they were used for food and for everyday necessities. Plants have been used in the manufacture of paper and infrastructure, for perfumes and spices, and to treat and prevent the microbial diseases (Hasheminejad *et al*., 2019). According to the World Health Organization, the herbal medicines are those medicines that contain herbal preparations, such as herbal materials; herbs, finished herbal products that contain plant parts, and\ or plant materials that are combined with other plants as active ingredients (Huang *et al*., 2019). It is well-known and acceptable that herbal therapies can be useful for treatment of chronic diseases in different countries, including the cancer and cardiovascular diseases (Zhang *et al*., 2016; Hare *et al*., 2017; Sundaram *et al*., 2019). In the 21st century, medicinal plants have gained increasing interests worldwide, because they have fewer reported side effects, are of low cost, easy to obtain, lack of the resistant bacteria, and are tolerated by patients especially those suffering from UTI (Das *et al*., 2015). There are many plant parts that can be used for treatment of UTI, including the flowers; leaves, bark, fruit, seeds, and even the whole parts of medicinal plants. These plant parts or their extracts are either consumed orally as a sole preparation, and\ or mixed with different other foods and drinks, such as water; honey, milk, juices, or black pepper; depending on the patient's sex, age, and current health status (Pattanayak *et al*., 2017). Based on the previous study conducted by Bag *et al*., (2008), java tea (*Orthosiphon spicatus*); horsetail (*Equisetum arvense*), Asparagus (*Asparagus officinalis*), couch grass (*Agropyron repens*), birch (*Betula* sp.), lovage (*Levisticum officinalis*), goldenrod (*Solidogo virgaurea*), nettle (*Urtica dioica*), and parsley (*Petroselinum crispsum*), were approved for use in the therapy of the UTI's patients, due to their abilities to help flush out the uropathogens.

Fig. 3: Ripe pomegranate fruit, adopted by Wang *et al*., (2018)

6. Treatments of the ESBL bacteria using *P. granatum*

Pomegranate (*Punica granatum* L.) (Fig. 3) that is commonly known as pomegranate, is a small tree of the family of *Lythraceae* (Shivsharan and Ravva, 2018). There are several factors that influence the chemical composition of the pomegranate fruit and the other plant parts, including the climate; geographical location, storage, and cultivars. Pomegranates are native to Iran and Northern India, and they are frequently cultivated in the Mediterranean, tropical, and subtropical climates (Yan *et al*., 2019; Patil *et al*., 2020; Patil *et al*., 2021). Each part of this plant can be used as a medical material, such as peels; seeds, seed oils, roots, trunk (barks), wood spout, leaves, flowers,

and fruit rinds (Pienaar, 2021). The pomegranate plant contains multiple constituents with therapeutic properties that have been identified and isolated from various parts. Among these are the flavonoids, such as kaempferol, quercetin, luteolin, and anthocyanins; including cyanidin, pelargonidin, and delphinidin. Moreover, *P. granatum* contains punicalin; punicalagin, ellagic acid and its derivatives (3,3´-tri-O-methylellagic acid, 3´-O- methyl-3,4-methylene, 3,3´-di-O-methylellagic acid), in addition to the phenolic compounds; mainly punicacortein A-D, gallocatechins, punicafolin, pedunculagin, corilagin, granatin A and B, corilagin, punigluconin and sterols, in addition to several fatty acids, triterpenes, and other tannins (Jacob *et al*., 2019). Numerous biological and pharmacological processes have been connected to *P. granatum*. The phenolic acids, flavonoids, and tannins' bioactive components of *P. granatum* play a major role its nutritional value (Coronado-Reyes *et al*., 2021). These phenolics possess a strong binding ability to the different molecular structures, such as the proteins or glycoproteins, thus they can antagonize the bacterial antibiotic resistance. In this regard, the *P. granatum* plant has been evaluated for its potential therapeutic effects, and has been shown to possess anti-radical; antimicrobial, anti-inflammatory, hypolipidemic, anti-proliferative, and hypoglycemic activities (Di Sotto *et al*., 2019). During the previous study of Zam and Khaddour, (2017), an aqueous pomegranate peel extract's expressed an inhibitory activity against the uropathogenic *E. coli*, which was dose- and pH dependent. Furthermore, this extract recorded a minimum inhibitory concentration (MIC) value of 0.6 mg\ ml, which caused a reduction of the adhesion index of the *E. coli* up to 80 %; accompanied by a decrease in the bacterial motility. Meanwhile, the *P. granatum* peel aqueous extract has exhibited a minimum bactericidal concentration (MBC) value of 1.2 mg\ ml. As a consequence, the peel extract has suppressed the *E. coli* biofilm formation and has reduced the bacterium adherence capability. Another recent study conducted by Elshafie *et al*., (2021) highlighted that an aqueous extract of the *P. granatum* leathery exocarp has demonstrated antifungal and antibacterial potentials against *E. coli* and several other microorganisms. This may be attributed to the antioxidant properties and the anti-acetylcholinesterase inhibitory effect of the *P. granatum* extract.

6.1. Antibacterial efficacy of the *P. granatum* peels

Punica granatum peel has been used by many cultures around the world for treating several health problems (Khanavi *et al*., 2013). The dried *P. granatum* peel powder is good for treating many health disorders, particularly the headache and the stomach problems (Gullon *et al*., 2016). In addition, the *P. granatum* peel powder has been widely used in treating the bleeding gums and plaques, in addition it has a promising antimicrobial activity (Elbatanony *et al*., 2019). Several previous studies reported by Abdel-Salam *et al*., (2018); Fahmy *et al*., (2020); Pirzadeh *et al*., (2021), that the aqueous and organic extracts obtained from the fruits and by-products of this *P. granatum* peel have antibacterial constituents, including the hydrolysable tannins (*i.e*., punicalagin, penicillins, ellagic acid, and gallic acid) that are synergized with the bioactive flavonols (*i.e*., myricetin, quercetin), and the anthocyanins (*i.e*., cyanidin-3-glucose and pelargonidin-3-galactose). By forming hydrogen bonds with the bacterial proteins, tannins in *P. granatum* peel interfere with the structure of the bacterial cell wall and prevent the protein synthesis; accordingly they suppress the bacterial growth (Hajoori *et al*., 2014). The *P. granatum* peel represents approximately 50 % of the fruit weight, and is reported to contain more vigorous antioxidant potential compared with the fruit juice (Pirzadeh *et al*., 2021). Numerous studies depicted in Table (1) have demonstrated the antibacterial potency of *P. granatum*.

6.2. Antibacterial potential of the *P. granatum* leaves

For several years, the experience-based traditional medicine has utilized *P. granatum* leaves as a therapy for treatment of the skin injuries and arthritis, which has been applied in the form of an ointment or as a paste (Shukla and Kashaw, 2019). There are several

reports of using *P. granatum* in the form of infusion and\ or decoction for treatment of the urinary tract infections; stomach disorders, renal colic, and sore throats (Mestry *et al*., 2020). Previous preclinical studies conducted by Al-Muammar and Khan, (2012); Rao and Krishnamurthy, (2019) have shown that using *P. granatum* ethanolic leaf extract as a dietary supplement can help in the management of obesity; hyperlipidemia, and hypercholesterolemia. Furthermore, there are indications that *P. granatum* leaf infusion lacks excitatory and cathartic components that have been observed in the conventional teas and weight-loss medicines, which implies the greater safety of this leaf infusion without expressing significant side effects, such as diarrhea, nausea, and vomiting (Al-Muammar and Khan, 2012). There are many benefits associated with using *P. granatum* leaves as a complementary and alternative therapy. Table (2) demonstrates several studies that revealed the antibacterial efficacy of *P. granatum* leaves.

Table 1: Antibacterial activity of the *P. granatum* peels

Findings	References
Pomegranate peel extracts are potent and efficient inhibitors of the *E. coli* growth. The diameter of zone of inhibition of *E. coli* has been higher in the ethanol extract than the acetone extract, recording 12 mm and 11 mm, respectively.	(Karthikeyan and Vidya, 2019)
The pomegranate methanolic extract presented MIC value of 250 µg\ ml to *E. coli*, which has been determined using a microdilution method.	(Bakkiyaraj *et al*., 2013)
The methanol and aqueous extracts of pomegranate peels have showed a good inhibitory activity against *E. coli*. The observed inhibition zone diameters are 25.7 mm and 18.3 mm; respectively, with MIC of 6.25 mg\ ml.	(Ibrahim *et al*., 2022)
The antibacterial effects of various parts of the pomegranate fruit have been tested against *E. coli* and *K. pneumoniae*. Compared to the other extracts, the peel extract has demonstrated the strongest antibacterial activity, expressing inhibition zone diameters of 20 mm and 18 mm, respectively.	(Dahham *et al*., 2010)
Several studies have revealed that the antimicrobial activity of Pomegranate peel extracts has been more potent than the other parts, which is related to the total flavonoids and tannins contents. Pomegranate peel extracts are well known for their antimicrobial activity against many bacterial and fungal pathogens.	(Casquete *et al*., 2015; Ismail *et al*., 2016)

The pomegranate extracts have been tested for their antibacterial properties against *K. pneumonia* and *E. coli*. The results showed that *E. coli* is more susceptible to the pomegranate extracts than *K pneumonia*. This may be attributed to the presence of a capsule as a virulence factor of the *K. pneumonia*, which has increased its resistance to the various antibacterial agents.	(Alnuri *et al*., 2022)
The methanol extract of the *P. granatum* peels has exhibited significant antibacterial activity against the MDR strains of 11 bacterial species, including *E. coli* and *K. pneumonia*. The recorded diameters of inhibition zones are 19 mm for *E. coli* with MIC value of 0.67 mg\ ml and MBC value of 1.51 mg\ ml, while it was 21 mm for *K. pneumonia* with MIC value of 3.41 mg\ ml and MBC value of 4.27 mg\ ml.	(Mishra *et al*., 2017)
The methanol extract of *P. granatum* has shown significant inhibitory potential against the MDR bacterial strains that cause urinary tract infections	(Jacob *et al*., 2019)

Table 2: Antibacterial activity of the *P. granatum* leaves

Findings	References
The antibacterial activity of *P. granatum* extracts has been assayed *in vitro* against the standard and resistant strains of *E. coli*. The inhibition zone diameters have varied from 8 to 19 mm, and the MIC values have varied from 0.625 to more than 5 mg\ ml.	(Trabelsi *et al*., 2020)
The extracts of *P. granatum* leaves have expressed antimicrobial efficacy, due to the presence of tannins and flavonoids in these extracts, which are known of having antimicrobial potency.	(Chung *et al*., 1998; Cushnie and Lamb, 2005)
The methanol leaf extract of *P. granatum* has showed antibacterial activity against *E. coli*, expressing an inhibition zone diameter of 18 mm.	(Balamurugan *et al*., 2020)
The antibacterial activity of ethyl acetate extract of pomegranate leaves has been tested using the microdilution assay, and has showed MIC of 7.5 mg\ ml against *E. coli*.	(Bisht *et al*., 2016)
A previous study has evaluated the antibacterial activity of *P. granatum* leaves extract against *E. coli*. The recorded diameters of zones of inhibition are 19 mm, 20 mm, 21 mm, 21 mm, and 21mm, for the corresponding concentrations of the extract of 2 %, 4 %, 6 %, 8 %, and 10 %, respectively.	(Falbo *et al*., 2016)
The secondary metabolites of *P. granatum* leaves extracts have significant antibiotic properties. They may interfere with specific steps in the homeostatic biosynthesis of the bacterial cell walls, which alter the shape and size of the cells, induce stress responses in the cells, and cause bacterial cell lysis.	(Burt, 2004)

Conclusion

A global issue of the MDR bacteria has prompted the scientists to develop novel bioactive compounds derived natural resources that can be used as safe phytotherapy. The presence of pomegranate's tannins; glycosides, alkaloids, resins, volatile oils, flavonoids, and gums makes it a valuable food product with a variety of potential therapeutic properties. The experimental data have showed that *P. granatum* contains several bioactive compounds that exhibit a wide range of pharmacological properties, including antioxidants; antimicrobials, antivirals, antidiabetics, anticancer, and antianxiety effects. In addition, the isolation of such compounds from *P. granatum* peels, which are considered as agro-industrial wastes, can also contribute to reducing the amounts of the produced wastes.

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Conflicts of interest

The authors declare that no conflict of interests exist.

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Ethical approval

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