Review Article

A Systematic Review on the Antibacterial Activity of *Punica granatum* L. against *E. coli*

Jeen Hernandez Magbuhat, Paulene Dinglasan Bool, Kristine Jane Rios Olit, Diana Rose Olavidez Sales, Axel An Lee Mangalonzo, Franzyn Evol Uy Montances, Sophia Galang Gallardo, Gilmar Chrys Parreño Salenga, Carl Vincent Del Rosario Arizala

Department of Medical Technology, Institute of Arts and Sciences, Far Eastern University, Manila, PHILIPPINES.

Submission Date: 23-08-2022; Revision Date: 14-09-2022; Accepted Date: 02-11-2022.

ABSTRACT

Background: The global crisis in shortage production and overuse of antibiotics resulted in the resurfacing of drug-resistant bacteria, posing a threat to the effects of conventional antibiotics. The medicinal plant *Punica granatum* L. (pomegranate) has been widely studied for its antimicrobial effects against bacteria, fungi, and helminths, because of the phytochemicals present in the different parts of the plant (leaves, petals, peels, seeds, and fruit). Studies show that pomegranate is effective against the potent gram-negative bacterium, *Escherichia coli (E. coli)*. Different strains of *E. coli* are highly drug-resistant and multidrug-resistant. Hence, the objective of this paper is to determine the antibacterial efficacy of phytochemicals found in the different parts of pomegranate against *E. coli*. **Materials and Methods:** To review the antibacterial activity of *Punica granatum* L. against *E. coli*, online databases such as PubMed, and ScienceDirect were used to search for journals and studies published between 2013-present. Search terms include "Pomegranate", "*Punica granatum* L.", "Antibacterial", "*E. coli*", and "Extract". And software named Zotero was used for the removal of duplicates. **Conclusion:** After the analysis, this review reveals that polyphenols, specifically flavonoids, and tannins, are the most common class of compounds present in many parts of pomegranate including the leaf, peel, and exocarp that exert antibacterial properties against *E. coli.*

Keywords: Pomegranate, *Punica granatum* L., Antibacterial, *E. coli.*

Correspondence:
Ms. Jeen Hernandez Magbuhat,
Department of Medical Technology, Institute of Arts and Sciences, Far Eastern University, Manila, PHILIPPINES.

Email id: jeen.magbuhat@gmail.com

INTRODUCTION

Punica granatum L. (pomegranate) is a fruit-bearing tree from the Punicaceae family found in some areas of Asia including Iran, Northern India, and Persia. This plant is being cultivated globally and is one of the medicinal plants being studied as a potential antibacterial alternative.[1] Due to its wide spectrum of bactericidal, anti-helminthic, and antifungal effects, *P. granatum* L. is being evaluated for its possible ability to regulate the immune system of the host and its effects on infections commonly affecting the human oral cavity.[2] Extracts from the leaves, petals, peels, seeds, and fruit part of the *P. granatum* L. may provide a natural alternative for antibiotics due to its potency against a wide range of species of microorganisms. Research shows that various active phytochemical contents of *P. granatum* L., such as hexane, ethanol, and aqueous extract, etc. (commonly found in leaves and peels) demonstrates antibacterial efficacy against a number of highly drug-resistant bacterial strains.[1]

Escherichia coli (E. coli) is characterized as a gram-negative, rod-shaped bacterium that belongs in the family Enterobacteriaceae.[3] It is noted to be the most resistant amongst all gram-negative bacteria that induces a wide array of illnesses targeting all age groups. Extensively drug-resistant, multi-resistant, and pan-drug-resistant strains of *E. coli* have been reported ubiquitously which deems it as a worldwide issue. Cephalosporins, a β-lactam antibiotic, is the most commonly used antibiotic for the treatment of gram-negative bacilli infection. However,

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2022.11.84

E. coli strains showed resistance against this antibiotic by producing extended spectrum beta lactamase (ESBL) which has the ability to hydrolyze and inactivate β-lactams such as cephalosporins and monobactams.[4] The global crisis of overusing drugs and the lack of new antibiotic solutions by the pharmaceutical industry has led to the resurgence of drug-resistant bacteria which threatens the effects achieved with the use of conventional antibiotics.[5] Given this instance, it is vital to search for plant extracts as an alternative for preventive and therapeutic options. The authors then directed their focus to review the centuries of evidence regarding the effectiveness of pomegranate extract against *E. coli*. This plant, having different parts such as its leaves, seeds, juice, and peel, gives out a high efficacy rate on combating the emergence of microorganisms. The authors of this study aim to determine the maximum potential of pomegranate through examining its parts, the different methods of extraction, and different phytochemicals that produce the most efficient results. This mini review aims to assess and summarize the antibacterial efficacy, particularly the phytochemical components of *P. granatum* L. from different classes of compounds. Classes of which include phenolic acids, tannins, flavonoids, and lectins, that exhibit antibacterial properties against different *E. coli* strains based on the evidence gathered from collated published and peer-reviewed literature articles. Ensured knowledge may provide future schemes for responsive aids for the practices of the medical technologist for the determination of the effect of the different parts of the pomegranate towards various *E. coli* strains. Furthermore, this study will help researchers gain applicable and more comprehensive information that is useful in their future practice as medical technologists.

MATERIALS AND METHODS

Literature Search

The research articles and journal studies that are utilized in the mini-review were retrieved from online databases such as PubMed, and ScienceDirect through the search terms used such as "Pomegranate", "*Punica granatum* L.", "Antibacterial", "*E. coli*", and "Extract". These databases are the basis for the selection process of the research articles and journals that are used in the mini review conduction. A software named Zotero was used by one of the researchers for the removal of duplicates. Built-in filters from online databases were then used to remove review articles and studies published beyond the range of year considered.

Eligibility Criteria

The inclusion criteria in this paper are experimental studies that encompass the following: (1) written in English, (2) published between 2013-present, (3) from credible sites such as PubMed, and ScienceDirect, (4) covers the antimicrobial activity of the phytochemicals present in leaves, petals, peels, seeds, and fruit of *Punica granatum* L. against *E. coli* bacterium. Whereas, the exclusion criteria are the following: (1) review papers, (2) articles and studies that were not written in English, (3) published before 2013, (4) from non-credible sites, (5) and studies that did not cover the antibacterial activity of *Punica granatum* L. against any *E. coli* strain. The selection process of the articles can be seen in the flowchart in Figure 1.

Selection Strategy

The eligibility of the studies were reviewed and selected by all of the authors individually according to the inclusion and exclusion criteria aforementioned. Initially, the title and abstract were assessed followed by its credibility. Full-text of the studies were then examined for further evaluation.

Data Extraction

All eight authors extracted eligible articles based on study characteristics such as the first author's last name, year of publication, part of pomegranate used, class of compounds and specific compounds extracted, targeted *E. coli* strain, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), the zone of inhibition (ZOI) of the extract, and the results of the study.

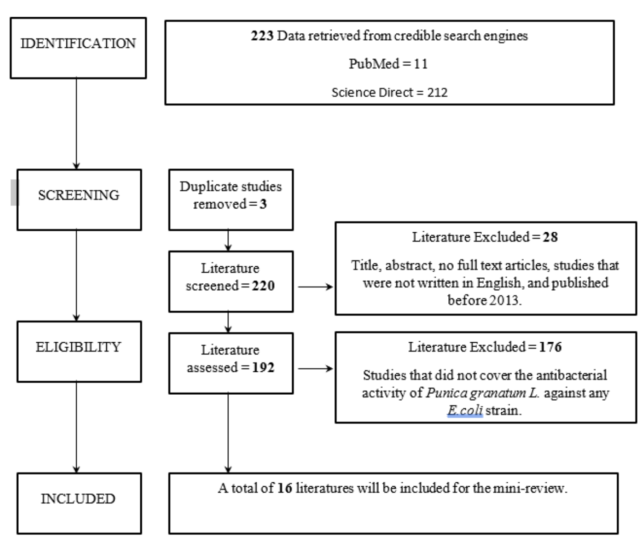

Figure 1: Schematic Diagram for Study Selection.

RESULTS

A total of 223 literature articles and journals were retrieved during the initial search, PubMed (11) and ScienceDirect (212). The literature articles and journals that were retrieved underwent data extraction which resulted in exclusion of data that does not meet the criteria. The excluded literatures and journals were removed through the utilization of ZOTERO, a software that manages the collection of research journals or articles through compilation, inclusion, and exclusion. A total of 220 articles and journals were retained due to the removal of 3 studies. The 220 literatures were screened through the assessment of title, abstract, relevance, availability of full text articles, the language the study is written, and year of publication. The screening procedure yielded 192 assessed studies and underwent exclusion with the basis of relevance of the literature to antibacterial properties of *Punica granatum* L. which produced a total of 176 excluded literature. After the screening, 16 studies were gathered. The retained articles and studies will be included and utilized for the mini-review. Data extracted for each article is present in Table 1.

Phytochemical compounds present in *P. granatum* plant

The phytochemical compounds present in the pomegranate vary in different portions of the fruit and are dependent on the geographical and environmental conditions that influence the bioactivity secondary metabolites.[6]

The phenols and polyphenols are mostly found in pomegranate peels but these can also be found in other parts such as exocarp, golnar, seed, leaves, and juice. The phenols which can be found in dried peels of pomegranate include phenolic acid (p-coumaric), flavan-3-ols (catechin and epicatechin), flavanone (hesperidin), flavonol (rutin), and ellagitannin (punicalin).[7] However, in another study, cinnamic acid is the major component of phenols followed by quercetin, p-coumaric acid, protocatechuic acid, punicalagin, then gallic acid.[6]

Flavonoids and ellagitannins are the principal constituents found on *P. granatum*. These compounds can be located on the peels, leaves, and exocarp. Different classes of compounds such as rutin, luteolin, flavones, and flavonoid glycoside are found as well. Seeds, leaves, and peels also consist of ellagic acid, a class of compound found on ellagitannins. Phenolic acid resides on the peel and leaf part of *P. granatum*. Phenolic compounds are composed of ellagic acid and gallic acid. Flavonoids, ellagitannins, and phenolics are compounds that facilitate cell lysis due to a damaged or ruptured cell membrane.[8]

Tannins are classified as polyphenols of plant origin which have been shown to have antibacterial effects. The association of tannins in the antibacterial activity of *P. granatum* L. peel extracts can be due to its high-molecular weight.[7] Hydrolyzable tannins are one of the distinct types of tannins. Peel contains a high quantity of phenolic compounds, with hydrolysable tannins as the primary components, when compared to other *P. granatum* L. fruit sections. It was shown in another study that the majority of bioactive compounds identified in the ethanol *P. granatum* L. peel extracts are from the group of tannins most specifically the ellagic acid derivatives and ellagitannins.[9] Anthocyanidins, also classified as polyphenols, is a naturally made plant pigment considered as one of the most beneficial compounds found in *P. granatum* L. peel and juice that exert therapeutic effects.[10] Anthocyanins is a specific source of phenolic compound in *P. granatum* L. juice when cyanidin glucosides and delphinidin is present. Plants produce coumarins by biosynthesizing p-coumaric acid. In terms of its antimicrobial activity, p-coumaric acid found in *P. granatum* L. peel methanolic extract has previously been shown to be effective as an antibacterial compound.[6]

Formulated metal oxide nanoparticles made from plant extracts become increasingly popular due to its effectiveness as therapy agents against infection caused by multi-drug resistant Gram-positive and Gram-negative bacteria. According to a study, the smaller the nanoparticle in size, the higher its surface to volume ratio, and the greater its antimicrobial efficacy. This is due to the fact that tiny nanoparticles may directly interact with the cell membrane of the bacterial pathogen. Zinc oxide nanoparticles are considered as one of the most researched inorganic nanoparticles, owing to their durability and stability in harsh environments and antibacterial characteristics.[11]

Minimum inhibitory and bactericidal concentrations of *P. granatum* L. extract against *E. coli* strains

The leaf part of the plant *P. granatum* L. is focused upon by the following three studies.[1,12,13] Getting the extract of leaves, and testing the standard strain produces MIC against *E. coli* of 0.625 mg/ml, 2.5 mg/ml for penicillin-resistant strain, and 5 mg/ml for the multidrug-resistant strain.[1] Another study resulted in MIC values of 0.050 ± 0.005 mg/ml and a ZOI value of 13 mm. This antibacterial activity is explained by the presence of tannins and flavonoids.[12] The third study reported an MIC value of 1250 μg/mL.[13]

The antibacterial activity of sarcotesta which resulted in MIC values of 12.5-50.0 ug/ml and MBC values of 25.0-100.0 ug/ml.[14] These results are correlated to the ability of the seed coat to withstand heat up to 100°C. Furthermore, it is capable of hindering biofilm proliferation by disrupting the viability of microbes, decreasing the expression of genes associated with the formation of biofilm, and the ability to destroy enzymes and polysaccharides that help in producing biofilm.

Freeze dried extracts of peels produce the highest antibacterial activity with an MIC value of 0.39 mg/ml. The compounds present include flavonoids, polyphenol, phenolic acid, and tannins, all of which are figured to be the reason for the inhibition of microbes.[7] In a similar focus, MIC/MBC values were discovered to be within the range of 0.8 to 6.4 mg/ml due to the compounds tannins and flavonoids.[9] In a another study, polyphenols including phenolic acid, coumarin, cinnamic acid, and flavonoids are also found in peels and the MIC value was 500 μg/mL while ZOI value was 15.6 mm.[16]

Punicalagins (α and β) and ellagic acid from the class of ellagitannins and polyphenol has the highest antibacterial activity as well as the highest concentration against *E. coli* CCM 3988, which has MIC_{50} of 171± 1 μgmL^{-1}.[15] Peel extracts were able to show less antibacterial effect against *E. coli* compared to other extracts from peels, yielding effectivity with MIC values of 0.039 – 0.156 mg/mL presumably due to the interference from AHL-based (acylhomoserine lactones) QS (quorum sensing) dysfunction. *P. granatum* L. peel extract contains punicalagins, punicalins, and other ellagitannins. All of the compounds have antibacterial action against *E. coli*, with MIC90 values ranging from 0.3 mg/mL to 2.7 mg/mL.[16] Its growth is totally reduced when the greatest concentration (2.7 mg/dl) of H2O peel extract and EtOH peel extract is added.[17] The phenols, particularly ellagic acid, have been demonstrated to have significant antibacterial properties against *E. coli*. with an MIC value of 100–1000 μg ml^{-1}.[18] While a study found zinc oxide nanoparticles to be bactericidal and inhibiting at concentrations of 64.53 μg/mL and 90.90 μg/mL (MIC).[11] Another study indicates an inhibitory zone of *P. granatum* L. peel of the aqueous extract valoneic acid dilactone and hexane extract coumaric acid at concentrations of 12 +- 0.7mm and 13 +- 0.3mm (ZOI) versus *E. coli*. This may be attributable to the presence of hydroxycinnamic acid derivatives and hydrolyzable tannins compounds in the peel of *P. granatum* L.[19] When tested against both *S. aureus and E. coli* strains peel extracts had more inhibitory activity than juice extracts, resulting in a larger inhibition zone (15-30 mm) and MICs of 20mg/disc, 8 mg/disc, 4 mg/disc, and 2 mg/disc, respectively.[10]

P. granatum L. exocarp extracts, which contain high amounts of compounds of tannins, phenols, and flavonoids, showed inhibitory activity with an MIC value of 5 mg/mL. The antibacterial activity can be attributed to the detected phytochemical constituents in the exocarp including punicalagin, catechin, and rutin.[20] Juice extracts were able to show less antibacterial effect against *E. coli* compared to extracts from peels, yielding effectivity with MIC values of 20 mg/disc in a crude extract and 4 mg/disc in a purified extract.[10] Juice extracts of pomegranate showed antibacterial activity against *E. coli DSM 498* in higher concentrations compared to peels with MIC values of 2.7 mg/ml and 0.3 mg/ml, respectively.[17] Pomegranate seeds were also studied and it showed inhibitory activity against the strain in the exact MIC value as the juice extracts. The antibacterial activity of compounds present in the golnars of *P. granatum* L. produced MBC values varying from 12.50 - 50.00 mg/ml depending on the fraction used. It showed most effectiveness in the methanol and water fractions with an MBC value of 12.50 mg/ml against *Escherichia coli ATCC 25922*. The Zinc oxide nanoparticles extracted from the flowers explored the antibacterial activity of the compound against *E. coli ATCC 25922* and with MIC values of 2500 μg/ml, *E. coli* growth was suppressed.[13]

DISCUSSION

Different parts of *P. granatum* L. consist of varying phenolic compounds that are responsible for its antibacterial effect towards *E. coli* targets specifically. Experimental studies identified the classes of compounds present in the plant including flavonoids, polyphenols, tannins, phenolic acids, and lectins through High Pressure Liquid Chromatography (HPLC) and Folin-Ciocalteu colorimetric method. Varying antibacterial mechanisms are suggested among the compounds found in each part.

The compounds present in peels include flavonoids, polyphenol, phenolic acid, and tannins, all of which are figured to be the reason for the inhibition of microbes. Flavonoid and Phenolic acids cause cell lysis through disruption of cell membrane which result to impaired DNA synthesis. Tannins specifically ellagitannins sequester metal ions which are essential for bacterial proliferation. The inhibitory mechanism of Polyphenols, specifically ellagic acid, remains undiscovered.[8] These polyphenols, specifically

Table 1: Experimental studies involving the antibacterial activity of different Pomegranate parts against *E. coli* strains.

Author	Plant Part	MIC/MBC/ZOI	Target Pathogen	Class of Compounds	Compounds Present	Other Remarks
[1]	Leaf	Standard strain – 0.625 mg/ml (MIC); Penicillin-resistant strain – 2.5 mg/ml (MIC); Multidrug-resistant strain – 5 mg/ml (MIC)	*E. coli* ATCC 25922	Phenolic acid, Flavonoids	Rutin, Luteolin, Gallic acid, Ellagic acid	The TOF extract was shown to be the most active extract against strains tested in the antibacterial investigation. For penicillin-resistant *E. coli*, TOF extract had the best synergistic interaction with amoxicillin.
[14]	Sarcotesta	12.5-50.0 ug/ml (MIC); 25.0-100.0 ug/ml (MBC)	*E. coli*	Lectin	PgTeL	PgTeL demonstrated antibacterial action against beta-lactamase-producing *E. coli* isolates by causing damage to its growth, cell structure, and biofilm formation.
[17]	Peel	Freeze dried: 0.39 mg/ml 40°C: 0.20 mg/ml 50°C: 0.20 mg/ml 60°C: 0.39 mg/ml Streptomycin: 0.02 mg/ml (MIC)	*E. coli* ATCC 11775	Flavonoids, Polyphenol, Phenolic acid, Tannins	Catechin and Epicatechin, Ellagic acid, Gallic acid, Punicalin	Antibacterial activity of PPEs can be associated with tannins that have a high molecular weight. Freeze dried peels produced the greatest amount of naturally occurring bioactive compounds inclusive of punicalin, catechin, flavonoids, and epicatechin.
[12]	Leaf	MIC against *E. coli*: 0.050 ± 0.005 mg/ml Reported MIC, *E. coli*: 0.016, 0.125. 0.64 ZOI: 13 mm	*E. coli* MTCC 40	Tannins, Flavonoid	Flavones, Apigenin, Luteolin, Flavonoid glycoside	Reported that AgNPs produce an antibacterial efficacy against the specific bacteria. The results of the zone inhibition were 14, 13, 14, 13 and 13 mm for *S. aureus, B. subtilis, P. aeruginosa, E. coli and P. vulgaris*, respectively, when 200 µg/mL of nanoparticles were used.
[9]	Peel	0.8 to 6.4 mg/mL (MIC/MBC)	*E. coli* ATCC 25922	Tannin, Flavonoids	Punicalagin, Casuarinin, Granatin, Ellagitannin, Ellagic acid derivatives, Punicalin, Digalloyl exose, Lergastannin C, Epigallocatechin, Pedcalangin II, Peducalangin III	Combining the biological tests, untargeted MS analysis, and afterwards chemometrics processing, the study was able to discover and identify specific compounds from PPEs that contributed the most to observed specific biological activities.
[20]	Exocarp	5 mg/ml (MIC)	*E. coli*	Tannins, Phenols, Flavonoids	Punicalagin, Catechin, Rutin	The distinguished antimicrobial effect of LEP is due to the high percentage of polyphenol, specifically flavonoids and tannins.
[6]	Peel	500 µg/mL (MIC) 15.6 mm (ZOI)	*E. coli*	Hydroxybenzoic acid derivative, Hydroxycinnamic acid derivative, Coumarin, Cinnamic acid, and Flavonoids	Protocatechuic acid, p-coumaric acid, Punicalagin, Gallic acid, Quercetin	The highest antibacterial activity against bacterial strains was noted to be achieved by using a methanolic extract. Peel extracts indicated an absence of hemolytic activity proving the biosafety of extracts.
[19]	Peel	12 +- 0.7mm and 13 +- 0.3mm (ZOI)	MDR *E. coli*	Hydroxycinnamic Acid Derivative, Hydrolyzable Tannins	Coumaric acid, Valoneic Acid Dilactone	Fraction 1 of the aqueous extract (Valoneic acid dilactone) and Fraction 2 of the hexane extract (Coumaric acid) showed inhibitory activity to *MDR E. coli* specifically.

continued...

Table 1: Cont'd.

Author	Plant Part	MIC/MBC/ZOI	Target Pathogen	Class of Compounds	Compounds Present	Other Remarks
[10]	Juice and Peel	20mg/disc, 4 mg/disc; 2 mg/disc, 4 mg/disc, and 8mg/disc (MIC)	*E. coli*	Hydroxybenzoic Acid Derivative, Hydrolyzable Tannins; Hydrolyzable Tannins, Anthocyanins	Gallotannins, Gallagyl Esters, Ellagitannins; Punicalagin, Ellagic acid, Digalloyl-triHHDP-diglucose, Pedunculagin, Delphinidin, Cyanidin glucosides	Peel extracts exhibited more inhibitory activity compared to juice extracts yielding a bigger inhibition zone (15-30 mm) when tested against both S. *aureus* and *E. coli* strains.
[11]	Peel	64.53 μg/mL 90.90 μg/mL (MIC)	*E. coli*	-	Zinc oxide nanoparticles	Smaller-sized S3 of about 32.98 ± 8.63 nm exhibited better inhibition activities against Gram-negative bacteria (*E. coli*).
[18]	Peel	100–1000 μg ml^{-1}(MIC)	*E. coli*	Phenol	Ellagic Acid	The synergistic activity of pomegranate extract with antibiotics. has shown improved efficacies against pathogens which. are resistant to antibiotics.
[21]	Golnar	12.50-50.00 mg (MBC)	ESBL-producing *E. coli*	Phenol	Rutin, Gallic acid, Ellagic acid	Pomegranate pericarp extract enhances the antibacterial activity of ciprofloxacin against extended-spectrum beta-lactamase and metallobeta-lactamase producing Gram-negative bacilli.
[17]	Peel, Seed, and Juice	0.3 mg/mL; 2.7 mg/mL; 2.7 mg/mL (MIC90)	*E. coli* DSM 498	Polyphenol	Punicalagins, punicalins, and other ellagitannins	The study proved that the peel extracts of pomegranate is a good *E. coli* inhibitor. Its growth is completely suppressed at addition of the highest concentration (2.7 mg/dl) of H_2O peel extract and EtOH peel extract. This yields a 100% of MGIR.
[13]	Leaf and Flower	1250 μg/mL; 1250 μg/mL (MIC)	*E. coli* ATCC 25922	-	Phenolic content with Zinc Oxide Nanoparticles	Depending on the bacterial strain, the minimum concentration of zinc oxide that could effectively suppress the growth of the microorganisms utilized in the study ranged from 0.6 μg/mL to 2500μg/mL.
[15]	Peel	PTO8: 171± 1 μgmL^{-1} (MIC_{50}), 190± 1 μgmL^{-1} (MIC90)	*E. coli* CCM 3988	Ellagitannins, Polyphenol	Punicalagins (α and β), Ellagic acid	The PTO8 cultivar (sour–sweet) presented the highest antimicrobial activity, and it also has the highest concentrations of punicalagins (α and β) and ellagic acid.
[16]	Peel	0.039 mg/mL 0.078 mg/mL 0.156 mg/mL	*E. coli*	Anthocyanins, Hydrolyzable tannins	Punicalagin	TFPR inhibits *E. coli* biofilm formation possibly by interfering with AHL-based (acylhomoserine lactones) QS (quorum sensing) dysfunction.

flavonoids, can directly kill the bacteria, disarray the pathogenicity of the microbe, and/or inhibit bacteria through the activation of antibiotics.[22]

The presence of gallic acid in leaves inhibits bacteria through synergistically acting with tetracycline thus inhibiting the efflux pump found in *E. coli*. Aside from this, tannins and flavonoids are also present.[12] Tannins inhibits extracellular microbial enzymes or deprives substrates needed for the bacteria to grow while flavonoids have the ability to inhibit the role of cytoplasmic membrane or disrupts the permeability of membrane.[23]

The antimicrobial activity of the leathery exocarp (LEP) extract of pomegranate is attributed to the significant amounts of polyphenols, especially flavonoids and tannins. Its antimicrobial mechanism is due to the chemical structure of the compounds of phenols. Since it is lipophilic, it can easily enter the pathogen's membrane and impede its enzymatic activity necessary to produce energy and proteins, hence resulting in cell death.[20] Flavonoids are also present and exert the same antimicrobial mechanisms similar to its presence in peels.

Lectin is a bioactive protein that consists of carbohydrate attachment. Its antimicrobial property present in sarcotesta is facilitated through reversible and non-covalent binding to the surface of attached proteins, polysaccharides and lipids found on gram-negative bacteria.[14] The class of compound seen in *P. granatum* is *Punica granatum* sarcotesta lectin (PgTeL). Synergistic action of lectin is also evident with some antimicrobial compounds such as ampicillin, carbenicillin, and cefuroxime.

Anthocyanins are a particular source of phenolic compounds in *P. granatum* juice especially when cyanidin glucosides and delphinidin are present. The degree of antibacterial property of most phytochemical compounds including anthocyanin is determined by factors such as the method used for extraction, the cultivar, and the geographical origin and seasonality of the *P. granatum* L. plant. p-coumaric acid is the third main phenolic component (19.85 mg/mL in concentration) in *P. granatum* L. peel extract using HPLC analysis.[15]

The rutin, gallic acid, and ellagic acid compounds detected in the golnar extract were deemed responsible for this assistive bactericidal action of ciprofloxacin in ESBL and MBL-producing *E. coli*.[21]

Punica granatum L. is a good source of bioactive components with a high antibacterial effect on the growth of *Escherichia coli* strains (e.g., ATCC 25922, ATC 11775, MTC 40, and so on), implying that the researched pomegranate cultivar could be a natural treatment to reduce the emergence of *E. coli* strains, which are frequently involved in food-borne disease. The number of total polyphenols varied depending on the part of the fruit, with the peel containing more than the juice extracts. The MIC/MBC values of pomegranate extracts determined in several types of research differ significantly. According to the review's summary, the authors perceive an exceptional potential, particularly in extracts obtained from various components of *P. granatum* (peels, seeds, golnar, and exocarp), which are now considered waste food or as a by-product in the manufacturing of pomegranate juice. The extraction of bioactive components, particularly those with antibacterial activity, could be used to produce medications and aid in the fight against antibiotic resistance.

CONCLUSION AND RECOMMENDATION

The study focuses on research that determines the antibacterial activity of the different parts of *Punica granatum* L. plant, particularly the phytochemical components present from different classes of compound. A review of multiple studies showed that polyphenols, specifically the flavonoids and tannins, are the most common class of compounds present in many *P. granatum* L. parts including the leaf, peel, and exocarp that exert antibacterial properties. The MIC value of 0.39 mg/ml in the peel part of the plant that contains a significant amount of polyphenols is shown to produce the highest antibacterial activity.

In conclusion, the phytochemical compounds of *P. granatum* L. exhibit an antibacterial activity. The classes of phytochemical compounds are the principal constituents that exert specific mechanisms to facilitate the antibacterial activity of *P. granatum* L. Cell membrane damage and bacterial growth inhibition are the most common mechanisms of action used by these phytochemical compounds while its degree of antibacterial activity is dependent on the environmental and geographical conditions of the plant. Nevertheless, more thorough research is necessary to determine the mechanism of action of these phytochemical compounds and further studies are needed to assess the efficacy and effectiveness of *P. granatum* L. in different strains of *E. coli.*

ACKNOWLEDGEMENT

The authors would like to express their heartfelt and sincere gratitude to their research professor, Mr. Bernardino Hagosojos for his constant guidance and regular consultation throughout the research work and to acknowledge Far Eastern University Manila for funding this research. The authors are also grateful for each member of the team that provided reliable sources and exemplary effort in making this paper. Lastly, the authors would like to thank their parents and families for their never-ending understanding and lastly, their encouragement throughout our study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MBC: Minimum Bactericidal Concentration; **MIC:** Minimum Inhibitory Concentration; **ZOI:** Zone of Inhibition; **TOF:** Total Oligomer Flavonoids; **PgTeL:** *Punica granatum* sarcotesta lectin; **AgNP:** Silver Nanoparticle; **PPE:** Pomegranate Peel Extract; **LEP:** Leathery Exocarp Extract; **TFPR:** Tannin-rich Fraction from Pomegranate Rind.

SUMMARY

The focus of this study was to determine the phytochemical components of *Punica granatum* L. that significantly possesses antibacterial properties. The antibacterial activity was tested against the gram-negative bacteria *E. coli*. The study investigates the antibacterial activity of extracts, specifically phytochemical compounds prepared from different parts of *P. granatum* L. The bioactive components present in *P. granatum* L. were identified through High-Pressure Liquid Chromatography (HPLC) and Folin-Ciocalteu colorimetric method. It is composed of various phenolic compounds responsible for its antibacterial effect against *E. coli*. The agar well diffusion method was used to evaluate the antibacterial activity of *P. granatum* L. peel extract. The compounds found on the peel exert an MIC value of 0.39 mg/ml which produce the highest antibacterial activity. Furthermore, pomegranate extracts' MIC/MBC values have been measured in various studies; however, they vary significantly. The phytochemical compounds found in *P. granatum* L. vary in various parts of the fruit. The degree of antibacterial property of the phytochemical compounds varies depending on the geographical and environmental conditions of the plant. Based from a review of multiple studies, this study concluded polyphenols, such as flavonoids and tannins, as the most common class of compounds present in *P. granatum* L. that exert an antibacterial property against the gram-negative bacteria *E. coli*.

Author's Contribution

The concept of the mini review was proposed by Author K.O. The initial draft of the manuscript was written by all authors. Authors F.M. and A.M. performed the literature search. Authors P.B., S.G., J.M., A.M., F.M., K.O., and D.S. contributed to the data extraction and assessment of the articles included in the study. Authors P.B., J.M., A.M., F.M., K.O., D.S., took part in the analysis of the data. Author J.M. led the group until the paper's completion. Author C.A. provided guidance and assistance for the study to progress. All authors read and approved the final manuscript.

REFERENCES

1. Trabelsi A, El Kaibi MA, Abbassi A, Horchani A, Chekir-Ghedira L, Ghedira K. Phytochemical study and antibacterial and antibiotic modulation activity of *Punica granatum* (pomegranate) leaves. Scientifica. 2020;1-7. https://doi.org/10.1155/2020/8271203
2. Aparecida Procópio Gomes L, Alves Figueiredo LM, Luiza do Rosário Palma A, Corrêa Geraldo BM, Isler Castro KC, Ruano de Oliveira Fugisaki L, *et al*. *Punica granatum* L. (Pomegranate) Extract: *In vivo* Study of Antimicrobial Activity against *Porphyromonas gingivalis* in *Galleria mellonella* Model. The Scientific World Journal. 2016;8626987. https://doi.org/10.1155/2016/8626987
3. Jang J, Hur HG, Byappanahalli MN, Yan T, Ishii S. Environmental *Escherichia coli*: Ecology and public health implications-a review. J Appl Microbiol. 2017; 123(3):570-81. doi: 10.1111/jam.13468. Epub 2017 Jul 3. PMID: 28383815.
4. Wu D, Ding Y, Yao K, Gao W, Wang Y. Antimicrobial Resistance Analysis of Clinical *Escherichia coli* Isolates in Neonatal W. Clinical Trial. 2021. https://doi.org/10.3389/fped.2021.670470
5. Ventola CL. The Antibiotic Resistance Crisis. Pharmacy and Therapeutics. 2015;40(4):277-83.
6. Yassin MT, Mostafa AAF, Al Askar AA. *In vitro* evaluation of biological activities and phytochemical analysis of different solvent extracts of *Punica g*ranatum L. (pomegranate) peels. Plants. 2021;10(12):2742. https://doi.org/10.3390/plants10122742
7. Mphahlele RR, Fawole OA, Makunga NP, Opara U L. Effect of drying on the bioactive compounds, antioxidant, antibacterial and antityrosinase activities of Pomegranate Peel. BMC Complementary and Alternative Medicine. 2016;16(1). https://doi.org/10.1186/s12906-016-1132-y
8. Makarewicz M, Drożdż I, Tarko T, Duda-Chodak A. The Interactions between Polyphenols and Microorganisms, Especially Gut Microbiota. Antioxidants (Basel, Switzerland). 2021; 10(2):188. https://doi.org/10.3390/antiox10020188
9. Peršurić Ž, Saftić Martinović L, Malenica M, Gobin I, Pedisić S, Dragović-Uzelac V, Kraljević Pavelić S. Assessment of the biological activity and phenolic composition of ethanol extracts of pomegranate (*Punica granatum* L.) peels. Molecules. 2020;25(24):5916. https://doi.org/10.3390/molecules25245916
10. Pagliarulo C, De Vito V, Picariello G, Colicchio R, Pastore G, Salvatore P, Volpe MG. Inhibitory effect of pomegranate (*Punica granatum* L.) polyphenol extracts on the bacterial growth and survival of clinical isolates of pathogenic *Staphylococcus aureus* and *Escherichia coli*. Food Chemistry. 2016;190:824-31. https://doi.org/10.1016/j.foodchem.2015.06.028
11. Mohamad Sukri SN, Shameli K, Mei-Theng Wong M, Teow SY, Chew J, Ismail N A. Cytotoxicity and antibacterial activities of plant-mediated synthesized zinc oxide (zno) nanoparticles using *Punica Granatum* (pomegranate) fruit peels extract. Journal of Molecular Structure. 2019;1189:57-65. https://doi.org/10.1016/j.molstruc.2019.04.026
12. Singhal M, Chatterjee S, Kumar A, Syed A, Bahkali AH, Gupta N, Nimesh S. Exploring the antibacterial and antibiofilm efficacy of silver nanoparticles biosynthesized using *Punica granatum* leaves. Molecules. 2021;26(19):5762. https://doi.org/10.3390/molecules26195762

13. Ifeanyichukwu UL, Fayemi OE, Ateba CN. Green synthesis of zinc oxide nanoparticles from pomegranate (*punica granatum*) extracts and characterization of their antibacterial activity. Molecules. 2020;25(19):4521. https://doi.org/10.3390/molecules25194521
14. Da Silva PM, Da Silva BR, De Oliveira Silva JN, De Moura MC, Soares T, Feitosa AP, *et al*. *Punica granatum* sarcotesta lectin (PgTeL) has antibacterial activity and synergistic effects with antibiotics against β-lactamase-producing *Escherichia coli*. International Journal of Biological Macromolecules. 2019;135:931-9. https://doi.org/10.1016/j.ijbiomac.2019.06.011
15. Rosas-Burgos EC, Burgos-Hernández A, Noguera-Artiaga L, Kačániová M, Hernández-García F, Cárdenas-López JL, *et al*. Antimicrobial activity of pomegranate peel extracts as affected by cultivar. Journal of the Science of Food and Agriculture. 2016;97(3):802-10. https://doi.org/10.1002/jsfa.7799
16. Yang Q, Wang L, Gao J, Liu X, Feng Y, Wu Q, *et al*. Tannin-rich fraction from pomegranate rind inhibits quorum sensing in *Chromobacterium violaceum* and biofilm formation in *Escherichia coli*. Foodborne Pathogens and Disease. 2016;13(1):28-35. https://doi.org/10.1089/fpd.2015.2027
17. Kupnik K, Primožič M, Vasić K, Knez Ž, Leitgeb M. A comprehensive study of the antibacterial activity of bioactive juice and extracts from pomegranate (*Punica granatum* L.) peels and seeds. Plants. 2021;10(8):1554. https://doi.org/10.3390/plants10081554
18. Bakkiyaraj D, Nandhini JR, Malathy B, Pandian SK. The anti-biofilm potential of pomegranate (*Punica granatum* L.) extract against human bacterial and fungal pathogens. Biofouling. 2013;29(8):929-37. https://doi.org/10.1080/08927014.2013.820825
19. Khan I, Rahman H, Abd El-Salam NM, Tawab A, Hussain A, Khan TA, *et al*. *Punica granatum* peel extracts: HPLC fractionation and LC MS analysis to quest compounds having activity against multidrug resistant bacteria. BMC Complementary and Alternative Medicine. 2017;17(1). https://doi.org/10.1186/s12906-017-1766-4
20. Elshafie HS, Caputo L, De Martino L, Sakr SH, De Feo V, Camele I. Study of bio-pharmaceutical and antimicrobial properties of pomegranate (*Punica granatum* L.) leathery exocarp extract. Plants. 2021;10(1):153. https://doi.org/10.3390/plants10010153
21. Mahboubi A, Asgarpanah J, Sadaghiyani PN, Faizi M. Total phenolic and flavonoid content and antibacterial activity of *Punica granatum* L. var. *Pleniflora* Flowers (Golnar) against bacterial strains causing foodborne diseases. BMC Complementary and Alternative Medicine. 2015;15(1). https://doi.org/10.1186/s12906-015-0887-x
22. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Current Medicinal Chemistry. 2015;22(1):132-49. https://doi.org/10.2174/0929867321666140916113443
23. Sieniawska Elwira. Activities of Tannins – from *in vitro* Studies to Clinical Trials. Natural Product Communications. 2015;10(11). 1934578X1501001. doi:10.1177/1934578x1501001118

Cite this article: Magbuhat JH, Bool PD, Olit KJR, Sales DRO, Mangalonzo AAL, Montances FEU, Gallardo SG, Salenga GC, Arizala CVD. A Systematic Review on the Antibacterial Activity of *Punica granatum* L. against *E. coli*. Asian J Biol Life Sci. 2022;11(3):631-9.