

Review Article

# Pomegranate as an Antibacterial Agent against Pathogens and at the same Time Advantageous to Beneficial Bacteria: A Review

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## A B S T R A C T

Pomegranate contains several categories of bioactive constituents such as anthocyanins, flavonols, flavanols, isoflavones, flavones, hydrolysable and non-hydrolysable tannins that possess strong antioxidant, antibacterial and anti-inflammatory properties. Researches showed that pomegranate's juice, extracts and isolated compounds are effective against various pathogens, as *Clostridium*, *Escherichia coli*, *Aggregatibacter*, *Acinetobacter*, *Bacillus*, *Helicobacter*, *Porphyromonas*, *Acinetobacter*, *Cryptococcus*, *Citrobacter*, *Alcaligenes*, *Prevotella*, *Bacteroids*, *Cronobacter*, *Achromobacter*, *Prevotella*, *Proteus*, *Enterococcus*, *Yersinia*, *Treponema*, *Serratia*, *Klebsiella*, *Mycobacterium*, *Listeria*, *Pseudomonas*, *Shigella*, *Streptococcus*, *Staphylococcus*, *Salmonella* and *Vibrio* giving results in many cases comparable or even better than the commercial antibiotics. Pathogens are causing several infections with different scale of severity. Pomegranate's phytochemicals possess more than one bioactivities and thus it can treat such health issues by more than one ways, affecting more than one parameters associated to them. For example, in cases of bacteria that are causing inflammation, pomegranate is not only killing the bacteria but also by its anti-inflammatory and antioxidant activity is treating the infections caused by them, achieving faster health rather than just removing the infection's reason (the bacteria). Even pomegranate is bactericidal towards pathogens, it is protecting and enhancing the growth of gut microbiota that is important for health maintenance. Safety tests showed that pomegranate is safe in contrast with antibiotics that are having side effects and are disturbing gut microbiota causing dysbiosis and other disorders indicating that in cases that the antibacterial activity of pomegranate is comparable to antibiotics' activity, it could be considered as a promising alternative antibacterial agent.

**Keywords:** Pomegranate, Phytochemicals, Antioxidant, Antibacterial, Anti-Inflammatory, Pathogens, Beneficial-Bacteria, Health, Probiotics, Antibiotics

## Introduction

Pomegranate, containing several phytochemicals each of them having more than one bioactivities, has various medicinal uses such as antibacterial, antiviral, antifungal, astrigent, styptic, antihelminthic, vermifuge, stimulant, immune modulation and it has been used since ancient years until now in traditional medicines in order to treat cardiovascular diseases, asthma, bronchitis, cough, diabetes, dyspepsia, disendery, diarrhea, malaria, fever, obesity, erectile dysfunction, male infertility, vaginitis, acquired immune deficiency syndrome, ulcers, wounds, bruises, denture stomatitis, bleeding disorders, Alzheimer's disease, prostate cancer, skin lesions, mouth lesions and hypoxic-ischemic brain injury.<sup>1,2,3</sup>

## Pomegranate Phytochemicals

Pomegranate is rich in polyphenols categorised in flavonoids (flavanones, flavones, flavonols, flavanols, isoflavones, anthocyanins), non-flavonoids (phenolic acids, hydroxybenzoic acids and hydroxy-cinnamic acids) and tannins (hydrolysable and non-hydrolysable) which possess strong antioxidant, antimicrobial and anti-inflammatory properties. Every plant part contains different phytochemicals and also same in different concentrations.<sup>4</sup>

The peel that is the 30-40% of the total fruit weight is very rich in phenolics such as gallic acid, gallotannins, ellagitannins, punicalagin, punicallagin, granatin A and B, anthocyanins, rutin, quercetin, catechin, epigallocatechin gallate, pelargonidin, casuarinin, luteolin and prodelphinidin. The juice is rich in phenolics as gallic acid, ellagic acid, anthocyanins, ascorbic acid, rutin, quercetin, catechin, epigallocatechin gallate and also contains amino acids and mineral elements. The seed oil contains punicic acid, fatty acids, ellagic acid and sterols. Ellagitannins as punicalagin and punicalin and flavonols as apigenin and luteolin are present in the leaves. The flowers contain gallic acid, triterpenoids as asiatic, maslinic and ursolic acid. Piperidine alkaloids and ellagitannins are found in roots and barks.<sup>5,6,7,8</sup>

## Antibacterial Properties of Pomegranate

*Salmonella*, a Gram negative pathogen causes a range of clinical diseases that can be categorised in three groups: localised intestinal infection (gastroenteritis), systemic infection of healthy host (typhoid) and systemic infection of immune compromised or selectively sensitive host (non-typhoid salmonellosis).<sup>9</sup> In a research, in vitro and in vivo tests were carried out in order to evaluate the antimicrobial properties of pomegranate peel ethanolic extract against 16 *Salmonella* strains including<sup>6</sup> antibiotic resistant strains. The strains used in the study were *Salmonella dublin* ATCC 39184, (JOL 409), *S. typhi* ATCC 19943 (JOL 380), *S. choleraesuis* ATCC 7001 (JOL 411), *S. derby* ATCC 6960 (JOL 410), *S. gallinarum* ATCC 9184 (JOL 423) and also local

isolates of *S. paratyphi* A (JOL 381), *S. Enteritidis* strains JOL 386, JOL 407, *S. typhimurium* strains JOL 387, JOL 388, JOL 389, JOL 408, *S. gallinarum* strains JOL 419, JOL 420, JOL 421 and JOL 422. The antibiotic resistant strains were JOL 389, towards ampicillin, chloramphenicol, sulfisoxazole, streptomycin, ticarcillin, JOL 411 towards ampicillin and rimethoprim/sulfamethoxazole, JOL 419 towards cephalothin, sulfisoxazole, trimethoprim/sulfamethoxazole, JOL 420 towards cephalothin, nalidixic acid, JOL 421 towards sulfisoxazole, nalidixic acid, streptomycin and JOL 423 towards ampicillin, amoxicillin/clavulanic acid, chloramphenicol, sulfisoxazole and streptomycin. The extract showed very high antibacterial activity against all strains tested including the antibiotic resistant strains. The in vivo tests have shown that inhibition of *S. typhimurium* growth decreased remarkably the mice mortality and that in the extract administrated mice, histological damage or clinical signs of infection rarely were observed while untreated mice had severe histological damage and clinical signs of infection in the tested organs. These results show that the ethanolic extract can efficiently treat salmonellosis. HPLC analysis of the extract showed that it contained punicalagin as main ellagitannin, punicallagin isomers, ellagic acid and gallic acid. It is referred that ellagic acid has antimicrobial properties, gallic acid shows antibacterial activity against intestinal bacteria, yet all above isolated compounds show weak activity against salmonella and this indicates that the high antibacterial activity was because of the presence of metabolic toxins that show antibiotic activity<sup>10</sup> or due to synergistic action among the extract's components.

*Escherichia coli* is an enterobacterium, part of the normal intestinal flora and it is not causing infections unless it goes to another body part as in the abdomen, the lower urinary tract or in the case of neonatals, the brain. Pathogenic *E. coli* can cause stomachic cramps, vomit, fever, nausea, diarrhea, that in some cases can be even fatal.<sup>11</sup> *E. coli* O104:H4, is a heat resistant enteroaggregative strain that can be found in the meat and causes diarrhea or bloody diarrhea. In order to see if high ellagic acid concentration commercial pomegranate could be used in the ground chicken as antibacterial agent against these strains by meat processors, pomegranate powder (70% ellagic acid) by 0.0, 1.0, 2.0 and 3.0 wt/wt% in temperatures 55.0, 57.5, 60.0 and 62.5 oC were tested in a full factorial design and was found that the rich in phenolics pomegranate powder increases the susceptibility of *E. coli* O104:H4 that as heat resistant could remain after heating process in the meat and thus cause infections. Pomegranate powder 1% reduced lethality time by 50% in comparison with the control where no pomegranate powder was added.<sup>12</sup>

Uropathogenic *Escherichia coli* is the main cause of over the 80% of the uncomplicated infections of the urinary

tract.<sup>13</sup> Research showed that exposure of the uropathogenic *Escherichia coli* strain CFT 073 in pomegranate rind extract, results in decrease of the expression of the flagellin gene (fliC) and of the flagelin production and as the flagellin-mediated motility helps bacteria to spread around, the bacteria's swimming and swarming motilities were found to be prevented and thus bacteria did not spread in the upper urinary tract. Pomegranate fruit powder and punicalagin, a hydrolysable tannin, were also evaluated and was found that the pomegranate rind extract showed the highest inhibitory effect on the expression of fliC, yet pomegranate powder and not the rind extract showed the highest inhibitory activity in swimming motility with the inhibitory activity percentage of rind extract, punicalagin and powder to be 50%, 14% and 70% respectively.<sup>14</sup> Even if punicalagin is one of the main pomegranate compounds, these results indicate synergic action among the phytochemicals of the rind extract and of the pomegranate powder so they have significantly higher antimicrobial activity than the isolated punicalagin.

*Staphylococcus epidermidis* is a multidrug resistant gram-positive bacteria that mostly colonizes human skin, is associated with acute and chronic pulpitis, dry socket, pericoronitis, angular stomatitis and periodontitis and also is involved in nosocomial infections.<sup>15</sup> The antibacterial properties of fresh pomegranate juice have been evaluated against 60 strains of *S. epidermidis*, resistant to the antibiotics: ampicillin, ceftriaxone, cephalothin, ceftazidime, ciprofloxacin, gentamicin, ethidium bromide, neomycin, norfloxacin, sulfisoxazole, polymyxin, ofloxacin, oxacillin, vancomycin, tetracycline and tobramycin. The results showed that the juice at 20% had on all the strains that were tested minimum inhibitory concentration equal to 100% something that is attributed to the high polyphenolic content and also to its' antioxidant activity.<sup>16</sup>

*Staphylococcus aureus* is found in the skin flora almost in 1/3 of human population without causing infections, yet depending on the immune system it is possible to infect the skin, the soft tissue in the body, the blood stream and the bones and also is referred as the most frequent reason of post-surgical infections. *Pseudomonas aeruginosa* is a resistant to last resort antibiotics as polymixins and carbapenems hospital pathogen causing to immunocompromised individual sepsis and death. It produces various exoproducts as alkaline protease, elastase, hemolysin, pyocyanin and rhamnolipids associated with pathogenesis and infections and also is referred that can cause denial for lung transplantation.<sup>11,17</sup>

Pomegranate peel and seeds ethanolic extracts were tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa* clinical isolates from Health Centers and was found that both extracts showed very good inhibitory

activity on the bacteria tested, with the peel extract to have stronger antibacterial properties, as in all tests, the minimum inhibitory and minimum bactericidal concentrations were remarkably lower than those in seed extract. The minimum inhibitory concentrations were 12.5 mg/ml for the peel and 25.0 for the seed extract and the minimum bactericidal concentrations were 25.0 mg/ml and 50.0 mg/ml for peel and seed extracts respectively. The main compounds found in both peel and seeds extracts with difference in percentage in favour of the peel extract were gallic acid, catechin, epicatechin, galocatechin, galocatechin-(4,8)-catechin, furfural, heptacosane, ellagic acid, ellagic acid, 3,3'-di-O-methyl, ellagic acid, 3,3', 4'-tri-O-methyl, 5-hydroxymethylfurfural, pyrogallol, ascorbic acid, linoleic acid, gamma-sitosterol, stigmasterol.<sup>18</sup>

Methicillin Resistant *Staphylococcus Aureus* (MRSA) are the strains that became resistant to beta-lactam antibiotics such as cephalosporins and penicillins. As they do not respond to the first line antibiotics, they can cause to people with weakened immune system serious infections that can be even fatal. Pomegranate ethanolic extracts were found to inhibit 35 MRSA hospital isolates with MIC values to be a range of 0.2-0.4 mg/ml. After pomegranate extract application, it is found by scanning electron microscopy that alterations have been caused on the bacterial cell walls.<sup>19</sup>

In a study the antibacterial activity of pomegranate skin methanolic and aqueous extracts has been evaluated against *Staphylococcus aureus* and the *Pseudomonas aeruginosa* and the results were compared to commercial antibiotics. Both, aqueous and methanolic extracts showed good antibacterial properties against both bacteria, with the methanolic extract to give better results. The observed activity against *Staphylococcus aureus* was comparable with cloxacillin's activity and the activity on *Pseudomonas aeruginosa* was similar with gentamycin's, showing that the pomegranate can be considered as a potent antibacterial agent against these bacteria.<sup>20</sup>

In another study, 95% ethanolic pomegranate extract was found in a research to show very strong and comparable activity against *Staphylococcus aureus* with the commercial antibiotics Clindamycin, Chloramphenicol, Vancomycin and Gentamycin.<sup>21</sup>

Furthermore, pomegranate peel extract and Cu(II) ions combination was found to indicate strong antibacterial activity against isolated Methicillin-Sensitive *Staphylococcus Aureus* (MSSA) and Methicillin-Resistant *Staphylococcus Aureus* (MRSA).<sup>22</sup>

In an in vivo experiment C3H/He mice were orally administrated with methanolic extract of pomegranate peel containing ellagic acid, punicalin and punicalagin and after 14 days infection has been induced by *Citrobacter*

*rodentium*, a pathogen that mimics several aspects of the *Escherichia coli* enteropathogenic human infections. Infection with *Citrobacter rodentium* causes strong Th1/Th17 immune response that result in overproduction of the pro-inflammatory interleukin cytokines IL-1 $\beta$ , IL-12, IL-17, IL-22, IL-23, IFN- $\gamma$  and TNF- $\alpha$ , that mean high risk of inflammation, autoimmune disorders, tissue damage, pain, allergies, pathogenesis processes and chronic diseases.<sup>23-27</sup> The results showed that the *C. rodentium* infection induced weight loss and the mortality of mice treated with extract were decreased in comparison with the controls which were treated with water instead of the extract. Has been observed that significant colonization of *C. rodentium* in spleen occurred only in the control group, as in pomegranate group there was a remarkably dose dependent colonization decrease. However, colonization of the bacteria in colon has not been affected despite the pomegranate treatment, but the damage of the colon was less in the pomegranate group.<sup>23</sup>

*Klebsiella pneumoniae* is an enterobacterium found in the digestive tract and it is associated with hospital infections, pneumonia, respiratory and urinary tract infections. In neonatals it can also infect the bloodstream.<sup>11</sup> Pomegranate juice, methanolic and aqueous peel extracts and the isolated pomegranate constituents ellagic acid, quercetin, epigallocatechin-3-gallate (EGCG) and caffeic acid were tested against *Klebsiella pneumoniae* clinical isolates from different sources that were resistant to ampicillin/sulbactam, aztreonam, ceftazidime, chloramphenicol, ceftriaxone, ciprofloxacin, cefuroxime, cefixime, doxycycline, ceftiofur, imipenem, gentamicin, levofloxacin, nitrofurantoin, norfloxacin, meropenem, ofloxacin, piperacillin/tazobactam and tetracycline. All isolated compounds which were dissolved in DMSO (2mg/ml), juice and extracts showed antibacterial activity, with most effective to be the isolated compounds quercetin (MIC 64-256 $\mu$ g/ml), ellagic acid (MIC 64-256 $\mu$ g/ml), EGCG (64-256 $\mu$ g/ml), caffeic acid (128-512 $\mu$ g/ml) than the juice and extracts (mixture of compounds) which gave higher MIC values. These compounds, extracts and juice were also effective against *Mycobacterium tuberculosis* strains resistant to ciprofloxacin, D-cycloserine, isoniazid, kanamycin, rifampicin and streptomycin. The best results were given by isolated compounds with MIC values to be 64-512 $\mu$ g/ml for quercetin, ellagic acid and EGCG and higher for caffeic acid, extracts and juice.<sup>28</sup>

*Streptococcus sanguis* is the main cause of dental plaque formation.<sup>29</sup> It is one of the bacteria that cause Recurrent Aphthous Stomatitis and also it is found to be associated with bacterial endocarditis<sup>30</sup> and gastrointestinal carcinoma.<sup>31</sup> Hydralcoholic pomegranate seed extract (70% ethanol) has been tested on *S. Sanguis* and the values of the Minimum Inhibitory Concentration (MIC) which is the lowest concentration of an antimicrobial agent that

prevents the bacteria's visible growth (bacteriostatic) and the Minimum Bactericidal Concentration (MBC) that is the lowest concentration of the antimicrobial agent that can kill a bacterium into specific conditions. The results showed that the extract inhibited efficiently the growth of *S. Sanguis* with MIC and MBS values to be 500 ppm and 2000 ppm respectively.<sup>32</sup> In an in vivo study, Swiss Webster mice were administered with pomegranate seeds ethanolic extract and has been observed that the extracts showed strong bacteriostatic and bactericidal activity against the bacteria *S. Sanguis* ATCC 10556. In the same study toxicity test has also been carried out and showed that the extract had no toxicity in the dosage needed for the antibacterial activity.<sup>33</sup>

*Cronobacter sakazakii*, an opportunistic pathogen causes severe, even sometimes fatal infections to infants. Pomegranate peel methanolic extract containing in high concentrations the ellagitannins  $\alpha$ - and  $\beta$ - punicalagin followed by punicalin, ellagic acid and its' derivatives has been found to possess bacteriostatic and bactericidal properties against *Cronobacter sakazakii* strains HPB 2855, HPB 2871 and HPB 3290. Significant decrease of the minimum inhibitory and bactericidal concentrations has been observed by reducing the temperature (37, 21, 10oC) and pH (6, 5, 4).<sup>34</sup>

Shigellosis is a common enteric infection including symptoms as fever, nausea, abdominal cramps, dysentery, vomiting, diarrhea or bloody diarrhea and it is caused by *Shigella* bacteria.<sup>35</sup> Pomegranate's ethanolic extracts were found to be highly effective against *Shigella* spp. Its' antibacterial activity was found higher than the antibiotic's chloramphenicol, and lower than trimethoprim's activity. The extracts were also very effective against *Shigella sonnei*. Pomegranate fruit pericarp extracts showed higher antibacterial activity against *Salmonella typhi* in comparison with ampicillin. Pomegranate peel extract was also found very effective against *Vibrio cholerae*, which is causing cholera infections.<sup>19</sup>

In a research, the antibacterial properties of punicalagins, ellagic acid, gallic acid and pomegranate extract were evaluated against the gram-positive intestinal pathogens *Clostridium ramosum*, *Clostridium clostridioforme* and *Clostridium perfringens*. Punicalagins inhibited the growth of all bacteria tested. Ellagic acid was found to inhibit completely the growth of *C. clostridioforme* and *C. perfringens* and the growth of *C. ramosum* by 26% compared to control. Pomegranate extract inhibited *C. ramosum* and *C. perfringens* while the *C. clostridioforme*'s growth was decreased by 60%. Gallic acid decreased not significantly the growth of *C. ramosum* and *C. perfringens*.<sup>36</sup>

The antibacterial properties of three different genotypes of pomegranate 17-67, 19-66 and 19-121 were evaluated against *Escherichia coli* (ATCC 25922), *Salmonella Enteritidis*

(ATCC 13076) and *Staphylococcus aureus* (ATCC 25923) and was found that all the pomegranate genotype extracts showed significant activity against the tested strains. It has also been observed that the genotype 19-66 despite having the highest total phenolic content it showed markedly lowest antibacterial activity than the other genotypes and an explanation that was given for this result was that there is synergistic activity of phenolics with organic acids and when the concentration of organic acids is increased, the antibacterial activity also is increased.<sup>37</sup>

*Acinetobacter baumannii* is a hospital pathogen, resistant to last-resort antibiotics as carbapenems and polymyxins that is responsible for various infections.<sup>11</sup> Pomegranate peel extracts (100 mg/ml) in methanol, ethanol, acetone and water were evaluated as antibacterial against *Acinetobacter baumannii* ATCC 27945, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29243. The aqueous extract had total phenolics  $83.17 \pm 0.003$  mg/g and total flavonoid content  $10.65 \pm 0.003$  mg/g, the ethanolic extract total phenolics  $94.14 \pm 0.02$  mg/g and total flavonoids  $14.48 \pm 0.005$  mg/g, the methanolic total phenolics  $105.6 \pm 0.005$  mg/g and total flavonoids  $20.99 \pm 0.004$  mg/g, the acetonic extract had total phenolics  $130.36 \pm 0.01$  mg/g and total flavonoids  $70.5 \pm 0.01$  mg/g. The inhibition zones of the acetonic, methanolic, ethanolic and aqueous extract against *S. aureus* were 28mm, 22mm, 21mm, and 13mm, result expected due to total phenolics and total flavonoids values for each extract. The inhibition zones against *P. aeruginosa* for aqueous and ethanolic extracts were 24mm, for methanolic 26mm and for acetonic extract were 28 mm. In the *A. Baumannii*'s case, despite the fact that less phytochemicals were extracted in the aqueous extract, the inhibitory zone of the aqueous was the same with this of the methanolic extract, 28mm. The inhibitory zone was 26 mm and 29 mm for ethanolic and acetonic extract respectively.<sup>38</sup> Phytochemicals have different solubility in the various solvents and these results may indicate that the compounds that have bigger solubility in the water, are those who play important role in the *A. Baumannii*'s inhibition or may indicate a possible synergic action among those compounds and other phytochemicals. Further experiments could give more information.

In an in-vitro study, the antibacterial properties of 95% ethanol, acetone and hot water extracts were evaluated against the food related bacteria *Salmonella typhimurium*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. All the extracts showed potent antibacterial properties against all the bacteria tested. Results have also shown bigger susceptibility of the gram-positive bacteria to the pomegranate extracts than the gram-negative.<sup>39</sup>

The antibacterial properties of pomegranate extracts have been evaluated in vitro (agar diffusion) and in situ (food)

against food-borne pathogens. *Yersinia enterocolitica*, the main cause of Yersiniosis, is a zoonotic bacteria causing infections such as gastroenteritis and is related to extraintestinal manifestations too, such as reactive arthritis. Pomegranate peel methanolic extract (80%) was found to be a strong inhibitor of *Yersinia enterocolitica*, *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*.<sup>40</sup>

In a study, pomegranate juice, peel and seeds extracts were found to possess good antibacterial properties against *S. aureus* with the peel extract to show the strongest activity.<sup>41</sup>

Pomegranate peel extracts were found to be very effective in inhibiting in test plates the growth of poultry meat isolated *Pseudomonas stutzeri* strain CTSP36.<sup>42</sup>

The antibacterial properties of pomegranate juice, ellagic acid, gallic acid, punicallagins, punicalins and also of mixture of tannin fractions in H<sub>2</sub>O, BuOH and EtOAc have been evaluated against *Escherichia coli*, *Cryptococcus neoformans*, *Mycobacterium intracellulare*, *Candida albicans*, *Pseudomonas aeruginosa* and methyliccin resistant *Staphylococcus aureus* and was found that all isolated compounds, mixture and juice showed very good antibacterial activity with gallic acid and punicallagins to show the strongest activity against *Cryptococcus neoformans*, *Pseudomonas aeruginosa* and methyliccin resistant *Staphylococcus aureus*.<sup>43</sup>

In order to see if pomegranate peel flour can be used as a natural antimicrobial agent in the food industry, a research has been carried out where HPLC polyphenol profile determined flour was tested against bacteria strains that are responsible for several infections and food poisoning. The strains were *Listeria innocua*, *Listeria monocytogenes*, *Salmonella sp*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Eight phenolic compounds have been identified with the main component to be punicalgalin (16.67 mg/g) followed by ellagic acid (0.12 mg/g). The minimum inhibitory concentrations were between 20 and 50 mg/ml. The antibacterial activity against the strains was *L. innocua* < *P. aeruginosa* < *L. monocytogenes* = *Salmonella sp* = *S. aureus* = *E. coli*.<sup>44</sup>

In a research, the antibacterial activity of pomegranate leaf extracts against standard *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) strains and clinical isolates resistant strains as well has been evaluated by microdilution method and disc diffusion method. The extracts used were aqueous, ethanolic, hexane, ethyl acetate, chloroform and also extract enriched with Total Oligomer Flavonoids (TOF) prepared using an acetone/water mixture to extract the phytochemicals from leaves in which the phenols and flavonoids were in the highest concentration among all extracts and with ellagic acid, gallic acid, luteolin and rutin to be determined by HPLC in

it. It was found that the TOF extract showed the strongest antibacterial activity against every tested strain. The ethanol extract showed antibacterial activity against both standard and resistant *Staphylococcus aureus* and also in the standard *Escherichia coli* strains. The aqueous extract showed antimicrobial activity especially against *Escherichia coli* and *Staphylococcus aureus* standard strains. In the same research, the synergic action between the pomegranate leaf extracts and the antibiotics amoxicillin and cefotaxime was also evaluated with the check board method and was found that amoxilin and TOF extract showed the best synergistic interaction against penicillin resistant E. Coli and penicillin resistant *S. Aureus*.<sup>45</sup>

Ethanol extract of pomegranate flowers with total phenolic content  $40.8 \pm 1.3$  mg gallic acid equivalent/g of extract and total flavonoid content ( $64 \pm 38$  mg quercetin equivalent /g of extract) has been tested against 8 strains of antibiotic resistant *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Shigella spp* and *Klebsiella pneumonia*. The bacteria strains were resistant to 1-12 of the antibiotics: amikacin, ampicillin, chloramphenicol, cephalothin, ceftriaxone, gentamicin, penicillin, tetracycline, azithromycin, cefoxitin, cefotaxime, erythromycin, norfloxacin and methicillin. The results showed a moderate activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* and weaker activity against the other antibiotic resistant bacteria.<sup>46</sup>

The antibacterial properties of pomegranate peel extracts in acetone-water (70%), methanol-water (80%) and ethyl acetate have been evaluated against *Achromobacter denitrificans* CECT 449, *Listeria innocua* CECT 910 and *Algaligenes faecalis* CECT 145, while the polyphenolic profile of each extract has also been determined by HPLC in order to correlate the phytochemicals that mainly were extracted by each solvent with the observed antibacterial activity. The extraction yield was highest in acetone extract by 86.5%. In methanolic extract was 67.5% and in ethyl acetate only 3.8%. All extracts showed very good antibacterial activity against all bacteria except the ethyl acetate extract which was found to be no effective against the *Achromobacter denitrificans* bacteria. The main compound that was identified in acetone and methanol extracts was punicalagin and in the ethyl acetate extract was ellagic acid. Three derivatives of ellagic acid have also been determined. The acetone extract, containing polyphenols in highest concentrations, showed the best antibacterial activity among all extracts.<sup>47</sup> It is referred that one of the reasons that make ellagic acid and also gallic acid to show strong antibacterial properties is their ability to precipitate membrane proteins and inhibit those enzymes which lead to cell lysis.<sup>48</sup>

In a research, pomegranate peel extracts in petroleum ether, methanol and water have been evaluated against the

gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* and the gram-positive bacillus subtilis and *Pseudomonas aeruginosa*. The phytochemicals that were extracted in each solvent were different. In the methanolic extract were found polyphenols, tannins (gallic and catechic), flavonoids and alkaloids. The aqueous extract was containing polyphenols, catechic tannins, flavonoids and Leucoanthocyanes. In the petroleum ether, sterols and polyterpenes were extracted. The antibacterial activities of the extracts and also of the commercial chloramphenicol have been evaluated by agar disk diffusion method. The methanolic extract inhibited significantly the growth of *S.aureus* which was inhibited most, *E. coli* and *P. aeruginosa*. The results were comparable to those of chloramphenicol especially in *P. aeruginosa* bacteria. The aqueous extract was also very effective against *S.aureus*, *E. coli* and *P. aeruginosa*. The best results were given by the methanolic extract obtained by ultra sound extraction. It is also referred that ethanolic pomegranate bark extracts show strong antibacterial properties against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.<sup>49</sup>

Pomegranate leaves' aqueous extracts were found to be very effective against Gram(+) and Gram(-) multidrug resistant strains of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia liquefaciens*, *Citrobacter freundii*, *Proteus mirabilis*, *Staphylococcus epidermidis*, *Streptococcus faecalis* and *Staphylococcus aureus*. The inhibition zones were found to be very large with the diameter in some cases to reach 23.7mm and the MIC and MBC values to be lower than 0.78mg/ml. It is important to be emphasized that the extracts were effective against *Acinetobacter baumannii*, that is a very difficult to treat hospital pathogen causing serious infections such as nosocomial meningitis and pneumonia, resistant to last resort antibiotics as polymyxins and carbapenems.<sup>50-52</sup>

Researches showed that the dried powdered peel exhibit strong antibacterial properties against dental plaque microorganisms.<sup>22</sup> *Rothia dentocariosa* and *streptococcus mutans* Clarke are considered to be the main cause of dental caries. Pomegranate's hydralcoholic peel extract and juice were evaluated against *R. dentocariosa* clinical isolate and *S.mutans* ATCC 25175, and the results showed that the peel extract inhibited sufficiently the survival and growth of both bacteria with MIC value to be  $10 \mu\text{g}/\mu\text{l}$  and MBC  $15 \mu\text{g}/\mu\text{l}$ . The juice inhibited significantly the *S. mutans* with MIC and MBC values to be  $25 \mu\text{g}/\mu\text{l}$  and  $40 \mu\text{g}/\mu\text{l}$  respectively and also showed inhibitory activity on *R. dentocariosa* with MIC and MBC values to be  $20 \mu\text{g}/\mu\text{l}$  and  $140 \mu\text{g}/\mu\text{l}$  respectively.<sup>53</sup> In another study, pomegranate mouthwash was found to be very effective antibacterial agent against the periodontal bacteria *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans* and

*Porphyromonas gingivalis* and against *Helicobacter pylori*, associated to deep periodontal pockets and also gastritis inflammation, chronic inflammation and gastric cancer.<sup>3,1,54</sup>

60 healthy patients aged 9-25 years old used as mouth rinse hydralcoholic pomegranate extract in order to evaluate its' antibacterial activity against dental plaque microorganisms, *E. coli*, *Staphylococcus*, *Proteus*, *Klebsiella* and *Streptococcus species*. Results showed similar efficacy with chlorhexidine, indicating that hydralcoholic pomegranate extract could be used as an alternative dental plaque treatment. The high antibacterial activity is attributed to the punicallagin and ellagitannin fraction.<sup>55,29</sup>

The pomegranate's antibacterial activity against periodontal bacteria is attributed to reactions of its' phytochemicals with sulphhydryl groups, oxidation of enzymes of the bacteria and through more nonspecific reactions with their proteins.<sup>56</sup>

In a study were used mono- and multi-species biofilms in order to evaluate the pomegranate juice effects on the periodontal pathogens' *Treponema denticola*, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* viability. The pomegranate juice decreased remarkably both mono- and multi-species biofilm mass, at all juice concentrations used and all incubation times (1h, 6h and 24h). The best results were obtained in case of *T. denticola* with 25% concentration (optical density of biofilm mass OD  $0.87 \pm 0.08$ ) in case of *A. actinomycetemcomitans* with 50% concentration (OD  $0.22 \pm 0.01$ ), in case of *P.gingivalis* with 100% (OD  $0.34 \pm 0.03$ ) and in case of multispecies film with concentration 50% (OD  $0.09 \pm 0.02$ ).<sup>57</sup>

Gingivitis is a gum's inflammation caused by bacterial plaque biofilms attached on tooth surfaces. 19 Gel containing pomegranate extract has been used for 21 days by chronic gingivitis patients. It was found that the gel use in combination with mechanical debridement resulted in remarkable improvement of plaque index, gingival index and papillary bleeding index.<sup>58</sup>

Biodegradable chips impregnated with pomegranate peel extract and also placebo chips were implanted in patients with periodontitis having gum pocket depths 5-8 mm in order to evaluate the extracts effects. The level of bacterial attachment, plaque indexes, gingival and bleeding were measured and compared to the measurement results after 3 and 6 months. The pomegranate treatment lead after 3 months to remarkable decrease of the pocket depth, bacterial attachment and also plaque decrease compared to the control. Moreover, the inflammatory marker interleukin IL-1beta has been decreased after 3 and 6 months treatment.<sup>19</sup>

In a research, punicallagin was found to enhance the growth of probiotic bacteria, mostly *Lactobacillus* and *Bifidobacterium species*, which seem to be able to treat

sufficiently periodontitis by decreasing the periodontal bacteria and also by strengthening the epithelial barrier function that leads to higher resistance to infections.<sup>56</sup>

*Helicobacter pylori* causes gastritis inflammation. Pomegranate shows good antibacterial activity against *H. Pylori* and kills the bacteria, yet the inflammation it created it remains or could in certain circumstances recur. Pomegranate constituents except bactericidal possess anti-inflammatory properties too and thus it contributes by more than one way- bioactivities to health achievement.<sup>1</sup>

Open wounds for a long time can be a favorable environment for tumor growth. Pathogens in wounds can cause serious infections and delay wound closure.<sup>59</sup> Research showed that 5% methanolic pomegranate peel extract showed significant antibacterial properties against the wound present bacteria such as *Escherichia coli*, *Salmonella anatum*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In an in vivo experiment, ointment containing methanolic peel extract 5% was applied on guinea pigs' wounds for 12 days. The wound closure was markedly enhanced as the extract showed strong antibacterial activity against wound bacteria, and also, the DNA and protein synthesis were increased<sup>19</sup> as the pomegranate contains several phytochemicals with more than one bioactivities leading most of the times to the desirable result by more than one way.

Several researches have also shown that pomegranate peel constituents have very good antibacterial activity against *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Streptococcus salivarius*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella enterica* serovars.<sup>60</sup>

Pharmacokinetic studies showed that the flavanoids naringin and pelargonidin in the peel have antibacterial properties<sup>7</sup>. Pomegranate fruit barks show antibacterial activity against *Bacillus anthracis* and *Vibrio cholerae*.<sup>61</sup>

### **Pomegranate Protecting and Promoting Useful Bacteria**

The gut microbiota is acting as it is a real body's organ, having important metabolic function, stimulating the immune system and thus regulating the inflammatory response, and by the symbiotic interactions among microorganisms and gastrointestinal tract it's helping into the gut homeostasis maintaining. They also inhibit urogenical and intestinal pathogens; growth. *Lactobacillus* bacteria, decrease the risk of urinary tract infections caused by pathogens, prevent and treat gastroenteritis and also with its' presence in vagina protects from vaginosis.<sup>62</sup> *Bifidobacteria* can treat gastrointestinal disorders as diarrhea, reduce Inflammatory Bowel Disease symptoms, inhibits rotavirus that is causing

sporadic diarrhea to neonatals, protects DNA from damage induced by carcinogenesis. It is also found to inhibit genotoxic carcinogen's effect preventing thus colorectal cancer.<sup>63</sup> Imbalance of gut microbiota, dysbiosis, as it happens with every other organ's imbalance leads to diseases, particularly to intestinal and extraintestinal pathogenesis and disorders such as Inflammatory Bowel Disease, allergies, obesity, asthma, cardiovascular diseases, coeliac disease, metabolic syndrome, Irritable Bowel Syndrome, inflammation, psoriasis and also it is associated with colorectal cancer<sup>64, 65, 66, 67</sup> indicating that the protection and enhancement of probiotic gut bacteria are important for the health maintenance.<sup>19</sup>

While pomegranate is inhibiting pathogens' growth, it is promoting the beneficial bacteria's growth. It is found that the pomegranate extracts are promoting the growth of *Lactobacillus* spp, *Bifidobacterium* spp, *Bifidobacterium infantis* and *Bifidobacterium breve*.<sup>19</sup> Pomegranate extract containing in high concentrations oligomers composed by 2-10 repeating units of ellagic acid, glucose and gallic acid in different combinations and also separately punicalagins, all compounds possessing antibacterial properties, were incubated with bacteria from fecal samples in order to find out their effect on gut bacteria growth. The results showed that with the pomegranate extract not only did not reduce the bacteria, but also remarkably enhanced their growth due to the dibenzopyranone-type urolithins production during incubation. Same results are also referred in in-vivo experiments where rats were fed with pomegranate extracts. Punicalagins also protected the gut bacteria but showed lower effect on their growth in the concentration used in the experiment.<sup>68</sup>

In a research, pomegranate juice has been tested in order to find out the way it is affecting the growth of the beneficial bacteria *Bifidobacterium animalis*, *Lactobacillus lactis* spp, *Lactobacillus casei* and also the growth of 4 strains of the multidrug resistant bacteria *Clostridium difficile* hypervirulent NAP1/027/BI which causes epidemic diarrhea, severe watery diarrhea, pseudomembranous colitis and also producing enterotoxins is responsible for damages in the target cells' actin that lead to inflammation, neutrophil infiltration, necrosis of epithelial cells, damages of the tight joints of epithelial cells, vascular permeability and hemorrhage. Results showed that the pomegranate juice inhibited the growth of the multidrug resistant *C. Difficile* with MIC and MBC values to be 309 µg/ml and 780 µg/ml respectively for all 4 strains tested while the growth of the beneficial bacteria was not affected.<sup>69, 70</sup>

In another study, the effect of pomegranate juice and pomegranate extract on the growth of the beneficial intestinal Lactobacilli and Bifidobacteria and also on the pathogens Clostridia, *Bacteroides fragilis* and Enterobacteriaceae

has been determined. Both pomegranate extract and juice stimulated the growth of the beneficial bacteria *Lactobacillus* and *Bifidobacterium*, while remarkable dose dependent growth inhibition of the pathogens has been observed.<sup>71</sup>

The antibacterial activity of HPLC determined pomegranate gel containing 0.234% punicalagin has been evaluated against the pathogens *Streptococcus sanguinis* and *Streptococcus mutans* and the beneficial *Lactobacillus casei* and was found that the pathogens were sufficiently inhibited having MBC 250 and 125 mg/ml and Total Kill Assay (TKA) 6 and 1 hour for *S. mutans* and *S. sanguis* respectively. Other studies have shown that pomegranate plant part extracts are inhibiting *S. mutans* sufficiently, with the results to be comparable with those of chlorhexidine.<sup>72</sup>

In an experiment, the probiotic *Lactobacillus casei* shirota was not affected by pomegranate peel ethanolic extract while pathogens were inhibited at a concentration range 25-100 mg/ml in the following order: *Escherichia coli* NCIM2065 < *Escherichia coli* < *Staphylococcus aureus* NCIM2079 ≤ *Proteus mirabilis* < *Pseudomonas aeruginosa* ≤ *Salmonella typhi* para B < *Staphylococcus aureus* < *Salmonella typhi* < *Shigella flexneri*.<sup>73</sup>

### Antibiotics' Side Effects

Antibiotics can affect the gut microbiota and cause several serious long-term consequences such as reduced microbial diversity, enhancement of the opportunistic pathogens *Klebsiella* spp, *Escherichia* spp and *salmonella typhimurium*, overgrowth of the difficult to treat *Clostridium difficile* and modulation of the ratio Bacteroidetes/Firmicutes.<sup>64, 74</sup>

Side effects that are mentioned to be caused by antibiotics are gastrointestinal discomfort, stomach pain, diarrhea, nausea, vomiting, rashes and fever, allergic reactions, arthropathies, anemia. They are mentioned to affect the liver, kidneys and ear functions.<sup>75</sup> Heart rate effects are also referred.<sup>76</sup> Bactericidal antibiotics are found to promote the formation of reactive oxygen species as ROS and induce alterations to the cellular redox state<sup>77</sup> that could increase the risk of the diseases associated to the oxidative stress.

### Pomegranate Safety

Several experiments that have been carried out to test pomegranate's safety showed that the pomegranate juice and extracts are safe at the dosages that have been used in traditional medicine. It has been found that 600 mg/kg body weight did not cause any side effects. After 90 days treatment, there were not any treatment related gross or histopathology findings.<sup>2, 78- 84</sup>

### Discussion

Pomegranate, rich in flavones, flavanols, flavonols, isoflavones, anthocyanins, hydrolysable and non-

hydrolysable tannins, possesses very strong antioxidant, antibacterial and anti-inflammatory properties and is effective against strains of several pathogens such as *Escherichia coli*, *Salmonella*, *Clostridium*, *Staphylococcus*, *Streptococcus*, *Aggregatibacter*, *Citrobacter*, *Pseudomonas*, *Lysteria*, *Shigella*, *Acinetobacter*, *Cryptococcus*, *Enterococcus*, *Cronobacter*, *Yersinia*, *Klebsiella*, *Achromobacter*, *Prevotella*, *Alcaligenes*, *Mycobacterium*, *Serratia*, *Proteus*, *Prevotella*, *Porphyromonas*, *Helicobacter*, *Bacteroids*, *Bacillus*, *Treponema*, *Vibrio*, that are causing infections with severity to vary from mild to even life threatening.

The beneficial bacteria on the other hand are enhancing health and contribute in its maintenance. Disturbed gut microbiota, dysbiosis, can lead into many intestinal and extraintestinal disorders.

Pomegranate juice, plant part extracts and isolated compounds as well, were found to be potent antibacterial agents against pathogens, in several cases giving results comparable or even better than commercial medicines while at the same time they are protecting and enhancing the growth of beneficial bacteria. It is important to be mentioned that no side effects have been observed at the safety tests that have been carried out for the pomegranate while antibiotics have various side effects that several times are serious.

Moreover, pomegranate not only protects the beneficial for the health bacteria while inhibiting the pathogens' growth but as it contains various phytochemicals each one to possess more than one bioactivity it is achieving the health maintenance affecting more than one parameter involved in that health issue.

Free radicals and oxidative stress can cause or enhance an already existing inflammation. Pomegranate's constituents are strong antioxidants and also possess anti-inflammatory properties and thus in cases where bacteria cause inflammation, the one action towards healing and health achieving is removing-killing the bacteria that cause the inflammation and then by anti-inflammatory and antioxidant properties the inflammation can also be inhibited. The reason of the whole health issue were the bacteria, but the inflammation itself is a problem too that should be treated, and this is also achieved, as the pomegranates phytochemicals have apart from antibacterial, antioxidant and anti-inflammatory properties too.

An example where more than one bioactivity is involved in order to achieve health is *Helicobacter pylori* that causes gastritis inflammation. Pomegranate as it possesses antibacterial activity, it kills the bacteria, but also treats the inflammation, the remaining problem that bacteria left as it has strong anti-inflammatory properties too and thus the healing process is enhanced.

Besides, pomegranate is beneficial to periodontitis treatment by more than one way, as it enhances the probiotic bacteria that decrease periodontal pathogens in the oral biofilm and the same time strengthening the epithelial barrier function, contributing thus by more than one ways into reducing periodontitis symptoms.

Except for the multiple ways of treating the health issue the bacteria created which is an overall treating, leading many times to health achievement rather than just removing the factor that created the infection (bacteria), but not treat or even create new health issues (new infections, inflammation, diseases), pomegranate is offering many other benefits to human body organs, while antibiotics may by side effects cause damages to them.

For example, bactericidal antibiotics promote the formation of Reactive Oxygen Species (ROS) that can lead to oxidative stress that is the reason of several diseases. Pomegranates' constituents are antioxidant too except for potent antibacterials and thus the pomegranate use as antibacterial is improving the antioxidant status of the patient, while the antibiotic's use is harming it. Antibiotics can cause gastrointestinal discomfort stomach ulcers cramps and pain while pomegranate is gastroprotective,<sup>1</sup> can cause allergies while pomegranate has antiallergic properties,<sup>85</sup> they can cause fever while pomegranate has been used since ancient years to treat fever,<sup>3</sup> they can affect the liver and kidneys while pomegranate is hepatoprotective and is protecting kidneys<sup>86</sup> and they also may affect heart rate while pomegranate offer benefits to the cardiovascular health.<sup>6</sup>

It is important to emphasize that pomegranate was found effective even against multidrug resistant bacteria, and as it is protecting and also enhancing the growth of beneficial bacteria without side effects as antibiotics do and moreover is offering various benefits to the general health, could be considered as a promising alternative antibacterial agent. Further researches and clinical studies can provide us with important information on the bactericidal activity of pomegranate constituents and the biochemical paths they are involved as isolated compounds or as mixture as they exist in the juice and extracts, where synergic activity may occur.

## Conclusion

Pomegranate is found to possess strong antibacterial properties against pathogens and to be sufficient even against multidrug resistant pathogens' strains, with results in many cases comparable and even better than those of commercial medicines. In contrast with antibiotics that disturb the beneficial gut microbiota which is important for the health maintenance, and also they can cause by side effects severe diseases and disorders, the pomegranate is

protecting and also enhancing the gut microbiota not only without having any side effects, but also as it possesses various categories of phytochemicals with more than one bioactivities it offers multiple benefits to the human health, approaching more than one parameters involved to the respective health issue and contributing by more than one ways to the treating and health achievement. In the cases where results of pomegranate and antibiotics are comparable, pomegranate could be considered as a preferable potential alternative antibacterial agent.

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