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Urolithins and their antimicrobial activity: A short review

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Abstract

In the last few decades, the rate of the production of new antibiotic has declined significantly. This is mainly due to the high costs needed for both research and development processes. On the other hand, antibacterial resistance developed by bacteria against the already present antibiotics has been increasing extensively. Thus, finding alternatives to synthesize new antimicrobial molecules is now a priority to fight against resistant bacteria. One of these alternatives that can be used as precursors for new antimicrobial molecules is secondary metabolites. Ellagitannins, abundantly found in walnut, pomegranate, and berries, are known as precursors of ellagic acid which possess antimicrobial, anticancer, and antioxidant activities. Ellagic acid is metabolized in mammalian gastrointestinal system via gut microbiota to form dibenzo [b, d] pyran-6-one metabolites, which are known as urolithins. Urolithins are the metabolites of ellagic acid which are responsible for its biological activities. There are many types of urolithins such as urolithin A, urolithin B, urolithin C and urolithin D that were detected in mammalian gastrointestinal tract. Urolithins were shown to possess antimicrobial activity against bacteria, viruses and fungi. In this article, it was aimed to review the antimicrobial activities of various natural and synthetic urolithins concomitant to their chemistry.

Keywords

Antimicrobial activity, ellagic acid, ellagitannins, gut microbiota, urolithins.

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INTRODUCTION

The discovery of antibiotics was one of the greatest achievements in the history of medicine (Gaynes, 2017). However, nowadays, the spread of antibiotic-resistant pathogens has become a major public health problem. The period in between 1960 and 1980 is known as the golden age of the antibiotic discovery because majority of the antibiotics which are currently available was discovered during this period. However, during the 21st century, the antibiotic drug discovery could not continue at the same frequency as the golden age. Famous pharmaceutical companies such as; Novartis, AstraZeneca, Sanofi, Bristol-Myers Squibb, and Allergan started to drop their antibiotic researches and turning away from any participation in the development of new antibiotics. The reason of this turning away can be estimated as economic because the costs needed for both research and development process, as well as the organization of clinical trials carries a big financial risk irrespective of the drug candidate. Moreover, antibacterial drugs can only offer modest returns in investments compared to other classes of drugs. Morever, the increase in the rate of antibiotic resistant bacteria resulted in the difficulty of the treatment of the infectious diseases (Junaid *et al.,* 2018; Gajdács, 2019).

Secondary metabolites are low molecular weight molecules that can be extracted from various plant species with numerous pharmaceutical activities. These phytochemicals turned out to be an important area for research due to their abilities to offer larger scale structural diversities and less adverse effects those of synthetic compounds. This direction toward phytochemicals led to the discovery of new sources of antimicrobials, which is essential to overcome the constant evolution of the microorganisms' resistance against existing antimicrobials (Simões *et al.,* 2009).

There are various mechanisms that can result in the development of antibiotic resistance mechanisms. These mechanisms include the modification of the antibiotics by chemical alterations of the antibiotic, destruction of the antibiotic molecule, decrease in the penetration of antibiotic via the change in the permeability, efflux of the antibiotics by cellular pumps, change in the target sites by the protection of targets, modification/mutation (Smith, 2017).

One of the phytochemicals which possess obvious antimicrobial activity is polyphenols or phenolic compounds that include ellagitannins (Figure 1). Ellagitannins are complex chemical structures which are able to release hexahydroxydiphenoic acid (HHDP), the

precursor of ellagic acid, by spontaneous lactonization. Ellagitannins exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. Morever, they have beneficial effects on health and protective effects against various chronic cardiovascular diseases (Clifford, 2000).

Ellagitannins and ellagic acid in nature

Ellagitannins and ellagic acid are known as polyphenolic structures which are present in some seeds, fruits, and nuts such as; walnuts, almonds, pomegranates, strawberries, and black raspberries. Thus, ellagitannins and ellagic acid are present in daily human dietary intake with various beneficial activities. Studies related with their metabolism within body were performed to understand their mechanism of action (Landete, 2011). Ellagic acid is converted to urolithins in the gastrointestinal tract via gut microflora by the conversion of free ellagic acid to dimethylated ellagic acid glucuronide, which is then converted via colon microbiota into hydroxy derivatives of dibenzopyran-6H-6- one, which are known as urolithins (Tomás-Barberan *et al.*, 2009). Urolithins are chemically known as dibenzo [b, d] pyran-6-ones, or 3,4-benzocoumarins, dibenzo-α-pyrones, and benzo[c]chromen-6-ones. These compounds were initially isolated from natural sources followed by sheep renal calculus and called urolithin A, and urolithin B. Urolithins are produced within the gastrointestinal tract via intestinal microbiota through the opening and decarboxylation of one of the two lactones in ellagic acid and subsequent removal of hydroxyls from various positions. Decarboxylation of ellagic acid forms the first metabolite which is urolithin M-5 that can be metabolized to urolithin D, and urolithin M-6 by the removal of a hydroxyl group from different positions. Urolithin C and urolithin M-7 can be formed by removal of second phenol hydroxyl, whereas urolithin A and isourolithin A are formed via removal of third hydroxyl. On the other hand, urolithin B, and isourolithin B were detected as a result of dehydroxylation of urolithin A, and isourolithin A, respectively (Figure 2). According to the number of hydroxyl groups on the structure, urolithins are categorized into pentahydroxyurolithin as urolithin M-5, tetrahydroxyurolithin as urolithin D, and urolithin M-6, trihydroxyurolithins as urolithin C, and urolithin M-7, dihydroxyurolithins as urolithin A, and isourolithin A, and monohydroxyurolithin as urolithin B, and isourolithin B (Garazd and Garazd 2016).

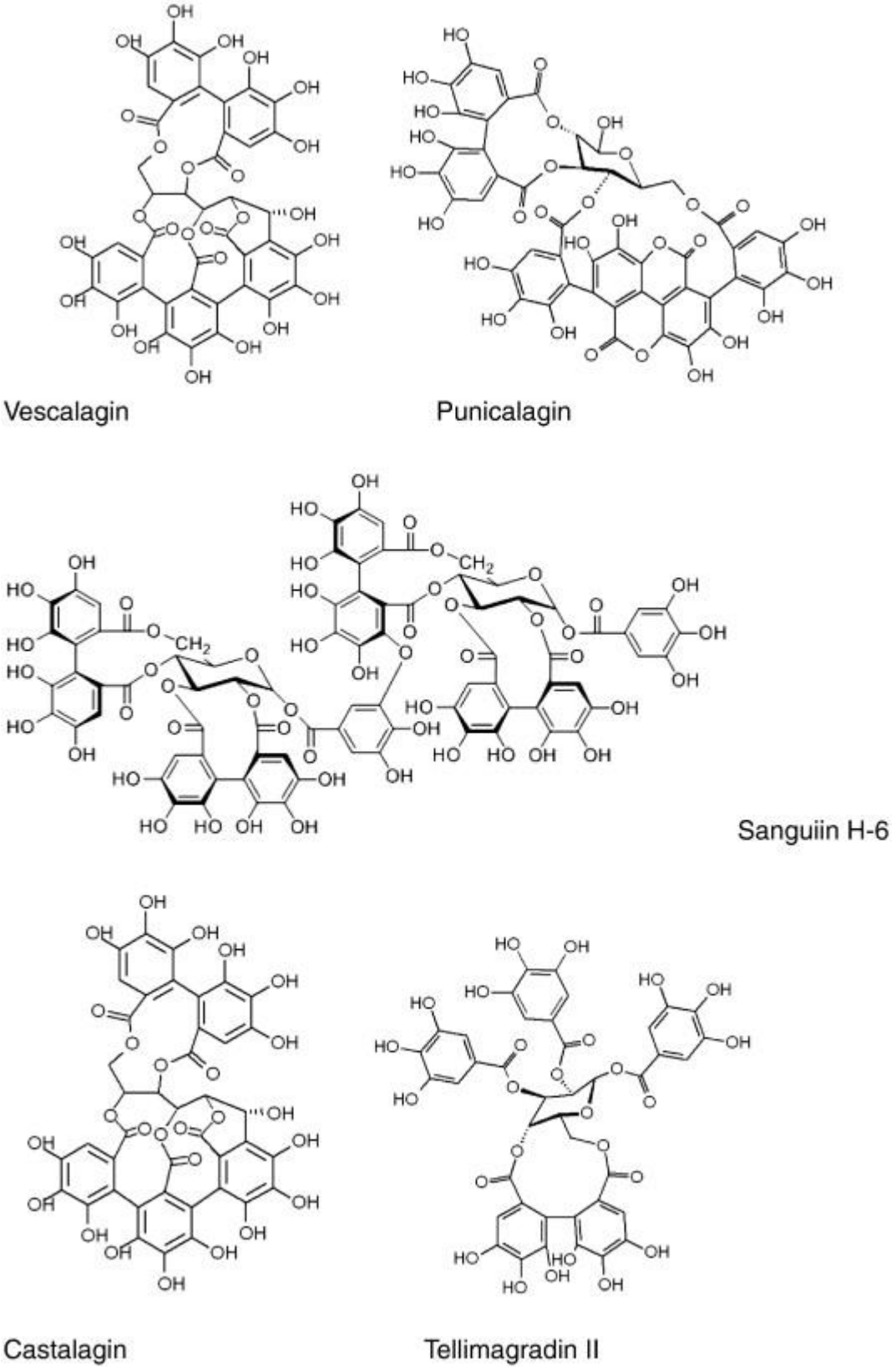

Figure 1: The main structure of ellagitannins (Landete, 2011).

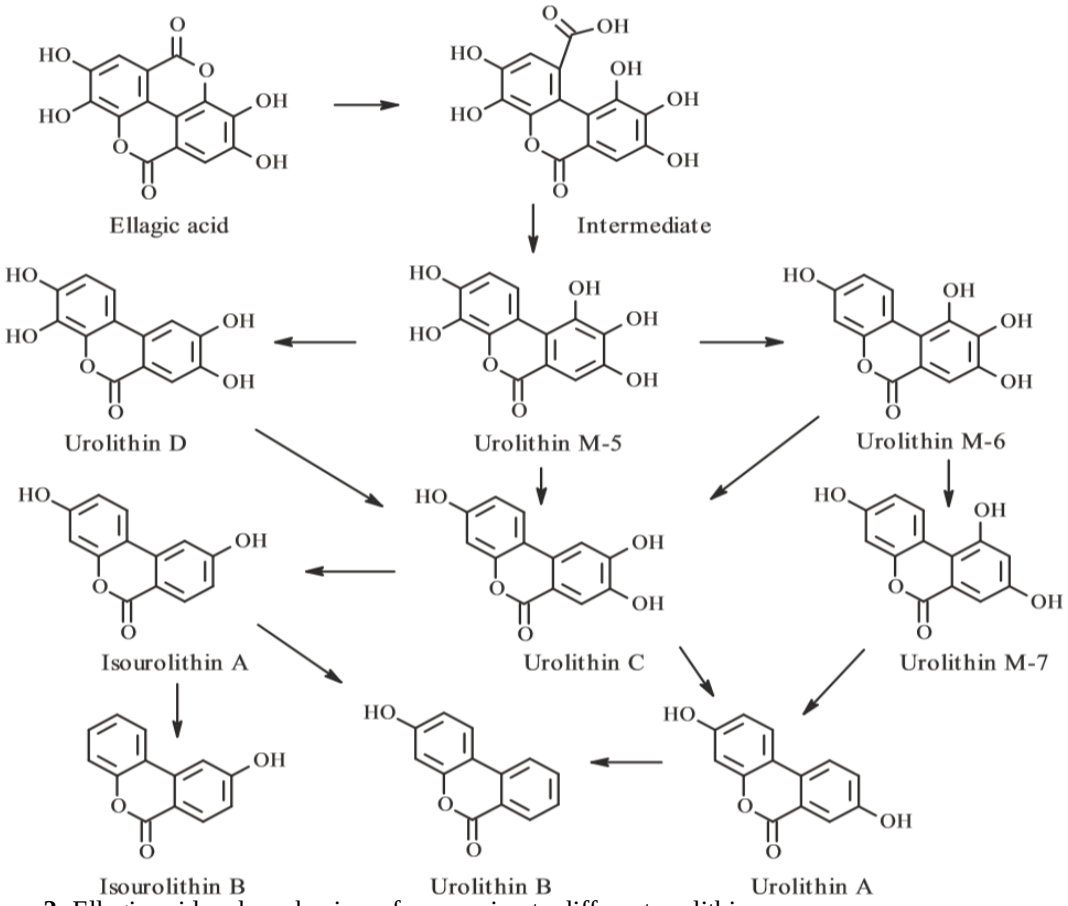

Figure 2: Ellagic acid and mechanism of conversion to different urolithins.

Urolithin production in different mammalian species

The production of urolithin from ellagitannins has been reported in different animal species. In the rumen of ruminants such as; cattle and sheep, the most observable urolithin derivatives are isourolithin A, and urolithin B. On the other hand, urolithin A was the most frequently detected urolithin derivative in the intestine. In monogastric animals such as; rat, mouse, and pig, the main urolithin derivative is urolithin A, its glucuronide, and sulfate conjugates, followed by urolithin B, urolithin C, and isourolithin A. Human being shows the same behavior as monogastric animals after eating ellagitannin-containing foods. These results are reflection of the fact that generally mammals can produce urolithins after ellagitannin-containing food intake. However, there are inter-individual variations because of the difference in the composition of gut microflora (Espín *et al.*, 2013).

Pharmacokinetic studies

In vitro studies indicate that ellagitannins show high stability under the acidic conditions of the gastric environment. Moreover, they are stable in the presence of

gastric enzymes such as pepsin, rennin and gastric lipase without any degradation or hydrolysis to free ellagic acid. Ellagitannins can be absorbed none or little amounts in the stomach can be absorbed because of their complex structures. Similarly, free ellagic acid molecules can be absorbed at very low percentage. During the following stages of digestion, ellagitannins and ellagic acid are metabolized via the intestinal microbiota to urolithin derivatives; especially to urolithin A and urolithin B. Afterwards, they are absorbed through the intestinal epithelia and undergo glucuronidation in liver (Lipińska *et al.*, 2014). Metabolism studies of urolithins showed that urolithin tends to undergo particularly phase II conjugation reactions forming glucuronide and the sulfate metabolites. Moreover, methyl ether metabolites catalyzed by catechol-O-methyl transferase (COMT) enzyme are observed (Yuzugulen *et al.,* 2019).

Antimicrobial activity of urolithins

Ellagitannins and ellagic acid have been investigated in many studies in order to understand their antimicrobial activities. These compunds exhibit antibacterial activities against both Gram positive and Gram negative bacteria, some viruses and fungi. It was shown that ellagic acid bears antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and some clostridia species (Bialonska et al., 2010). Moreover, dose-dependent antimicrobial activity of urolithins against *Helicobacter pylori* isolated from a peptic ulcer patients was observed (Chung, 1998).

It was reported that ellagitannin extracts can inhibit the growth of *Vibrio cholera*, *Shigella dysenteriae*, and *Campylobacter* species (Scalbert, 1991). In a study that was conducted on *Yersinia enterocolitica*, the antibacterial activity of urolithin A, and urolithin B were investigated related to their anti-Qorum Sensing (QS) effects. As a result, it was reported that urolithin A and B possess antimicrobial effect at the concentration of 4 μM via three mechanisms which are growth inhibition, a significant reduction in biofilm biomass, and a notable reduction in the bacterial motility. These effects were found to occur due to the reduction of the level of acylated homoserine lactone (AHL) autoinducers, especially 3-oxo-C6-HSL and C6-HSL, which are essential for lactone and flagella synthesis (Giménez-Bastida *et al.*, 2012). It was also reported that ellagitannins have bactericidal effect against antibiotic-resistant bacteria such as; methicillin-resistant *S. aureus* (MRSA) and carbapenem-resistant *Acinetobacter baumanii*. Inhibitory effects on the growth of some fungi such as; *Candida albicans* and *Cryptococcus neoformans* were also reported (Yoshida *et al.*, 2009).

Urolithins were also reported to have potent antibacterial effect against *Bacillus subtilis*, *E. coli*, *Bacillus cereus* and *Bacillus polymyxa* with minimum inhibitory concentration (MIC) of 20 ppm. Moreover, these compounds were also shown to exhibit strong antibacterial activities against *Salmonella* Paratyphi, *Salmonella* Choleraesuis, and *Salmonella* Enteritidis with MICs of 20 ppm, 10 ppm and 15 ppm, respectively (Hayriye, 2011).

CONCLUSION

Finding new alternatives of secondary metabolites is a priority nowadays because they can be used as precursors for the design of new antibiotic agents. Ellagitannins and ellagic acid have been shown to possess antimicrobial activity in many studies. Clarifying the mechanism of the antimicrobial activity is essential. Urolithins, which are produced via gut microbiota, have been demonstrated to be one of the main metabolites of ellagic acid in the gastrointestinal system of mammals. There are several types of urolithins such as; urolithin A, urolithin B, urolithin C, urolithin M5, urolithin M6, and urolithin M7. Especially urolithin A and urolithin B were reported to possess antimicrobial activity against *Vibrio cholera*, *S. dysenteriae*, *Campylobacter* species, MRSA and carbapenem-resistant *A. baumanii* via inhibiting QS system, a communication system that is essential for the virulence of bacteria.

As a conclusion, urolithin A and B are thought to be attractive precursors for the discovery of new antibacterial agents.

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