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The metabolites of ellagitannin metabolism urolithins display various biological activities

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Abstract

Dietary consumption to various nuts, berries, and particularly pomegranate is an important source of ellagitannins. These molecules are particularly subject to gastrointestinal metabolism producing urolithins as their metabolites. Urolithins (i.e., hydroxylated benzo[c]chromen-6-one analogues) have a greater absorption than the ellagitannins thus; greater bioavailability is of great significance. Therefore, the biological activities obtained through the use of ellagitannin rich foods are mainly attributed to urolithins. These compounds possess a good peripheral distribution. In addition, some of their further metabolites can penetrate to the central nervous system which, is of a topic of interest for CNS related pathologies. This review has aimed to introduce the structure and metabolism related formation of different urolithins concomitant to their biological activities discovered so far in the literature.

Keywords

Antioxidant, anticancer, antimalarial, anti-inflammatory, cholinesterases, ellagitannins, metabolism, urolithins.

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INTRODUCTION

Ellagitannins are present in a large number of dietary sources (Clifford *et al*., 2000; Abe *et al*., 2010). Nuts, berries, and pomegranate are rich sources of ellagitannins (Garcia-Villalba *et al*., 2015). So far, numerous studies have been conducted to investigate nutraceutical potential and biological actions of ellagitannin rich foods (Okuda *et al*., 1989; Lipińska *et al*., 2014). Some of these studies have mainly exploited different extracts of related plants, particularly the fruits.

Ellagitannin is a macromolecule condensed with glucose units and upon metabolism releases ellagic acid (Quideau *et al*., 1996; Seeram *et al*., 2006). Regarding the nature of the chemical composition of ellagitannin, it is quite difficult to attribute the resulting biological actions for such a big molecule (González-Barrio *et al*., 2010). Indeed, metabolism studies have shown that ellagitannins are subject to gastrointestinal system metabolism yielding out the disintegration of sugar units to produce ellagic acid (Seeram *et al*., 2004). It is known that ellagic acid has almost no absorption potential (Lei *et al*., 2003). In other words, it has negligible bioavailability. From this perspective, it is very difficult to relate the biological actions of ellagitannin rich food to ellagitannins and ellagic acid.

So far, numerous studies have been conducted for the investigation of the metabolism of ellagitannins and ellagic acid in various living things including mammalian and non-mammalian species. These studies point out an ellagitannin initiated metabolism cascade that leads to the microbiota dependent formation of urolithins in the gastrointestinal tract (Tomas-Barberan *et al*., 2014; Landete, 2011; Selma *et al*., 2014; García-Villalba *et al*., 2013).

Urolithins are hydroxylated benzo[c]chromen-6-one derivatives (Figure 1). Regarding the metabolism pathway, poly-hydroxylated urolithins are produced first, and then they are further subject to produce less hydroxylated metabolites ending up with the main compounds such as urolithin A (i.e., 3,8-dihydroxy-6H-benzo[c]chromen-6-one) and urolithin B (i.e., 3-hydroxy-6H-benzo[c]chromen-6-one) (Giménez-Bastida *et al*., 2012; Zhao *et al*., 2018). This cascade, although it may vary on the amount and the type depending on the metabolism differences among living things, is consistent including in humankind (Bialonska *et al*., 2010). Since urolithin A and B are the major

metabolites found in systemic circulation, they are considered as biomarkers of ellagitannins (Cerdá *et al*., 2005; Tomas-Barberan *et al*., 2018).

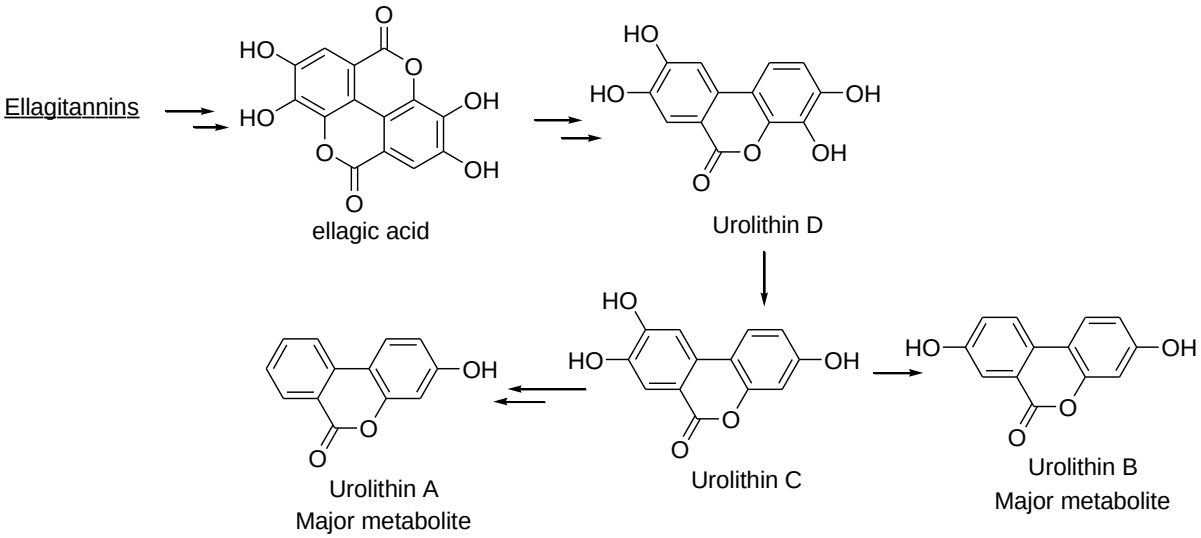


Figure 1: The formation of major urolithins through gastrointestinal tract metabolism reactions.

Metabolism studies have also pointed out that urolithin also tends to undergo further metabolism reactions, particularly phase II conjugation reactions (González-Sarrías *et al* 2014; Piwowarski *et al*., 2017; Pfundstein *et al*., 2014; González-Sarrías *et al*., 2017) (Figure 2).

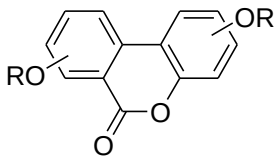


Figure 2: The phase II conjugates of urolithins.

The glucuronide and the sulfate metabolite formation through the hydroxyl groups are very common. Thus, it is not surprising to find urolithin in feces and urine. In addition, catechol-O-methyl transferase (COMT) catalyzed reactions are also observed and subsequently, methyl ether metabolites are observed within the central nervous system (Espín *et al*., 2013; Sala *et al*., 2015).

Regarding these basic features, it has been of interest for the identification of biological activities of urolithins and their metabolites for the last two decades. Therefore, the aim of this review is to focus on the key concepts of urolithins, with an emphasis on their reported biological effects. Some of these beneficial effects include antioxidant, anti-

inflammatory, anticancer, and antimicrobial effects.

Antioxidant activity

Poly-hydroxylated phenols, also considered as natural phenols found abundant in nature, are known to act as antioxidant compounds. They have been shown to be involved particularly in the prevention of certain diseases, mainly including metabolism disorders and central nervous system diseases (Scalbert *et al*., 2005; Manach *et al*., 2004). Although their mechanism of action is not proven, metal chelation and radical scavenging activities have been linked to their antioxidant activity (Hadi *et al*., 2007; Eghbaliferiz and Iranshahi, 2016). Recent findings have also pointed the significance of sulfate and glucuronide conjugates, even having function (Heleno *et al*., 2015). Since, urolithins are also hydroxylated phenolic compounds, they have been shown to act as antioxidants in various antioxidant assay systems (Bialonska *et al*., 2009; Kallio *et al*., 2013). In an earlier study by Cerdá *et al.* (2004), when urolithins were compared to ellagitannins they were reported as poorer antioxidants. It is noteworthy to mention that, radical scavenging activities have been linked to the number of hydroxyl groups. Therefore, the major urolithins (i.e., urolithin A and B) that contain only one and two hydroxyl groups, respectively are poorer antioxidants when compared to a greater number of hydroxyl groups present in urolithin C and D (Bialonska *et al*., 2009). As implied previously, the last studies on urolithins indicated the possible physiological roles of glucuronide and sulfate conjugates of urolithins.

Anti-inflammatory activity

Several studies have been conducted to evaluate the anti-inflammatory effects of urolithins on the gastrointestinal system upon the use of pomegranate juice or extract (Larrosa *et al*., 2010; Espín *et al*., 2013). Although there is no mechanistic study indicating the role of urolithins on some important inflammatory cascades, including the arachidonic acid pathway derived formation of prostaglandins, there are only a few research studies which examined the level of some inflammatory responses upon the use of urolithins. It is important to note that urolithin A has been found to be an inhibitor on the activation of nuclear factor kappa b and mitogen activated protein kinase (González-Sarrías *et al*., 2010). Moreover, it was also shown that both urolithin A and B have the potential to down-regulate the expressions of inflammation markers; COX-2 and prostaglandin E synthase (Larrosa *et al*., 2010; González-Sarrías *et al*., 2010).

Inflammation of the blood vessel wall plays a role in the development of atherosclerosis. Urolithin A glucuronide

conjugate was found to down-regulate chemokine ligand 2 and plasminogen activator inhibitor 1 thereby, inhibiting monocyte adhesion to endothelial cells (Giménez-Bastida *et al*., 2012).

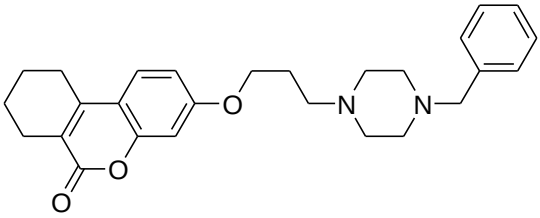
Anti-cancer activity

Anticancer activities of urolithins have been tested in various assay systems. Particularly, they were found to inhibit cancer-cell proliferation in colon, kidney, prostate, liver, breast, and bladder cancer cell lines (Tomás-Barberán *et al*., 2017). The mechanism is mainly associated with the blockage of cell cycle and the induction of apoptosis. However, it is noteworthy to state that these studies do not cover each urolithin and urolithin metabolite produced through metabolism. Furthermore, the dose used in these studies is also a topic of debate regarding the actual concentrations of urolithins found upon ellagitannin exposure.

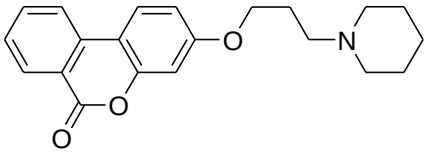
Cholinesterase inhibitory activity

Emerging role of polyphenols in neurodegenerative disorders, particularly in Alzheimer's Disease, has also been studied using pomegranate juice. In this concept, it is already known that phenolic compounds (such as ellagitannins) generally have poor potential to penetrate through blood-brain barrier. Therefore, their basic effect within the central nervous system is a question that remains to be clarified. Using in silico computational methods, Ahmed *et al.* (2014) have reported that urolithins, particularly methylated urolithin A and B, have shown possible penetration. From this point of view, methyl ether derivatives or methyl ether urolithin derived novel metabolite formation in central nervous system might be responsible for the effects within the central nervous system (Ahmed *et al.*, 2014; Yuan *et al*., 2015).

Our previous findings with urolithin B have indicated the low potential of this compound to inhibit cholinesterase enzymes A and B (i.e., around 50 µM IC_{50}) (Gulcan *et al*., 2014). The compound was shown to be more selective for acetylcholinesterase (Norouzbahari *et al*., 2018, Gulcan *et al*., 2014). Urolithins are more successful cholinesterase inhibitors, possessing low IC_{50}s. Some of these examples are shown in Figure 3.



0,9 and 3,1μM IC_{50}s, respectively for acetylcholinesterase and butyrylcholinesterase (Gulcan, HO et al 2014)



2,1 and 11,5μM IC_{50}s, respectively for acetylcholinesterase and butyrylcholinesterase (Gulcan, HO et al 2018)

Figure 3: Representative cholinesterase inhibitors derived from urolithins.

Antimalarial activity

As folk medicine, dried *Punica granatum* rinds (i.e., a source of ellagitannins and punicalagins) have long been used to treat malaria (Dell'Agli *et al.*, 2010). Recent studies have indicated that urolithins act on MMP-9 which is a proteolytic enzyme that degrades matrix proteins and associated in the pathogenesis of malaria. MMP-9 was shown to be up-regulated in haemozoin (malarial pigment) or trophozoite-fed human monocytes (Prato *et al.*, 2008). Urolithin A and B inhibited the release and expression of MMP-9, pointing out the significance of urolithins as active constituents in the traditional treatment of malaria (Dell'Agli *et al.*, 2010).

CONCLUSION

It is apparent that the research on urolithins is relatively new and more data is required to explain their preventive and protective potential in disease states, particularly at the molecular level. Many xenobiotics have the potential to act on the inhibition or activation of many proteins. From this perspective, trying to explain some of the activities of urolithins through the induction or inhibition of the expression of related protein synthesis cascades does not thoroughly display the certain mechanistic background. On the other hand, some research studies have used high concentrations of urolithins either in *in vivo* or *in vitro* experiments which are practically impossible to be reached with regular ellagitannin rich food exposure. From the medicinal chemistry perspective, urolithins are also important scaffolds to be utilized in drug design studies. Our focus continues to make

research on the design of novel urolithin derivatives with diverse biological actions, particularly focusing on the treatment of Alzheimer's Disease.

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