Review

Selected phenolic compounds in the modulation of biochemical pathways involved in ageing

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1. Abstract
The use of phenolic compounds, derived by plants, has recently emerged as a promising approach to prolong the lifespan by modulating metabolic pathways involved in ageing. Phenolic compounds possess a broad spectrum of biochemical and pharmacological effects beneficial to human health such as modulating cellular senescence processes by interacting with molecular targets that regulate ageing-related pathways. Phenolic compounds represent the major phytochemicals in our diet and possess several biological activities such as antioxidant and anti-inflammatory effects; protection against ageing-related diseases (cancer, diabetes and cardiovascular diseases) with potential therapeutic applications and this could suggest that these compounds could be used as anti-ageing nutraceutical support. In this review, we have considered the possible effects of some phenolic compounds in different ageing pathways, to provide an overview of recent knowledge on their anti-ageing mechanism of action.

Graphical abstract

2. Introduction
Ageing is a time-dependent physiological process characterized by the accumulation of biological changes leading to the functional decline of the organism over time representing an important risk factor for common conditions such as cardiovascular diseases, cancer, diabetes and neurodegenerative disease (Kaeberlein, 2013). In modern society, advanced healthcare can keep people alive longer, so the consequences of age and physiological decline become even more important to understand. In the last decade, research has contributed to a better understanding of how ageing occurs and its regulation by cellular signals and molecular pathways providing the possibility to developing therapies to delay ageing, as reported in the literature (Keshavarz et al., 2023).

The hallmarks of ageing include accumulations of genetic instability, epigenetic alterations, impairment of proteome and metabolome homeostasis, mitochondrial dysfunction and cellular senescence, which has also been associated with ageing and age-related diseases (López-Otín et al., 2023).

Cellular senescence is essential to repress tumorigenesis; on the other hand, the excessive accumulation of senescent cells increases the negative effects of aging. Due to this complex link, finding anti-aging strategies is difficult (Di Micco et al., 2021).

Finally, stem cells loss or dysfunction such as mesenchymal stem cell decline, which leads to osteoporosis and fractures, or intestinal epithelial stem cell depletion, which causes decrease in intestinal function, and alteration in intracellular communication are common features of ageing (Aunan et al., 2016).

Increased reactive oxygen species (ROS) levels, generated metabolically within cells or from exogenous sources, lead to macromolecular damage correlated to age-associated functional losses of tissue and organs (Liguori et al., 2018).

Throughout the years, several approaches for improving health and lifespan have been proposed, including the use of phenolic compounds (Pinto et al., 2023).

The main ways to increase healthy lifespan include lifestyle modifications and pharmacological (or genetic) manipulations (Liu, 2022). First, balanced diet and caloric restriction are crucial in healthy
aging (Zia et al., 2021). Key nutrients such as defined vitamins, minerals (as micronutrients), essential and branched amino acids, polyunsaturated fatty acids (PUFA), probiotics and plant metabolites such as polyphenols and terpenoids, are crucial in healthy aging (Corrêa et al., 2018). Certain drugs are pharmacological agents that can decrease the rate of aging and extend lifespan as demonstrated in different animal models such as yeast, rodents, non-mammals model organism and in vitro human cells model. Plant-derived compounds such as stilbenes, anthocyanins, epigallocatechin gallate, curcumin, and rosmarinic acid play a significant role in limiting aging processes through their antioxidant activities, inhibiting the influence of free radicals (Bjørklund et al., 2022).

Phenolic compounds could protect organisms against the effects of excessive accumulation of damage occurring in senescent cells but further studies of their specific activities on ageing processes are required. In particular, in spite of much evidence being present in several experimental models, the relevance of phenolic compounds in delaying aging in humans is still limited. In this context, here we discuss the effects of different classes of natural compounds on several ageing metabolic pathways, with particular attention to the most studied phenolic compounds. The identification of the effects of specific phytochemical on a metabolic process, depend on several parameters: the used experimental approaches (from chemical interaction of purified molecules and metabolites to clinical trials), concentration and interaction with other physic or -chemical parameters. Moreover, the nutraceutical properties of phenolic compounds are an issue of great interest described by hundreds of scientific papers, even when the area of interest is limited to phenolic compounds. Therefore, our aim is not to give a complete overview of the mechanisms through which specific phytochemicals contribute to healthy aging but give some examples of the interaction between specific phytochemical with promising properties and the metabolic pathways improving healthy lifespan.

3. Biochemical pathways implicated in aging

3.1 Signaling cascades

Multiple signaling cascades can modulate longevity and one of the most important is the mammalian target of rapamycin (mTOR) signaling pathway, that controls lifespan and influences aging-related processes, such as cellular senescence. Indeed, in animal models inhibition of mTOR has been demonstrated to increase lifespan (López-Otín et al., 2016). AMP-activated protein kinase (AMPK) has been defined as the ‘cellular energy regulator’. Its activity declines in ageing skeletal muscle of mammals, while overexpression of AMPK directly activates DAF-16/FOXO by phosphorylation (Greer et al., 2007) and extends C. elegans lifespan (Apfeld et al., 2004). Furthermore, AMPK has a pivotal role in autophagy, an important cellular process associated with homeostasis and the extension of lifespan. Through autophagy, molecules and subcellular components are degraded via the lysosomal pathway and the products are recycled. Sirtuins (SIRT) are NAD⁺-dependent deacetylases that are implicated in longevity. SIRT1, the most investigated member of this family, promotes the activation of AMPK playing a positive role in autophagy and longevity (Takeda-Watanabe et al., 2012) whereas mTOR is a negative regulator of lifespan, and the relationship between SIRT1 and mTOR is a reciprocal inhibition. Hence, AMPK is a negative regulator of mTOR, thus inducing autophagy and mitochondrial biogenesis (Mihaylova et al., 2011).

3.2 Inflamm-aging

Inflamm-aging is a theory that explains how the aging process would be due, at least in part, to a low-grade chronic systemic inflammation established during physiological aging (Franceschi et al.,...
Cellular senescence is a critical mechanism that contributes to ageing and ageing-related diseases, whereby cellular stress derived from proliferation or differentiation process results in a replicative arrest, apoptosis resistance, and the onset of a pro-inflammatory tissue-destructive senescence-associated secretory phenotype (SASP), leading to secretion of high amounts of immune modulators, growth factors, inflammatory cytokines, and proteases (Justice et al., 2019). This response is implicated in the pathogenesis of various chronic diseases associated with ageing, representing a link between cellular senescence and inflamm-aging (Childs et al., 2017).

### 3.3 Cellular senescence

Cellular senescence contributes to preserving cellular/tissue homeostasis and favorites tumor suppression and embryonic development, but chronic senescence, which results in the accumulation of senescent cells and their SASP, exerts deleterious effects on physiological processes (He et al., 2017). SASP expression is induced by multiple transcription factors, the most important of which is Nuclear Factor-kB (NF-κB). This is a transcription factor that has a pivotal role in inflammatory and immune responses, as well as transcriptional regulation of several chemokines and cytokines, and that modulates cell proliferation and apoptosis. Furthermore, NF-κB activation is associated with several signaling pathways that are known as lifespan regulators including insulin/IGF-1, mTOR, and FOXO. NF-κB activity progressively increases during aging and is connected to age-associated degenerative disorders such as Alzheimer disease (Tilstra et al., 2011).

Finally, the proteasome is the major cellular proteolytic machinery responsible for the physiological protein turnover as well as for degradation of damaged proteins, therefore its action avoids metabolic alteration due to the presence of dysfunctional proteins. Consistently, alterations of proteasome function have been recorded in various biological phenomena including ageing and replicative senescence. Natural substances that possess proteasome-activating properties have been also shown to promote lifespan extension (Katsiki et al., 2007).

Figure 1 describes the main pathways related to ageing and the possible interaction of natural compounds with them.

## 4. Phenolic compounds as a nutraceutical resource in ageing

Phenolic compounds can increase lifespan and improve health and quality of life by reducing the risk of some age-related chronic diseases such as diabetes, cancer, neurodegeneration, and cardiovascular illnesses (Bjørklund et al., 2022).

Polyphenols are bioactive compounds widely present in plants, mainly in fruits, vegetables, tea, wine, cocoa and aromatic plants. There are different classes of polyphenols, classified by their chemical structures in phenolic acids, flavonoids, stilbenes and lignans. Chemically, they contain one or more aromatic nuclei with several hydroxyl groups (Tsao et al., 2010).

Phenolic compounds possess several biological activities: antioxidant, anti-inflammatory, antibacterial, antiviral, anti-tumor, and anti-atherogenic action by improving endothelial barrier function and for these reasons have been investigated for their anti-aging properties considering the biological activities mentioned above (Zhang et al., 2022). In addition, these compounds can induce selective apoptosis of senescent cells (Hernandez-Segura et al., 2018) as well as they decrease the production of advanced glycation end-products, which are involved in both natural aging and several age-diseases such as diabetes, renal failure, and chronic inflammation (Spagnuolo et al., 2021).

Specifically, in the next paragraphs we will discuss the potential of three phenolic compounds, quercetin, resveratrol and curcumin, to summarize their anti-aging mechanism of action (Figure 2).
4.1 Biological activities of quercetin

Flavonoids possess a wide range of pharmacological properties and are promising candidates in anti-ageing research, improving lifespan and other markers of senescence directly or indirectly and these effects are related to maintaining SASP, inducing apoptosis in senescent cells, and activating different protective cellular mechanisms (Yi et al., 2017; Schonhofer et al., 2021). Among flavonoids, quercetin shows antioxidant, anti-apoptotic and anti-inflammatory properties, taking an important role in the treatment of aging-related diseases.

Quercetin has been shown to be a powerful *in vitro* antioxidant potent scavenger of ROS and RNS, including O$_2^\cdot$-, NO and ONOO$^-\cdot$ (Boots et al., 2008). In addition, quercetin possesses proteasome activating properties with antioxidant activities that consequently influence cellular lifespan, survival and viability of HFL-1 primary human fibroblasts (Chondrogianni et al., 2010). Quercetin is also a known enhancer of nuclear factor-erythroid-2-related factor 2 (Nrf2), a transcription factor regulating the genes responsible for the transcription of the 26S proteasome complex, thus explaining its effects on proteasome activation (Tanigawa et al., 2007). Several studies have shown therapeutic potential of quercetin associated with dasatinib (drug used to treat chronic myeloid leukemia) with a decrease of senescence biomarkers and fibrosis burden in the lungs of pulmonary fibrosis mice models (Schafer et al., 2017).

In addition, quercetin exerts neuroprotective effects against chronic aging-related diseases via targeting SIRT1 to regulate cellular senescence and multiple aging-related cellular processes such as SIRT1/NF-$\kappa$B mediated inflammatory response and and SIRT1/FoxO mediated autophagy (Cui et al., 2022).

Several phenolic compounds are poorly absorbed and/or extensively metabolized within enterocytes and liver. In addition, they undergo intensive transformation by gut microbiota. It is considered that less than 5% of the total phenolic compounds intake is absorbed and reaches the plasma unchanged (Luca et al., 2019). For example, after absorption, quercetin suffers biotransformation in the small intestine, colon, liver and kidney and non-metabolized quercetin and its metabolites are further secreted from the small intestine into hepatic portal circulation. The amount of quercetin that has not been intestinally absorbed, will be further subjected to colon microflora metabolism and in part eliminated. The bioavailability of quercetin in humans was estimated at 44.8%, too low to justify their potential biological activity (Wang et al., 2016).

4.2 Effects of resveratrol on certain aging pathways

Among the phenolic compounds, resveratrol is perhaps one of the most extensively studied because of its multiple biological activities leading to a reduction of negative aging-related modifications...
Resveratrol is present in many foods and plants such as dark grapes and derived red wine, peanuts, blueberries, strawberries, hop, cranberries and tomatoes. Resveratrol has antioxidant, anti-inflammatory, and immunomodulatory activities, and it has also proven to be effective in the prevention of cancer, cardiovascular diseases, neurodegenerative diseases and metabolic disease in several model systems as reviewed by Koushki and co-workers (Koushki et al., 2018). Knowing its chemical structure is essential for understanding its anti-aging properties: planar stilbene structure gives hydrophobic characteristics to the molecule, that interacts with the hydrophobic domains of target protein molecules (e.g., SIRT1, Nrf2). As mentioned previously, targeted protein regulation is the mechanism behind some of the aging processes, so resveratrol exerts its anti-aging effects through intracellular signal transduction. SIRT1, represents one of the proteins involved in the regulation of autophagy and inflammation and directly inhibits the mTOR pathway to activate autophagy and activates AMPK. The activation of AMPK pathway induces the expression of SIRT1 and promotes autophagy activation. Resveratrol enhances SITR1 and AMPK expression playing an important role in the aging pathways (Giovannini et al., 2017).

Studies conducted on non-mammals model organisms (Saccharomyces cerevisiae, Caenirhabditis elegans, Drosophila melanogaster) and mice have shown that resveratrol increases the lifespan primarily by activating SIRT1 and by positively regulating AMPK. AMPK is a negative regulator of mTOR, thus inducing autophagy and mitochondrial biogenesis. This causes a reduction in transcription of the pro-inflammatory gene and inhibition of ROS and cytokine production, leading to anti-aging effects (Howitz et al., 2003; Wood et al., 2004).

The limitation in the use of resveratrol in humans depends on its bioavailability: when orally administered, the rate of absorption of resveratrol is approximately 75%. However, due to rapid metabolism into sulfate and glucuronide metabolites in the intestine and liver that are eliminated in urine, the amount of resveratrol remaining bio-active is much lower than the amount uptaken with diet (Walle et al., 2011; Cottart et al., 2014).

Moreover, most of the studies on resveratrol have been conducted in vitro. When extended in rat, no adverse effect was observed at low doses (0–300 mg/day), while high doses (>1000 mg/day) caused kidney damage and body weight loss. In human studies an oral dose <1g/day showed no major adverse effects in a short period (<1 month), but some slight side effects like abdominal pain and diarrhea, could appear also when >0.5 g of resveratrol was administered (Almeida et al., 2009; Cottart et al., 2010). Considering the positive effects of phenolic compounds on ageing pathways, it is important increase the bioavailability. Resveratrol has rapid metabolism and low bioavailability: this has therefore been addressed by the use of bio-enhancers and nano-formulation to increase resveratrol’s solubility and tissue absorption. Different methods such as enhancement in solubility, administration, prevention of metabolism have been attempted and have been tested on animal models but their effect have not extensively been studied in humans (Pannu et al., 2019).

4.3 Effect of curcumin on age-related pathways

Curcumin (diferuloylmethane) is the main bioactive compound extracted from Curcuma longa (turmeric) rhizomes, which belongs to Zingiberaceae family and is broadly cultivated in Southeast Asia and India. The chemical name of curcumin is 1,7-bis(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione with a chemical formula of C_{12}H_{20}O_{6}; it is formed by two aromatic rings with a methaxy phenolic group, linked with a linear carbon chain with an α,β-unsaturated β-diketone moiety. Curcumin is used as dietary spice and coloring agent, but it has been also used for centuries in Indian traditional medicine, Ayurveda, and in traditional Chinese medicine for its anti-inflammatory properties to treat several illnesses such as anorexia, hepatic disorders, and arthritis (Shishodia et al., 2005). In the latest decades several studies revealed that curcumin modulates
multiple transcription factors, inflammatory cytokines, enzymes, growth factors, receptors, adhesion molecules, antiapoptotic proteins, and cell cycle proteins, exhibiting anti-inflammatory, antioxidant, and anti-cancer activities in *in vitro* and *in vivo* animal models (Lelli et al., 2017; Patel et al., 2020). Given its pleiotropic activities, curcumin has been studied for its probable role on influencing aging and lifespan, suggesting a potential role of this compound in slowing down senescence (Salvioli et al., 2007). The main effects of curcumin seem to be on cellular senescence, inflam-maging, but also can influence pathways implicated in aging. Indeed, curcumin is a potent antioxidant and can reduce age-related cellular damage induced by ROS. The curcumin’s phenolic groups have a powerful hydrogen-donating antioxidant activity (Kocadam et al., 2017). Furthermore, *in vivo* animal studies documented that curcumin activates the nuclear Nrf2/heme oxygenase-1 (HO-1) signaling pathway: curcumin upregulates Nrf2, enhancing the expression of HO-1, that in turn activates multiple antioxidative enzymes, including thioredoxin reductase, Hsp70, and sirtuins (Ren et al., 2019). Using *Saccharomyces cerevisiae* as aging model organism, Stepien et al. reported that curcumin-treated SKN-1 (homologous to the vertebrate Nrf protein) mutants did not exhibit a lifespan extension, which demonstrates that SKN-1 has an essential role in curcumin-mediated effect on lifespan (Stepień et al., 2020). Another study documented that in a *D. melanogaster* aging model, curcumin extended lifespan by enhancing superoxide dismutase activity (Suckow et al., 2006), thus suggesting that curcumin influence cellular senescence by modulating different pathways. Considering others signaling cascade in ageing, curcumin rapidly phosphorylation of mTOR, at physiological concentrations (2.5 mM), and its downstream effector molecules, p70, S6 kinase 1, and eukaryotic initiation factor 4E binding protein 1 in a panel of cell lines (Rh1, Rh30, DU145, MCF-7 and HeLa). Curcumin also inhibits phosphorylation of Akt in these cells, but only at high concentrations (440 mM) (Beegers et al., 2006). Curcumin can also activate AMPK and suppress mTOR signaling pathways, as documented in an ischemia-induced cardiomyocyte injury model (Yang et al., 2013) but also in other models, such as oxidative stress-induced intestinal barrier injury (Cao et al., 2020). Finally, curcumin restores autophagy via the SIRT1/AMPK/mTOR pathway in a model of senescent cardiomyocytes: this compound increases the expression of SIRT1, phosphorylates AMPK and decreases phosphorylation of mTOR inducing autophagy in a dose-dependent manner (Yang et al., 2022). Furthermore, curcumin can reduce oxidative stress in diabetic cardiomyopathy in both *in vitro* and *in vivo* mouse model (oral administration). This effect is mediated by the modulation of the SIRT1-FOXO pathway (Ren et al., 2020).

Finally, curcumin can improve inflam-maging. In fact, this compound both in *in vitro* and *in vivo* models can reduce inflammation by downregulation of NF-κB: for example, in a mouse model of pulmonary inflammation intra-tracheal instillation of curcumin reduces alveolar damage, decreases immune cell infiltration, and reduces proinflammatory cytokine production in both lung tissue and broncho-alveolar lavage. To understand the underlying mechanism, the authors used mouse macrophage cell line RAW264.7. Pretreatment with curcumin prevented the production of proinflammatory cytokines by inhibiting NF-κB through the suppression of MAPK signaling pathways. Furthermore, curcumin attenuated oxidative stress through the activation of Nrf2 and downstream antioxidant signaling (Lee et al., 2023). Curcumin can improve inflam-maging also by inhibiting p65: in mesenchymal stem cells lines, curcumin reduces production of IL-6 and IL-8, key components of SASP. In this model, p65 inhibition prevents also the transmission of paracrine senescence between mesenchymal stem cells and the proinflammatory message through small extracellular vesicles (Mato-Basalo et al., 2021). However, it has been suggested that this phenolic compound could have different effects in concentration higher of a certain threshold value (from positive to toxic effect- M. Maffei personal communication).
The bioavailability of curcumin has been assessed in numerous animal models and human studies. Recent studies have revealed that curcumin, similarly to other polyphenols, undergoes an alternative metabolism by intestinal microbiota. Curcumin is a poorly water-soluble drug and susceptible to degradation, particularly under alkaline conditions (Prasad et al., 2014). One strategy to avoid this is the use of nano-formulations or liposome vesicles, which provide an alternative to protect the bioactive substance and facilitate its optimal absorption, promoting the interaction of compounds with biological membranes, thereby enhancing its bioavailability (Ciuca et al., 2023).

5. Conclusions
Growing evidence shows the potential role of some phenolic compounds in promoting health and increasing lifespan by modulating multiple pathways implicated in different aspects of senescence, such as SASP and inflamm-aging. However, most of the studies are in vitro, and the very few in vivo human studies showed contrastant results, that could be a consequence of the low bioavailability of some of these compounds when administrated orally. In fact, curcumin and resveratrol have a rapid hepatic and intestinal metabolism via glucuronidation and sulfation (Dei Cas et al., 2019; Walle et al., 2011), while quercetin presents wide inter-individual bioavailability variation, probably due to genetic polymorphisms and to inter-individual variations in gut microbiota metabolism of quercetin (Almeida et al., 2018).
To overcome this limitation, in the latest years advanced extraction technologies, followed by encapsulation in microemulsion and nanoemulsion systems, are being used to improve bioavailability of these compounds (Chimento et al., 2019; Ciuca et al., 2023), with promising results in clinical trials (Abdolahi et al., 2019).
Thus, further studies are needed to understand if a diet rich in these phenolic compounds is sufficient to provide an efficacious blood concentration or if nutraceutical formulations are required, in order to improve bioavailability and stability of these molecules. The numerous positive evidences of beneficial effect of phenolic compounds on human health in several pathways of ageing or other pathologies certainly seems to indicate a strong nutraceutical potential, worthy of being further investigated.

6. Acknowledgements (optional)

7. Declaration of interest
We declare the absence of conflicts of interest that could be perceived as prejudicial to the impartiality of the reported research.

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9. References


49. Ren BC, Zhang YF, Liu SS, Cheng XJ, Yang X, Cui XG, Zhao XR, Zhao H, Hao MF, Li MD, Tie YY, Qu L, Li XY 2020 Curcumin alleviates oxidative stress and inhibits apoptosis in


Graphical abstract
Figure 1. Overview of pathways implicated in aging and effect of polyphenols age-related. Inflammation stimuli and ROS activate SIRT1 that is involved in the regulation of autophagy and inflammation. SIRT1 directly inhibits the mTOR pathway to activate autophagy and activates AMPK. The activation of AMPK pathway induces the expression of SIRT1 and promotes autophagy activation. Additionally, SIRT1 regulates NF-κB that is the most important factor involved in the expression of SAPS and cellular senescence. ROS activate also nuclear factor-erythroid-2-related factor 2 (Nrf2) by inactivating its negative regulator (Keap1). Antioxidants cause the dissociation of Nrf2 from Keap1, allowing for accumulation of Nrf2 enhancing the expression of 26S proteasome that promotes lifespan extension. Polyphenols as Curcumin, Epigallocatechin (EGCG), Quercetin and Resveratrol play an important role in the aging pathways: curcumin enhances Nrf2, SIRT1 and AMPK action and inhibits mTOR and NF-κB signaling; quercetin is involved in the activation of Nrf2 pathway; resveratrol enhances SITR1 and AMPK expression; EGCG inhibits mTOR pathway.
Figure 2. Chemical structure of different class of phenolic compounds

Figure 2