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Natural compounds with anti-ageing activity

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Ageing is a complex molecular process driven by diverse molecular pathways and biochemical events that are promoted by both environmental and genetic factors. Specifically, ageing is defined as a time-dependent decline of functional capacity and stress resistance, associated with increased chance of morbidity and mortality. These effects relate to age-related gradual accumulation of stressors that result in increasingly damaged biomolecules which eventually compromise cellular homeostasis. Nevertheless, the findings that genetic or diet interventions can increase lifespan in evolutionarily diverse organisms indicate that mortality can be postponed. Natural compounds represent an extraordinary inventory of high diversity structural scaffolds that can offer promising candidate chemical entities in the major healthcare challenge of increasing healthspan and/or delaying ageing. Herein, those natural compounds (either pure forms or extracts) that have been found to delay cellular senescence or *in vivo* ageing will be critically reviewed and summarized according to affected cellular signalling pathways. Moreover, the chemical structures of the identified natural compounds along with the profile of extracts related to their bioactive components will be presented and discussed. Finally, novel potential molecular targets for screening natural compounds for anti-ageing activity, as well as the idea that anti-ageing interventions represent a systemic approach that is also effective against age-related diseases will be discussed.

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1 Introduction

Multicellular organisms are extremely complex biological entities which depend on energy production in order to preserve their homeostasis, compartmentalization and fitness in a rather hostile oxidative environment. Almost invariably, a lifetime includes an initial highly programmed (in terms of both gene expression repertoire and duration) period, namely embryogenesis, and the lifetime after birth (Fig. 1). Following birth, organisms are constantly exposed to both internal (metabolism-related) and external (diet- or environment-derived) stressors that damage in a stochastic fashion all cellular biomolecules. Nonetheless, organisms, for a relatively long time (at least up to maturation) retain low levels of damaged biomolecules *via* the action of a modular, yet integrated quality control system which, apart from neutralizing stressors, also recognizes and either repairs or removes dysfunctional biomolecules. This fight is, however, condemned to be lost, since, as the organism gets older, these mechanisms are gradually compromised, resulting in impaired signalling and repair or clearance pathways that eventually deteriorate cellular functions, promoting the accumulation of high levels of stressors and tissue ageing which correlates with increased disability, morbidity and inevitably

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death (Fig. 1);[1,2] these processes can be accelerated by certain lifestyle habits; *e.g.* smoking. In accordance with this view, age is the major risk factor for several life-threatening diseases, including cancer, cardiovascular disease, neurodegeneration and diabetes.[3,4]

It is nowadays evident that both healthspan (the disease-free period of life) and/or lifespan (maximum longevity) can be prolonged by genetic and/or diet interventions (see below), suggesting that animals have the latent potential to live longer than they normally do. Understanding the molecular basis of these findings and of ageing is much needed in order to tackle the growing problem of the seemingly irrevocable trend (at least in the western world) of ageing societies.[5] To this end, cellular senescence is studied in experimental models, which include the single-cell budding yeast *Saccharomyces cerevisiae* and normal mammalian cells. The latter lose their replicative potential and inevitably stop proliferating as a result of serial passaging in tissue culture; this process is referred to as replicative senescence (RS) and in normal human cells relates to a progressive telomere shortening.[6] Young normal human cells possessing long telomeres can also senesce prematurely if exposed to various types of stress during a process termed as Stress-Induced Premature Senescence (SIPS);[7–9] it is assumed that a combination of both RS and SIPS contributes to human cells' senescence *in vivo*.[6] At the whole organism level, the experimental models that are mostly used for screening age-related effects of genetic and/or dietary interventions are the nematode *Caenorhabditis elegans* (lifespan of ~2 weeks), the fly *Drosophila melanogaster* (lives for ~2 months) and the mouse *Mus musculus* (lives for ~2 years). Invertebrate organisms have acted as engines for the identification of the molecular mechanisms that determine longevity, while studies in mice have established if homologous processes and genes can modulate

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ageing in mammals;[10,11] more recently lifespan extending dietary interventions are also tested in primates.[12,13]

Reportedly, the most effective intervention in extending longevity at model organisms is caloric restriction (CR), which refers to a reduction (without malnutrition) in food intake by ~10–50% below the level in mammals fed *ad libitum*. CR not only increases longevity but it also reduces risk for most (if not all) age-related diseases, including diabetes, cardiovascular disease, neurodegeneration and cancer.[10,12,13] At the genetic level it all started when it was observed that mutations in the nutrient sensing plasma membrane receptors could increase the adult lifespan of *C. elegans*.[14] Since then, several studies have demonstrated that lifespan can increase by mutations in genes that sense and transmit the presence of nutrients.[15] It is assumed that these mutations reduce the activity of nutrient cell signalling pathways, inducing a physiological state similar to that resulting from periods of food shortage. Thus, it is not surprising that either a true (CR-mediated) or a genetically (by mutants) induced downregulation of nutrient signalling results in the activation of stress responsive pathways and consequently in lower rates at which stressors and/or damaged biomolecules accumulate in cells;[16,17] notably, most of the other pathways known to affect ageing, like sirtuins, the rate of respiration or signals from the gonads, also converge in the activation of stress responses.[16] These phenotypes can be understood if we consider ageing as the outcome of a balance between biomolecule damage and repair (Fig. 1). Indeed, biomolecule integrity and proper function is constantly threatened by environmental-, diet- or metabolism-originating stressors (*e.g.* oxidants, reducing sugars or reactive aldelhydes) which, although at physiological levels are essential for normal development and metabolism, at high levels promote oxidative stress and non-enzymatic irreversible chemical modifications that damage cellular biomolecules.[18–21] Organisms counteract these threats *via* significant energetic effort and the coordinated action of a number of conserved stress responsive and damage repair or clearance signalling pathways. A first line of cellular defence against stressor fluctuations depends on a network consisting of antioxidant compounds and/or antioxidant enzymes, which are produced by oxidative stress sensors and ensure efficient recycling of oxidants *via* prevention of accumulation and/or neutralization.[18,21,22] A second line of defence refers to a modular, yet integrated cell- and subcellular compartment-specific network of DNA (DDR) and proteome (PDR) damage responses, which ensures genome and proteome stability.[21,23–26]

As genetic interventions cannot be applied in humans and it is challenging to implement CR, many studies have been devoted to the identification of natural compounds that can prolong healthspan and/or lifespan. It is well established that natural compounds possess a broad range of biological activities, and therefore, they constitute the ultimate reservoir for seeking novel structures capable of diverse and sometimes extraordinary effects. Natural compounds can be used as pharmacological modulators of the signalling pathways involved in ageing regulation by, for example, dampening signalling from nutrient sensing pathways, thus mimicking the systemic effect(s) of CR. Alternatively, protection from age-related damage of biomolecules can be implemented by natural compounds that either directly neutralize stressors or provide a mild sustained activation of the stress responsive pathways. In addition, natural compounds can be used for local skin rejuvenation purposes (*e.g.* in cosmeceuticals) but, obviously, this approach has no effect at a systemic level and thus is not anticipated to exert any true anti-ageing effect at the organismal level.

Herein, we review the known molecular causes of ageing and critically summarize the natural compounds (or extracts) that reportedly delay cellular senescence or prolong *in vivo* longevity along with their mechanisms of action and molecular targets. We should emphasize that natural compounds (or extracts) that have shown merely an antioxidative effect without clear evidence of a healthspan/lifespan extension are not within the scope of the present review and have therefore been excluded. Also, we provide suggestions of possible novel targets for screening natural compounds (or extracts) with healthspan and/or lifespan extending activity and we discuss the idea that tackling ageing would be an effective approach to combat age-related diseases.

2 Overview of the molecular basis of ageing

As mentioned previously, ageing can be postponed by either diet (*e.g.* CR) or genetic interventions. At the molecular level longevity responses to CR are actively regulated by nutrient sensing signalling pathways;[15] additional modulators of longevity are sirtuins, the rate of respiration, the telomere length and signals from the gonads.[10] Notably, most of these pathways have not been evolved as direct regulators of ageing as, for instance, nutrient signalling is critical in promoting growth effects during embryogenesis and early development (Fig. 1).[27] Interestingly, most (if not all) of the longevity regulating pathways converge to the modulation of stress responsive pathways (Fig. 2) and thus they affect, either directly or indirectly, the lifetime related rate of stressors and biomolecule damage accumulation. Therefore, this latter parameter has emerged as the key factor that fuels the appearance of ageing and age-related diseases.[16,20,21]

2.1 Nutrient and energy sensing pathways

The main nutrient and energy sensing signal transduction pathways that are implicated in CR-mediated longevity extension are the Insulin/Insulin-like Growth Factor-1 (INS/IGF-1); the Target of Rapamycin (TOR)/ribosomal protein S6 kinase (S6K) and the AMP-activated kinase (AMPK) signalling pathways (Fig. 3, 4).[10,15]

The INS/IGF-1 receptors are modulated by their cognate ligands, namely INS or IGF-1, respectively, while in mammals positive regulation is also exerted by growth hormone (GH).[28] CR reduces the INS/IGF-1 signalling by lowering the levels of available growth factors and these effects can be also mimicked by genetic means, since loss-of-function mutations in *Daf-2*,

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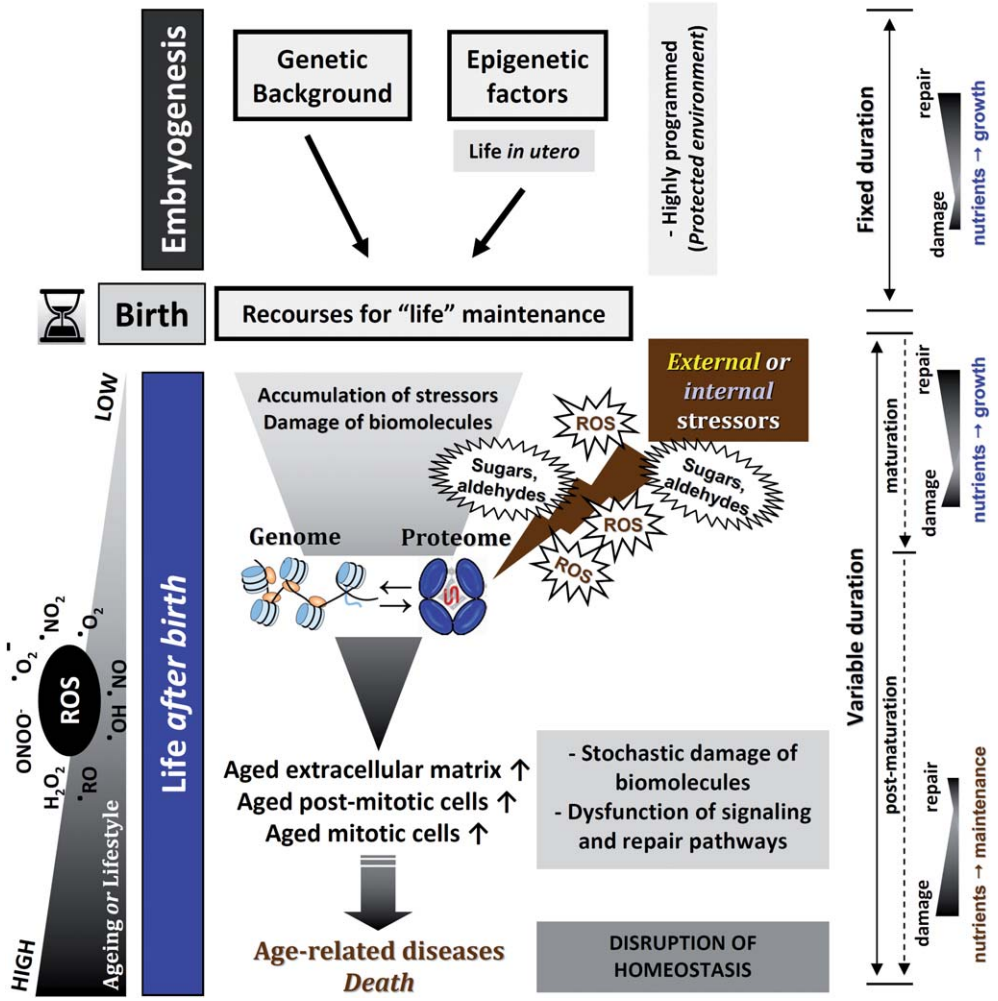

Fig. 1 The lifetime of an organism includes the strictly programmed (in terms of gene expression repertoire and duration) period of embryogenesis and the period after birth. Embryogenesis, which for most organisms takes place in a relatively protected environment (*e.g.* in the uterus), is defined by the genetic makeup of the individual and the epigenetic changes that occur during life *in utero*. On the other hand, following birth, organisms are constantly exposed to both internal (*e.g.* mitochondria-derived ROS) and external (*e.g.* pollutants, radiation, diet-derived reducing sugars or reactive aldehydes) stressors that promote the stochastic damage of all cellular biomolecules; within this context, nutrients are critical in supporting proper growth during early life, whereas later on they are mostly used for maintenance. Organisms, for a relatively long time (at least up to maturation) retain low levels of dysfunctional damaged biomolecules *via* the action of a modular, yet integrated quality control system which removes stressors and dysfunctional damaged molecules. Nevertheless, as the organism gets older these mechanisms are gradually compromised resulting in impaired signalling and repair or clearance pathways; which then trigger a vicious circle of further accumulation of stressors and molecular damage and so forth. Eventually, the aged tissue correlates with increased disease and death rates.

(a homolog of mammalian INS/IGF receptors in *C. elegans*) extends the lifespan of the worm by ~2-fold.[10,29] Also, an inverse correlation between IGF-1 levels and lifespan was noted in mice inbred strains, strongly implicating IGF-1 in lifespan regulation.[30] In the worm (like in most other model organisms), inhibiting INS/IGF-1 signalling, either by CR or by genetic means, upregulates autophagy [a catabolic proteolytic mechanism that maintains cellular energy levels and removes damaged organelles or protein aggregates (see section 2.3)], as well as several other cell defence mechanisms *via* the activation of the DAF-16 [a Forkhead box O (FOXO)-like transcription factor], Heat Shock Factor-1 (HSF1) and SKN-1 [the *C. elegans* ortholog of the mammalian NFE2-related factor 2 (NRF2)] transcription factors (Fig. 4).[16,31,32] FOXO transcription factors have been functionally implicated in stem cell maintenance, cell cycle regulation, apoptosis, metabolism regulation and resistance to both metabolic (starvation) and oxidative stress;[33] the anti-ageing effects of FOXOs are also evident in flies as enhancing the activity of FOXO specifically in adipose tissue of *Drosophila* increases lifespan.[34] Similar to the protective role of FOXO, HSF1 exerts a protective function against age-related proteotoxicity[35] and NRF2 is central to cellular antioxidant responses (see also section 2.3).[22,36] Mechanistically, it seems that a decrease in the nutrient sensing signalling pathways activity is perceived by the organism as a mild type of stress, providing beneficial hormetic effects;[16] hormesis refers to the phenomenon where low-level exposure to stress induces a long-term protective stress response.[37] In support, long-lived GH-deficient mice show increased stress resistance in muscle cells and fibroblasts and increased expression of antioxidant enzymes.[38] The protective effects of these pathways against ageing are, most likely, also present in humans as recent genetic

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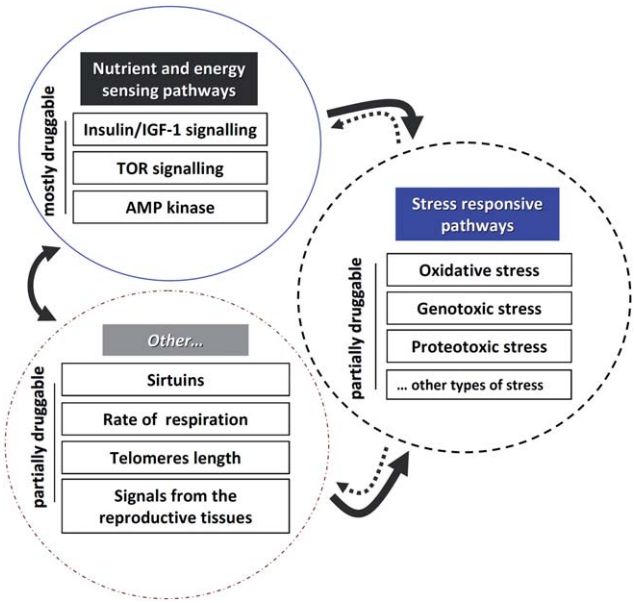

Fig. 2 Healthspan and/or lifespan duration is affected by various signalling pathways, including nutrients sensing pathways, sirtuins; the rate of respiration, telomere length and signals from the gonads. In most (if not all) cases cues from these pathways are transmitted to stress responsive pathways which retain homeostasis and regulate the rate of organisms' survival in a rather hostile oxidative environment. In all three categories there are druggable targets (*e.g.* enzymes or stress responsive transcription factors) for screening natural compounds.

studies have found inherited Single Nucleotide Polymorphisms in INS/IGF-1 signalling and FOXO genes that correlate with longevity in centenarians.[39,40]

TOR is a cytoplasmic-nuclear shuttling protein kinase that functions as a sensitive sensor of redox and nutrient balances to enhance protein synthesis and ribosome biogenesis, and to suppress autophagy.[41] TOR activity is reduced under conditions of nutrient shortage and thus decreasing TOR signalling by genetic means mimics aspects of CR; indeed, TOR inhibition extends lifespan in most model organisms.[42,43] In support, partial inhibition of its downstream targets, like S6K or protein synthesis, extends lifespan in yeast, worms, flies and mice,[44–47] while $S6K1^{-/-}$ mice have reduced fat stores and are protected from weight gain on a high-fat diet.[48] The underlying mechanism of TOR inhibition positive effects on longevity does not solely rely on metabolic effects, as in the worm the lifespan-extending effects also require the activation of the antioxidant factor SKN-1/NRF2, indicating that TOR inhibition increases resistance to environmental stress (Fig. 4).[49]

Similar to TOR, AMPK is a nutrient and energy sensor that represses anabolic pathways when the cell's ATP levels decrease; thus AMPK functions as a rheostat for cellular energy status.[50] Additional stressors that deplete cellular ATP and lead to AMPK activation are ischemia or hypoxia, glucose deprivation and exercise.[51] AMPK activity declines in ageing skeletal muscle of mammals,[52] while overexpression of AMPK directly activates DAF-16/FOXO by phosphorylation[53] and extends *C. elegans* lifespan even when CR starts in middle age animals.[54]

2.2 Sirtuins, respiration, telomere length and signals from the gonads

Sirtuins are a group of proteins that play distinct roles in the regulation of metabolism, stress resistance and survival and their overexpression has been reported to extend lifespan in most invertebrate model organisms.[55–57] Sirtuins possess ADP-ribosyltransferase and/or NAD-dependent protein deacetylase activity and thus, the requirement for NAD is one mechanism by which sirtuins sense and respond to metabolic status by triggering stress response pathways and changes in energy metabolism.[58] The mammalian Sirtuin 1 (SIRT1) activates key transcription factors involved in stress resistance, including p53[59] FOXO[60] and HSF1,[61] indicating that stress resistance is central to SIRT1-mediated longevity. As for AMPK, SIRT1 is downregulated in a number of tissues during ageing and can also be inhibited by a high-fat diet; conversely, SIRT1 activity is increased following nutrient deprivation caused by fasting or calorie restriction.[62,63] Notably, overexpression of SIRT1 in transgenic mice fed a normal diet does not extend lifespan,[64] raising the possibility that SIRT1 function in mammals is most relevant to lifespan regulation under stress-related conditions.

Reportedly, lifespan duration is also influenced by the rate of respiration as a modest inhibition of respiration increases longevity in a wide variety of species.[65,66] These findings explain the generally observed correlation between metabolic rate (which decreases with size) and lifespan (which tends to increase with size) meaning that perhaps larger mammals live longer partly because their metabolic rates are lower. Additionally, in several organisms as well as in mammalian cells, lowering of respiration activates alternative energy-generating pathways and cell-protection pathways.[67]

Because telomeres shorten with age in human mitotic cells, they have been seen as candidates for ageing determinants.[68] In support, overexpressing the catalytic subunit of telomerase in Human Diploid Fibroblasts (HDFs) restores telomerase activity, suppresses telomere shortening and virtually induces HDFs immortalization in the absence of cancer-associated changes.[69] Although mice that are genetically engineered to have longer telomeres live longer, they must also be genetically modified to resist cancer.[70] Interestingly, although telomere-mediated lifespan extension may relate to alterations in various physiological states, including the prevention of stem-cell loss;[70] findings from a recent study indicated that telomere dysfunction associated with impaired mitochondrial function decreased gluconeogenesis and increased oxidative stress.[71]

Finally, it is evident that signals from the reproductive system can significantly modulate longevity.[72,73] Specifically, germ cells loss (in the presence of the somatic reproductive tissues) in *C. elegans* increased lifespan by (among others) activating DAF-16/FOXO.[74] Also, it was recently reported that forced re-investment of resources from the germ line to the soma of the worm resulted in elevated somatic proteasome (the main proteolytic mechanism of the nucleo-cytosolic compartments; see section 2.3) activity, clearance of damaged proteins and increased longevity.[75] In support, we and others have found that *Drosophila* reproductive tissues age at much lower rates as they exhibit high capacity to

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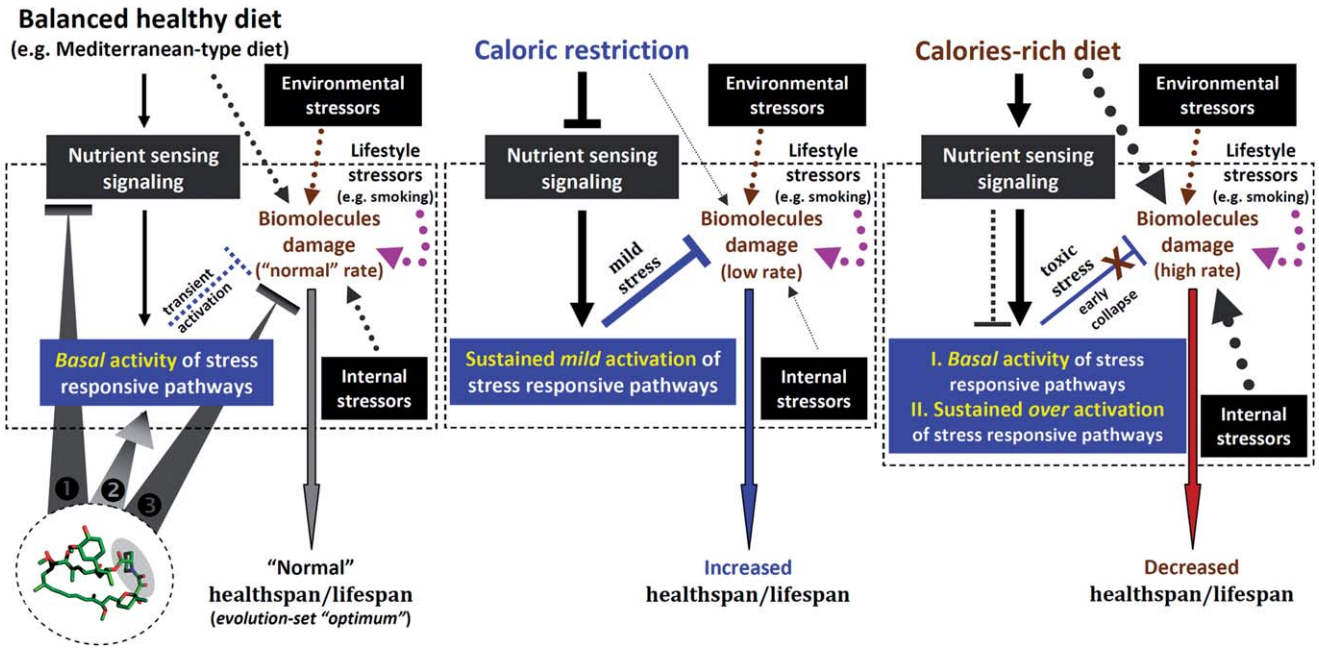

Fig. 3 The effects of nutrient sensing signalling and rate of biomolecule damage on healthspan and/or lifespan. An enriched in antioxidants, low-fat, balanced diet (*e.g.* Mediterranean-type) induces physiological activation of nutrient sensing signalling that correlates with basal expression levels of the stress response pathways; the latter remain, however, fully functional to counteract transiently increasing stress. Diet-, internal (*e.g.* metabolism) or environment-derived stressors damage biomolecules in a stochastic mode, resulting in a gradual decline of homeostasis and physiological ageing close to evolution-set "optimum". CR lowers the activity of nutrient signalling pathways triggering a sustained mild activation of the stress response pathways that effectively neutralize stressors and remove damaged molecules. As CR is also accompanied by reduced input of diet- or metabolism-derived stressors it correlates with lower rates of biomolecule damage, increased healthspan and/or lifespan. Conversely, a calorie-rich (*e.g.* high fat-glucose) diet, although it may initially retain normal activities of the stress response pathways (due to pour supply of nutrients), it may eventually trigger the sustained over activation of stress responses due to high (or toxic) levels of accumulating stressors. The accelerated rates of biomolecule damage correlates with premature collapse (or deregulation) of the cell defensive mechanisms, premature ageing and/or diseases; notably, high doses of glucose may also directly inhibit stress responsive pathways.[292] As the environmental doses of stressors do not fluctuate significantly within a lifetime of even long-lived organisms (like humans), the stressors that mainly impact (in a dose-dependent manner) on healthspan and/or lifespan are those derived from diet, metabolism and lifestyle habits (*e.g.* smoking). Natural compounds can readily affect these processes by suppressing nutrient signalling (❶), triggering a hormetic effect that results in mild activation of the stress responsive pathways (❷) or by directly neutralizing stressors and thus reducing stochastic damage of biomolecules (❸); [→, pathway activation; ⇥, pathway (or biomolecule damage) inhibition]. Dotted arrows indicate a pro-damaging effect on biomolecule damage; the thickness of the arrow correlates with the anticipated rate of damage.

prevent accumulation of damaged proteins and they retain high proteasome activities.[76,77] Thus, the anti-ageing strategies of the gonads converge (among others) to high activities of stress responsive and damage clearance pathways.

2.3 Damage of biomolecules as a central cause of ageing and stress responsive pathways

As is evident from the aforementioned research findings most (if not all) of the longevity extending pathways converge in the (assumed mild) sustained activation of the cellular stress responsive pathways (Fig. 2–4). This can be comprehended following the realization that although oxygen and nutrient usage (along with the integrated action of stress response pathways) has been optimized during evolution to maximize fitness in early life (Fig. 1), in the long term it undermines longevity as it correlates with the accumulation of stressors and damaged biomolecules. These deleterious effects are mostly promoted by an age-related decline in the effectiveness and integration of stress responses.[21,37]

But where do damaging stressors come from? In fact, they can be of exogenous environmental (*e.g.* radiations, pollutants or UV light) or diet (*e.g.* reducing sugars of reactive aldehydes) related origin, as well as from internal sources, including various inflammatory processes, excessive stimulation of NAD(P)H oxidases and mitochondria malfunction.[21] Reactive oxygen species (ROS) for instance, although much needed (at physiological concentrations) for various cellular functions, at abnormally high levels damage stochastically all molecules, including both the genome (causing, among other effects, mutagenic single and double strand breaks[78]), as well as the proteome where they promote protein oxidative modifications, such as carbonylation,[79] formation of peroxyl radicals,[80] glycation and glycoxidation.[81] Among the spontaneous non-enzymatic processes that start under hyperglycaemic and/or oxidative stress conditions and cause significant damage to all cellular biomolecules is also the formation of Advanced Glycation- (AGEs) or Lipidation- (ALEs) End Products;[82–84] AGEs/ALEs are also derived from exogenous sources that mostly relate to a heat processed diet with high lipid and protein content.[83,85] High levels of extracellular AGEs activate a number of plasma membrane receptors, including the receptor for AGEs (RAGE), resulting in oxidative stress and inflammation.[86,87] Interestingly, despite the fact that various studies found a correlation between oxidative damage

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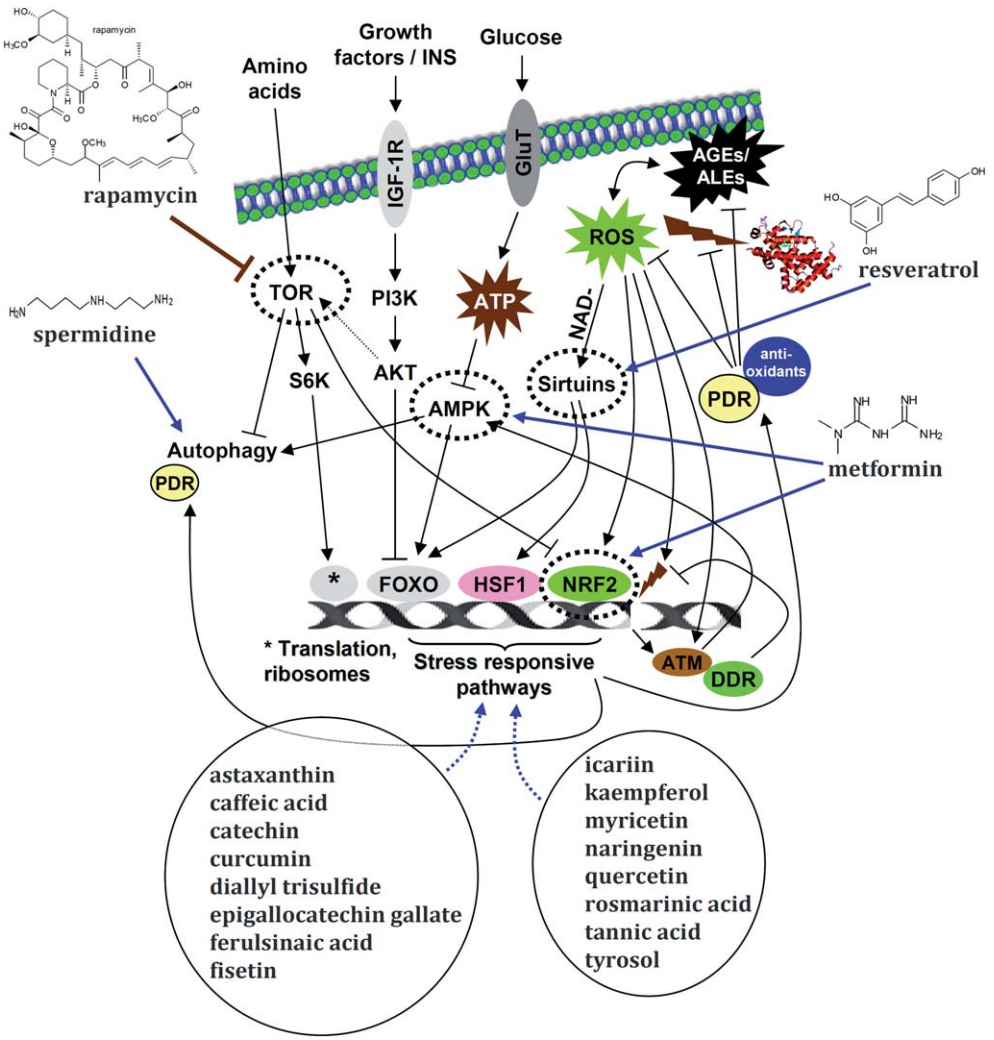

Fig. 4 The main signalling pathways affecting ageing along with natural compounds that reportedly delay *in vivo* ageing. Shown pathways include amino acids, growth factors/INS and glucose signalling (nutrient sensing pathways), as well as stress responsive pathways that trigger DDR or PDR; main stressors like ROS or AGEs/ALEs and their effect(s) on either the genome or the proteome are also shown. The compounds with anti-ageing activity along with the molecular pathways affected are indicated. Interestingly, most (if not all) of the compounds that delay ageing seem to converge in the mobilization of the stress responsive pathways, thus conferring increased protection against the various stressors that damage cellular biomolecules; (→, pathway activation; ↛, pathway inhibition).

and lifespan[88] or age-related diseases,[89] there are enough exceptions and contradictions (mostly in *C. elegans*)[90] to rule out simple causality. Studies in mammals, however, indicate a more direct link between ROS generation and lifespan, as overexpression of catalase (a detoxification enzyme) targeted specifically to mitochondria reduced oxidative stress and extended lifespan,[91] while a mutant of the $p66^{SHC}$ gene increased resistance to oxidative stress and prolonged lifespan[92] or reduced early atherogenesis in mice fed a high-fat diet.[93] In line with the severe impact of accumulating stressors in humans' healthspan, high levels of AGEs (*e.g.* in diabetic patients) have been correlated with increased risk for several age-related diseases.[94–96]

But then how can long-lived animals, like humans, survive for long periods free of disease? This is basically achieved by a strict homeostatic control of both the *in vivo* redox status and the tissue levels of damaged biomolecules.

A first line of defence against fluctuations in stressors (*e.g.* free radicals) is achieved by the action of antioxidants, like vitamins, natural flavonoids, carotenoids, melatonin or α-lipoic acid, that (among others) function as free radical-scavengers.[97] In support, vitamin E delays RS in HDFs[98] and it also prevents the degradation of collagen and consequently cartilage ageing in activated chondrocytes.[99] In the same first line of defence towards the neutralization of stressors are a number of antioxidant enzymes, like superoxide dismutase, catalase and glutathione peroxidase; the stress-induced synthesis of some of these enzymes is mainly triggered by the transcription factor NRF2, which plays a central role in the protection of cells against oxidative and xenobiotic damage.[22,36] In the absence of stress NRF2 is retained in the cytosol in an "off" position, while under conditions of elevated oxidative stress it is activated and translocates to the nucleus in order to stimulate the expression of its downstream gene targets, namely phase II detoxifying enzymes, intracellular redox-balancing proteins and multidrug resistance-associated proteins.[22,36,100]

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A second line of cellular defences to stressors relate to a network of sensors that signal biomolecules damage and mobilize the downstream effectors that either repair or remove damaged biomolecules.

In the case of the genome, these sensors identify DNA damage (*e.g.* single or double stand breaks) and activate a massive signalling network, namely DDR.[26] The primary transducer of the double stand breaks alarm is the protein kinase ataxia telangiectasia mutated (ATM), which phosphorylates numerous key players in the various branches of the DDR.[101] A number of genetic disorders known as segmental progerias (*e.g.* Werner syndrome)[102] involve impaired sensing or repair of DNA damage,[103] while, on the other hand, increased longevity may be associated with more efficient DNA repair.[104] Interestingly, in a recent mouse model of a human progeroid syndrome the transcriptional profiling of the liver showed reduced expression of genes in the INS/IGF-1 signalling pathway and increased anabolic metabolism and antioxidant defences[105] suggesting a shift of cellular resources (in the setting of genomic instability) from growth to repair. Furthermore, ATM functions as an important ROS sensor in human cells[106] that directly inhibits oxidative stress[107] and enhances autophagy *via* AMPK activation.[108]

At the proteome level, proteotoxic stress-derived PDR activation mobilizes the so-called proteostasis network (PN), which ensures proteome stability. PN comprises folding enzymes, trafficking components that influence compartmental localization, chaperones and degradation machineries like the autophagy-lysosome (ALS) and the ubiquitin-proteasome (UPS) systems.[21,24,25] Key modules in proteotoxic stress activated PDR are the endoplasmic reticulum-related unfolded protein response, which regulates proteostasis in both the secretory pathway and extracellularly, as well as the heat shock response, which maintains proteostasis in the cytosol and the nucleus.[21] Heat shock response relies on a small group of transcription factors and, among these; HSF1 is a highly conserved dominant transcription factor that controls cellular responses to heat and many other proteotoxic stressors. Under basal conditions HSF1 cytosolic molecules remain in a dormant state, while under conditions of proteome instability HSF1 molecules are activated and translocate to the nucleus to trigger the expression of molecular chaperones.[35] Molecular chaperones represent key effectors of the heat [and, thus, are also called Heat Shock Proteins (HSPs)] and proteotoxic stress insult responses, as they catalyze the correct folding of nascent polypeptides, prevent protein misfolding and aggregation, refold misfolded proteins and target damaged proteins to proteolytic systems, namely ALS and UPS for degradation.[21,25] ALS is mainly involved in the degradation of protein aggregates and damaged organelles and *via* TOR regulation (see above), it also participates in cellular protein catabolism, namely the turnover of cellular material under nutrient deprivation and growth factor depletion.[109,110] Short-lived nucleo-cytosolic regulatory proteins (marked for degradation by ubiquitination), as well as irreparably damaged proteins, are targeted for degradation by the 26S (or by the 20S) proteasome which thus controls numerous cellular processes, including signal transduction, cell death, immune responses, metabolism, cell cycle progression and development.[21,111]

A consistent observation in cell culture and animal studies has been the age-dependent decline of all PN modulator activities.[21,111,112] Also, lifespan extension from reduced nutrient signalling is suppressed in animals lacking HSF1 activity, while inhibition of HSF1 activity in *C. elegans* reduces lifespan by ~40%.[113,114] Similarly, reducing autophagy or proteasome activities either in human cells or *in vivo*, accelerates proteome damage and decreases lifespan.[9,115,116] On the other hand nematodes expressing additional copies of the HSF1 gene are resistant to hyperthermia and oxidative stress and they live longer compared to their wild-type counterparts,[113] while HSF1 and the chaperone sHSP-16.1 mediate cytoprotection under heat stroke and extend lifespan.[117] Furthermore, increasing autophagy in flies' neurons extends lifespan[116] and proteasome activity enhancement in HDFs delayed the appearance of RS.[118] Conclusively, the long-term sustained functionality of stress responsive pathways is central to organisms' optimum homeostasis and longevity assurance.

3 Natural compounds that exert anti-ageing effects

Natural compounds that were reportedly found to delay ageing at the organism level (*i.e.* exhibiting *in vivo* anti-ageing effects on model organisms) are grouped and presented according to their source of isolation (marine organisms, microorganisms and plants) and the structural categories to which they belong. Compounds that have been found to extend mammalian cells' replicative lifespan are also reported since they have the potential to be developed or to be tested *in vivo* as anti-ageing modulators in model organisms. Special emphasis was given to the studies where, besides the effect on healthspan/lifespan, the activity of a compound was related to a specific signalling pathway and in this regard the present review can be considered exhaustive.

3.1 Natural compounds isolated from marine organisms or microorganisms

The enormous biodiversity of marine ecosystems offer multiple opportunities for bio-prospecting, exploitation and use as it is commonly accepted that marine organisms possess the capacity to produce a huge diversity of molecules with unique structural features and biological potency due to the physicochemical properties of the marine environment. Thus, marine environment will definitely be an interesting source of natural compounds with promising skeletons and decoration patterns with potent anti-ageing properties. Several natural compounds isolated from marine organisms were found to prevent cellular senescence and to exert anti-photoageing or photoprotective effects.[119,120] One such example is dieckol (**1**), a phlorotannin isolated from *Eckloina cava* that was found to inhibit melanogenesis *via* a tyrosinase inhibition assay and to protect cells from UV-B radiation.[121] Similarly, sargaquinoic acid (**2**) and sargachromenol (**3**) from *Sargassum sagamianum*,[122] as well as porphyra-334 (**4**) and shinorine (**5**), isolated from the red alga *Porphyra rosengurttii*[123] and collemin A (**6**), a mycosporine isolated from the lichenized ascomycete *Collema cristatum* have

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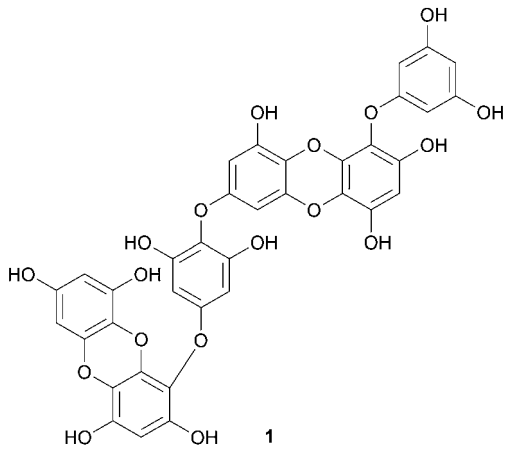

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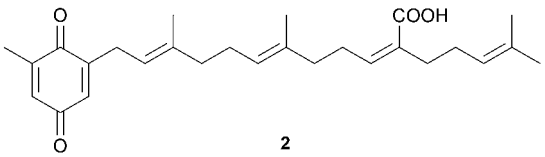

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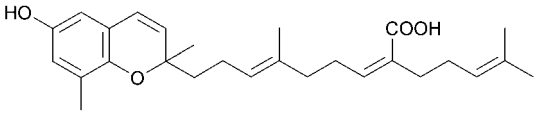

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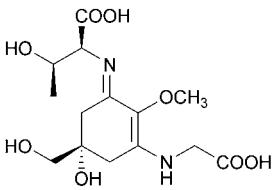

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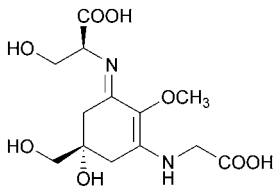

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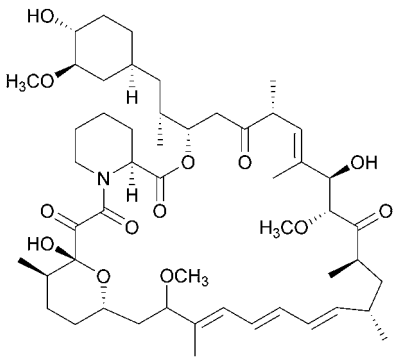
shown significant photoprotective activities.[124] Astaxanthin (**7**) is a carotenoid that is found in some marine organisms and it extends *C. elegans* mean lifespan by ~16–30%, when administered (at 0.1–1 mM) during pre-reproductive and young adult stages, by enhancing the expression of antioxidant enzymes (*e.g.* superoxide dismutases and catalases) and activating the DAF-16/FOXO pathway.[125]

A promising natural product in relation to its anti-ageing properties is the anti-fungal agent rapamycin (**8**). Rapamycin is currently used as an immunosuppressant and was first isolated from the bacteria *Streptomyces hygroscopicus* strain AY B994. Rapamycin was proven to be a potent inhibitor of the TOR pathway and it extended median and maximal lifespan of both male (~9%) and female (~14%) genetically heterogeneous mice at a food concentration of 14 mg kg^{-1}.[126] More recently it was reported that age-related alterations in various body organs (including heart and liver), as well as the age-dependent decline in spontaneous activity occurred more slowly in rapamycin-treated long-lived mice;[127] notably, in this study despite any assumed beneficial effects in relation to neoplastic disease, a number of side-effects were noted, including significantly higher incidence of testicular degeneration and cataracts. Rapamycin was also found to enhance longevity of *C. elegans* in a SKN-1/NRF2 (but not DAF-16/FOXO) dependent fashion at 100 μM,[128] while feeding adult *D. melanogaster* flies with 200 μM rapamycin resulted in lifespan extension that was associated with increased resistance to both starvation and oxidative stress. Analysis of the underlying mechanisms revealed that rapamycin increased longevity through (among others) the inhibition of the TOR pathway and the downstream induced alterations to both autophagy and the rate of protein synthesis.[129] Several studies indicate the protective effects of rapamycin against various age-related diseases as it significantly suppressed tumor onset in transgenic cancer-prone mice[130] and halted the progression of Alzheimer's-like deficits in the human amyloid precursor protein mouse model.[131]



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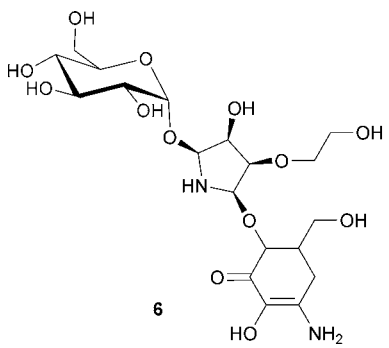
3.2 Natural compounds derived from plants

The age-modulating properties of the polyphenolic bioactive compound, curcumin (**9**) that derives from the rhizome of the plant *Curcuma longa* (Zingiberaceae) have been demonstrated in



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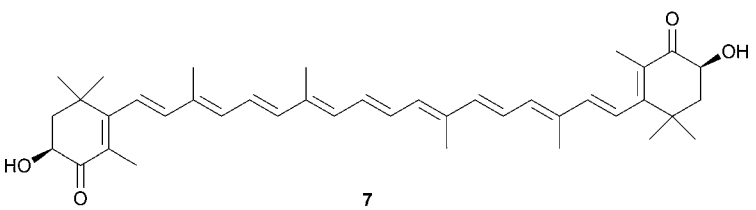

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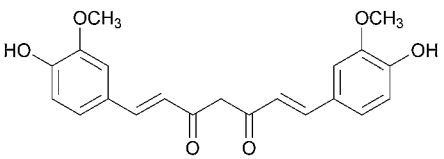
most model organisms tested. Specifically, exposure of *C. elegans* to 20 μM curcumin extended worms' mean lifespan by ~21% *via* effects relating to body size and the pharyngeal pumping rate but not reproduction. At the molecular level it was found that curcumin upregulated several stress responsive pathways, including sirtuins and (most importantly) the antioxidant responses transcription factor SKN-1/NRF2.[132] Likewise, 0.5–1.0 mg g^{-1} of curcumin in the culture medium extended by more than 10% the mean lifespan of *Drosophila* flies by suppressing oxidative stress and lipid peroxidation, reducing accumulation of malondialdehyde and improving locomotor performance; these effects have been attributed to the modulation of a number of stress-responsive genes, including the antioxidant enzyme superoxide dismutase.[133,134] Interestingly, high concentrations of curcumin did not extend the lifespan of CR flies, suggesting that curcumin and CR operate *via* the same nutrient sensing signalling pathways.[135] In support of the beneficial *in vivo* effects of curcumin[136] this compound was found to significantly increase both the healthspan and lifespan (up to 75%) at a concentration of 0.01% (w/w) when tested in five different *Drosophila* models of Alzheimer's disease; this effect was explained by the finding that curcumin promoted amyloid fibril conversion by reducing the pre-fibrillar/oligomeric species of amyloid-beta, thus resulting in reduced neurotoxicity.[137] In mammals, curcumin administration in an Alzheimer's disease trangenic mouse model suppressed indices of inflammation and oxidative damage in the brain and it also decreased the overall amyloid content and plaque burden.[138–140] Reportedly, curcumin activates signalling pathways downstream of the anti-ageing modulators AMPK and NRF2 and suppresses inflammatory processes mediated by NF-kB signalling;[141,142] because of these promising findings curcumin was tested in humans as a drug candidate for Alzheimer's disease.[143,144] Interestingly, a metabolite of curcumin, namely tetrahydrocurcumin (**10**), increased healthspan (but not maximum lifespan) when administered in *Drosophila* flies at a concentration of 50 μM and suppressed oxidative stress by regulating sirtuins- and FOXO-responsive pathways. In support of the beneficial effects of tetrahydrocurcumin, mice that received diets containing this compound at a concentration of 0.2% (w/w) had significantly longer average lifespans by 11.7% (compared to control mice) when the administration started at the age of 13 months.[145,146]



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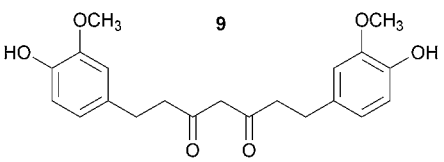

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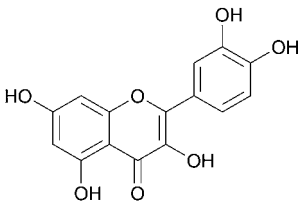
Quercetin (**11**) is a well known flavonoid of the flavonol class, which is commonly found, among others, in fruits and vegetables. It was shown that 330 μM quercetin increased oxidative stress resistance and longevity by ~60% in *S. cerevisiae*;[147] likewise at concentrations up to 200 μM it increased resistance to thermal and oxidative stress and extended the lifespan of *C. elegans* worms by up to 18%. At the molecular level quercetin seems to exert highly antioxidative properties and also modulates a number of genes, mostly related to nutrient sensing pathways (*e.g. daf-2*) but not DAF-16/FOXO or sirtuins.[148–151] Quercetin was also shown to reverse cognitive deficits in aged and ethanol-intoxicated mice[152] and it has been demonstrated that either quercetin or its derivative quercetin caprylate (**12**), enhanced proteasome activity, conferred resistance to oxidative stress and extended the replicative lifespan of HDFs.[153] The potential anti-ageing activities of two glycosides of quercetin from onion cultivars, namely quercetin 3′-*O*-β-D-glucopyranoside (**13**) and quercetin 3-*O*-β-D-glucopyranoside-(4→1)-β-D-glucopyranoside (**14**), have also been evaluated in *C. elegans*. At concentrations of 41 μM and 32 μM, respectively, both compounds have been shown to have lifespan extending properties by modulating stress tolerance responsive pathways with quercetin 3-*O*-β-D-glucopyranoside-(4→1)-β-D-glucopyranoside being more active.[154] In support of the potent anti-ageing activity of this category of compounds the methylated derivatives of quercetin, isorhamnetin [quercetin 3′-*O*-methylether)] (**15**) and tamarixetin (quercetin 4′-*O*-methylether) (**16**) were found (at 200 μM) to prolong the mean lifespan of *C. elegans* by 6–12%, and increase the worms' resistance against both thermal stress and juglone (a potent oxidant)-induced oxidative stress.[155]



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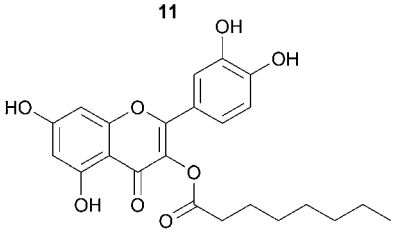

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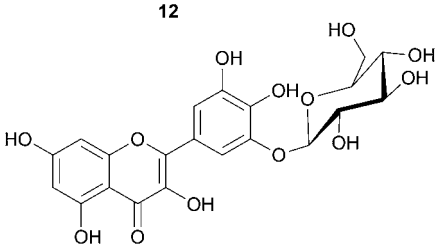

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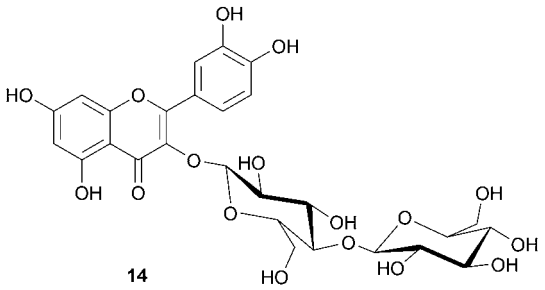

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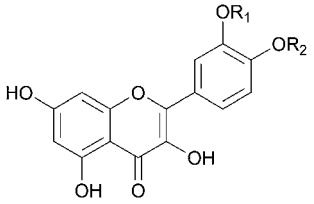
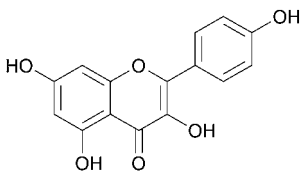


15 $R_1 = CH_3$, $R_2 = H$
16 $R_1 = H$, $R_2 = CH_3$

Kaempferol (**17**) is a natural flavonol, which like quercetin is a flavonoid found in dietary products. It was shown that at 100 μM this compound increased the survival of *C. elegans* by 10%, protected them from thermal stress and suppressed the accumulation of intracellular ROS and lipofuscin (a biomarker of ageing that relates to protein aggregation); these protective effects were likely induced by the activation of the DAF-16/FOXO stress responsive pathway.[156] Similar studies on the activity of another flavonol that is present in edible plants, namely fisetin (**18**), revealed that it also protected against both thermal and oxidative stress[157] and that it promoted the activation of the DAF-16/FOXO stress-responsive signalling pathway.[156] In support, fisetin (at a concentration of 10 μM) promoted a 55% lifespan lengthening in yeast.[158]



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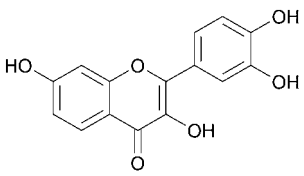

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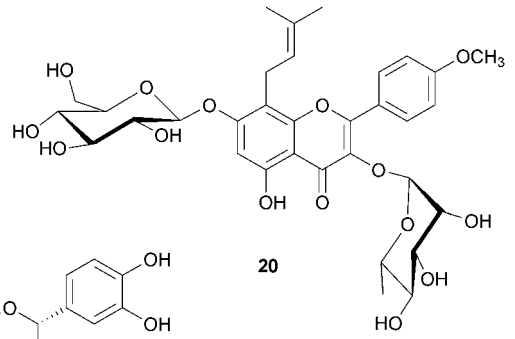
Increased longevity of *C. elegans* (up to 14%) was also observed after treatment with 200 μM of a compound belonging to the class of flavanols, namely catechin (**19**). These lifespan extending effects were also accompanied by enhanced resistance to oxidative and thermal stress and most probably relate to a broad spectrum of detoxification rather than a simple antioxidative action.[159] Intriguingly, however, in other studies neither catechin (at 200 μM) nor its isomer epicatechin (at 224 μM) extended the lifespan of *C. elegans*.[155,160] Icariin (**20**) is a flavonol diglycoside extracted from several plant species of the *Epimedium* genus that at 45 μM reportedly extended *C. elegans* mean lifespan by ~21%. Since pharmacokinetic analysis showed a high level of icariside II in the animals treated with icariin it was suggested that icariside II (**21**) was the predominant bioactive form *in vivo*. This latter compound was also tested in *C. elegans* and at the concentration of 20 μM was found to increase thermo and oxidative stress tolerance; a decrease in the rate of locomotion decline in late adulthood and it also extended worms' lifespan by 20%.[161] It was postulated that the lifespan extension caused by icariside II was dependent on the INS/IGF-1 and DAF-2/FOXO (and likely HSF1) signalling pathways, since *daf-16* and *daf-2* mutants failed to show any lifespan extension upon icariside II treatment.[161] Interestingly, icariside II seems to also exert a potent protective activity against age-related diseases as it was found to delay the onset of paralysis mediated by polyQ and Aβ(1–42) proteotoxicity in two *C. elegans* models of human proteotoxic diseases.[161]



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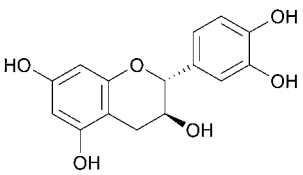

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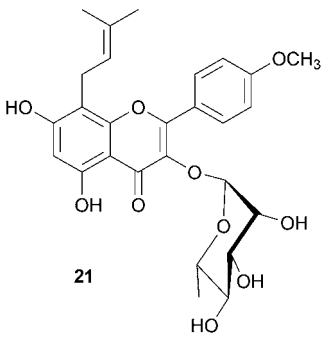

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Considering that sirtuins activity has been positively related to lifespan extending effects (see above) several studies have attempted to isolate sirtuin activators. More specifically, in a screening of compound libraries for SIRT1 modulators, butein (**22**), a plant derived tetrahydroxychalcone, was found at 10 μM to increase lifespan of the yeast *S. cerevisiae* by 31%.[158] In the same category of sirtuin activators a natural compound that has attracted significant interest (but has also been the subject of considerable debate and controversy) is resveratrol (**23**); a stilbene found in various berries, nuts, and other plants sources. The anti-ageing potential of resveratrol has been impressive since it has demonstrated biological effects in both eukaryotic cells and in yeast, as well as in the full spectrum of metazoans where by being tested in various doses it exhibited lifespan increase by up to 56%.[162] At cell based assays (*e.g.* in HDFs) resveratrol delayed the appearance of RS markers[163,164] and showed protective effects against DNA oxidative damage that were accompanied with a reduction in the levels of acetylated forms of the H3 and H4 histones and p53.[165] In the budding yeast *S. cerevisiae* resveratrol stimulated *sir2* sirtuin activity, increased DNA stability and cell survival and extended lifespan by ~70% at a concentration of 10

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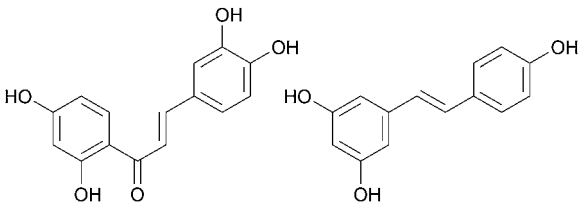
μM.[158] In support, wild-type adult worms showed an increase in lifespan upon resveratrol treatment[166] and it has been proposed that this effect was dependent upon *sir-2.1* (but not DAF-16/FOXO) activity.[166,167] Similarly, 200 μM of resveratrol increased longevity in flies by ~20% in a *sir2* dependent fashion.[166,168] Interestingly enough, although resveratrol exerted no lifespan prolonging effects in mice fed with a normal diet,[169] when administered to middle-aged mice fed with a high-calorie diet it shifted the physiology of treated mice towards that of mice on a standard diet and significantly increased their survival; this latter intervention also restored normal insulin sensitivity, reduced IGF-1 levels, increased AMPK activity and improved mitochondria number and function.[170,171] Nonetheless, according to other studies sirtuins may not be the direct target of resveratrol as this compound cannot promote the deacetylation of native SIRT1 substrates,[172,173] while in *C. elegans*, *sir2* extends lifespan through DAF-16/FOXO contrary to the fact that resveratrol does not (see above[167]); thus resveratrol may not only act as a simple sirtuin activator. Despite this ongoing debate, there is robust evidence showing that resveratrol exerts beneficial effects in healthy ageing and that this effect may engage sirtuins- and, most likely, stress-related pathways.



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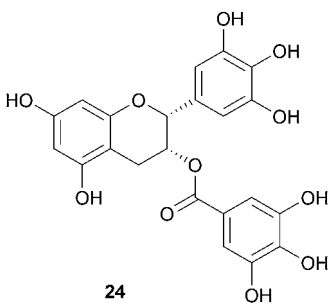
Administration of 220 μM epigallocatechin gallate (**24**), a major green tea polyphenol, in *C. elegans* increased the mean lifespan of the worm by ~10%, significantly attenuated intracellular oxidative stress and decreased formation of lipofuscin; most likely *via* the activation of the DAF-16/FOXO signalling pathway.[174,175] However, two other independent studies showed that at lower concentrations the increase in lifespan was marginal and it was only improved under heat and, especially, under oxidative stress.[176,177] The authors claimed that the free radical-scavenging effects of the compound along with the induction of stress-resistance-related proteins like superoxide dismutase-3 and heat shock protein-16.2 could be responsible for these effects.[177] Interestingly, epigallocatechin gallate has been also proposed as a candidate agent for the prevention and/or treatment of skin photoageing as it was found to inhibit the activation of matrix metalloproteinases and the destruction of collagen in UV-B irradiated human dermal fibroblasts.[178]



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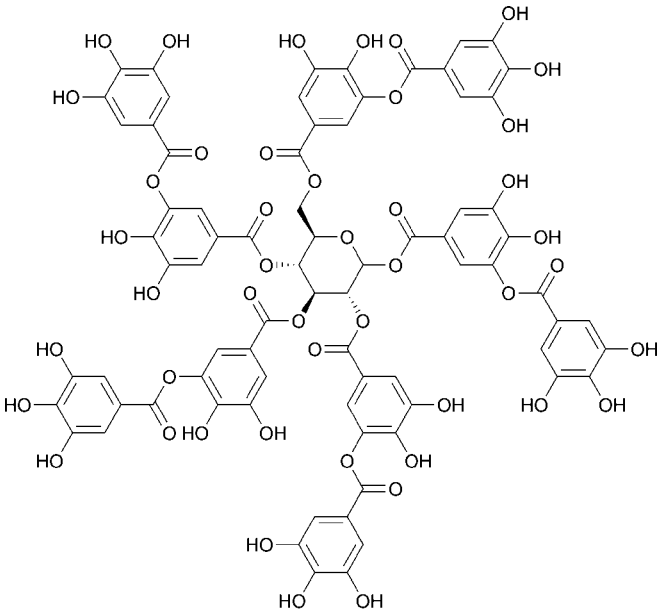

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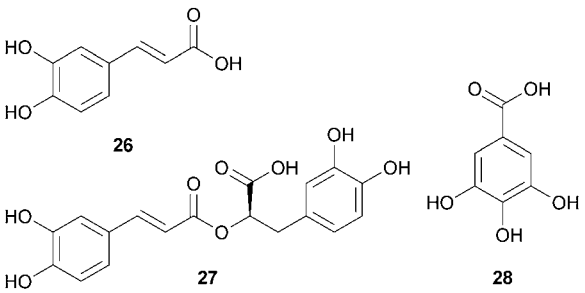

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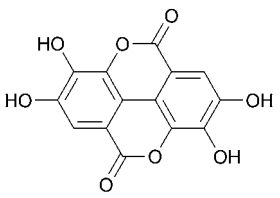
Exposure to 100 μM of the polyphenol tannic acid (**25**) induced potent life-prolonging properties in *C. elegans*, enhanced thermal stress resistance and slightly increased oxidative stress resistance in the absence of reproductive capacities and pharyngeal pumping rate modulation. It was postulated that tannic acid may mimic pathogenic stressors, most probably by inducing a hormetic effect.[179] Moreover, the phenolic acids, caffeic acid (**26**) (at 300 μM) and rosmarinic acid (**27**) (at 200 μM) extended lifespan (by ~11%) and enhanced thermotolerance of *C. elegans via* their antioxidative properties,



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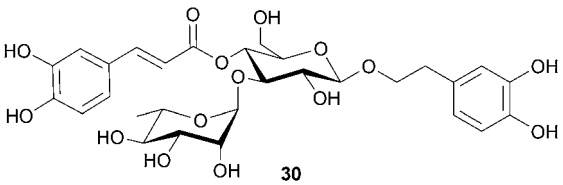

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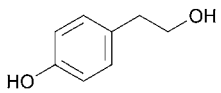
which are reportedly based on (among others) sirtuins and the DAF-16/FOXO (in the case of caffeic acid) signalling pathways.[180] In addition gallic acid (**28**) and ellagic acid (**29**) (at 25 μM and 800 μM, respectively) increased lifespan in *C. elegans*[181] by ~10%, while supplementation of 0.64 to 2.56 mg mL^{-1} of the herbal phenylethanoid glycoside acteoside (**30**) in *Drosophila* flies culture medium increased fly lifespan by 9 to 15%.[182]

Tyrosol (**31**) is a simple phenylethanoid, present in a variety of natural sources but mostly related to olive oil. Significant lifespan extension (~11%) was observed in the nematode *C. elegans* at a moderate concentration (250 μM) of the compound, which was likely mediated by an enhanced thermotolerance and resistance to oxidative stress with no notable effects on the overall growth in the nematodes. Apart from the known antioxidant action of tyrosol, lifespan experiments with several *C. elegans* mutant strains revealed that components of the heat shock response (HSF-1) and the INS/IGF-1 and DAF-16/FOXO signalling might also be implicated in the observed phenotypes; thus it was postulated that the compound may act by promoting a hormetic effect.[183]



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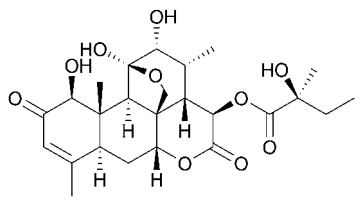
Glaucarubinone (**32**), a quassinoid known from different species of the tropical plant family Simaroubaceae, was found at 100 nM to significantly extend (~80%) lifespan in *C. elegans*; glaucarubinone also reduced the body fat content of *C. elegans* indicating that it may act through the nutrient sensing pathway.[184]



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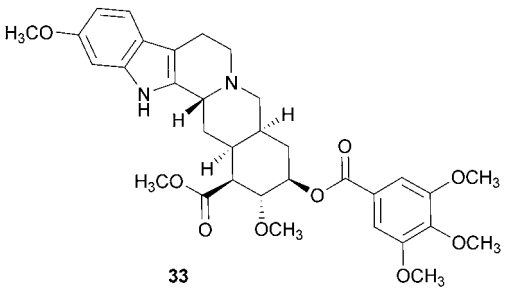
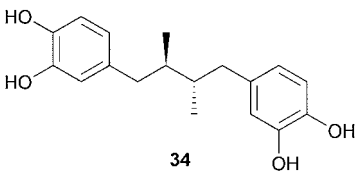
Reserpine (**33**), an indole alkaloid isolated from the dried root of *Rauwolfia serpentine*, constitutes an FDA-approved antihypertensive drug. It increased *C. elegans* lifespan by 31% upon chronic treatment from embryo till death at a concentration of 30 μM, and it also provided stress tolerance and prolonged mobility of the worms. Reserpine seemed to exert its effects independently of the INS/IGF-1 signalling pathway,[185] while in a *C. elegans* model of Alzheimer's disease it significantly delayed paralysis, improved movement and thermotolerance and increased mean lifespan by 46% when added at 60 μM in the worms culture medium.[186]



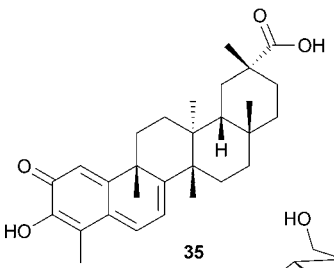
33

Nordihydroguaiaretic acid (**34**), a plant lignan, increased median survival by 12% when added at 2.5 g kg^{-1} of food in genetically heterogeneous male mice.[187] Older studies had also shown healthspan increasing effects in fruit flies, mosquitoes, and rats;[188,189,190] nordihydroguaiaretic acid was also found to extend, at 2500 ppm, the lifespan of a mutant mouse model of amyotrophic lateral sclerosis by 32%.[191]



34

In the category of terpenoids, celastrol (**35**); is a pentacyclic triterpenoid that extended lifespan by 9.4% and 13% when administrated in a transgenic mouse model of amyotrophic lateral sclerosis at 2 mg kg^{-1} day^{-1} and 8 mg kg^{-1} day^{-1} doses, respectively;[192] Crocin (**36**), a carotenoid isolated from Kashmiri saffron (*Crocus sativus*), increased the lifespan of Dalton's lymphoma-bearing animals by 44%.[193] Moreover, the garlic constituent diallyl trisulfide (**37**) reportedly increased *C. elegans* longevity at a concentration of 10 μM by 12.6% (mean lifespan) independently of the INS/IGF-1 and DAF-16/FOXO pathways *via* the activation of the antioxidant transcription factor SKN-1/NRF2.[194]



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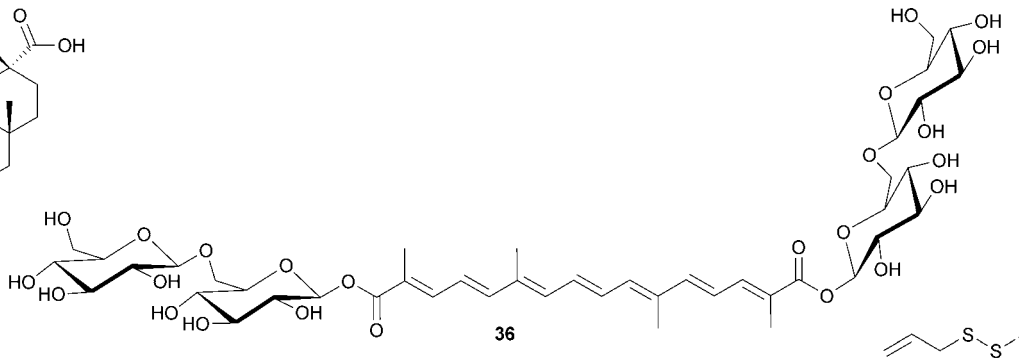

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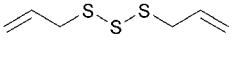

37

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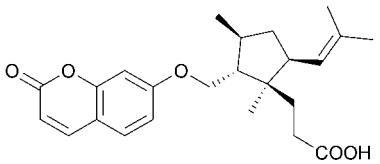
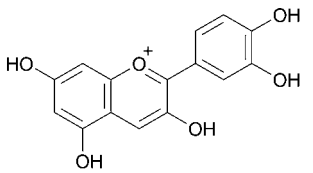
Ferulsinaic acid (**38**) is the first member of a new rearranged class of sesquiterpene coumarins of the genus *Ferula* that increased longevity of *C. elegans* in a dose-dependent manner as 500 nM, 10 μM and 100 μM lengthened mean lifespan of the worms by 1.93, 11.37 and 18.03%, respectively. The observed improvement in the resistance to heat and oxidative stress and the attenuation of lipid peroxidation and protein glycosylation suggested that the lifespan extension might be attributed to the antioxidant effect of the compound.[195]



38

The cell protective effect of the pigment cyanidin (**39**), at 1.7 μM and 35 μM applied concentrations, was investigated in human fibroblasts where this compound was found to enhance replicative lifespan and protect against SIPS by ameliorating oxidative stress and lipid peroxidation.[196] Similarly, oleuropein, (**40**) the major constituent of *Olea europea* leaf extract, when studied for its cell protective effects in human fibroblasts at a concentration of 1.0 μM was found to suppress oxidative stress, reduce protein oxidation and activate the proteasome throughout the entire cellular lifespan; it also delayed the appearance of cellular senescence as it increased replicative cellular lifespan by ~15%.[197] Two isomers of 4-hydroxy-5-hydroxymethyl-[1,3]dioxolan-2,6′-spirane-5′,6′,7′,8′-tetrahydro-indolizine-3′-carbaldehyde (HDTIC), HDTIC-1 (**41**) and HDTIC-2 (**42**), extracted from the herb *Astragalus membranaceus* var. *mongholicus* were also investigated for their effects on cellular RS in normal human fibroblasts. The results suggested that both the HDTIC-1 (at 0.1 μM) and HDTIC-2 (at 1.0 μM) delayed RS of cells, while HDTIC also showed positive effects towards cell growth and proliferation. This study nicely illustrated how the structure of the compounds affects their activity, as was evident by the differences of optimum concentrations of the isomers in delaying senescence.[198]



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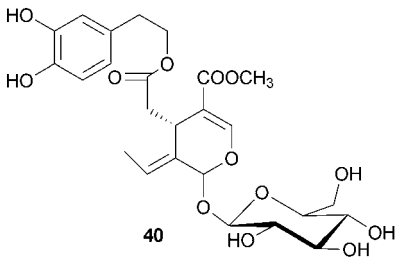

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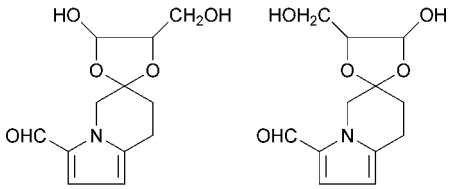
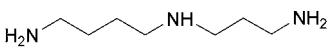


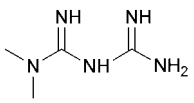
41 **42**

Spermidine (**43**) is a polycationic polyamine involved in multiple biological processes and it is reported herein because of its exceptional anti-ageing activity. Specifically, spermidine has attracted the interest of the scientific community because of its action as an inducer of autophagy both in cultured cells and *in vivo*,[199] as well as because an exogenous supply of millimolar concentrations of spermidine improves both the chronological and the replicative lifespan of yeast cells and it causes lifespan extension (while inducing autophagy) in both *C. elegans* and in *Drosophila* flies; additionally spermidine significantly reduces oxidative damage in mice, indicating that this agent may act as a universal anti-ageing compound.[200,201] Interestingly, it seems that spermidine activates autophagy not *via* sirtuins (histone deacetylases) activation (as in the case of resveratrol) but through the inhibition of cellular histone acetylases, which by promoting protein hypoacetylation result in autophagy activation.[200–202]



43

Metformin (**44**) belongs to the class of biguanides, which although they have never been reported in plants, derive from guanidine, which has been reported in *Galega officinalis*. This compound is an oral antidiabetic drug and it is referred to herein as it has been found to (among others) activate AMPK resulting in the dampening of the nutrients signalling pathways by suppressing TOR and S6K activity.[203,204] Studies on *C. elegans* proved that at a concentration of 50 mM it increased healthspan, delayed lipofuscin accumulation, increased locomotion and significantly extended median lifespan by 40%. It was shown that its action was independent of the INS/IGF-1 signalling pathway and DAF-16/FOXO since metformin still extended lifespan of a *daf-16* null mutant strain. In the same study it was shown that the healthspan benefits of metformin could also be mediated through the activation of the SKN-1/NRF2 transcription factor.[205] The anti-ageing effects of metformin have been also studied in mammals, where it has been shown that 100 mg kg^{-1} in drinking water provoked ~38% extension of mean lifespan in female outbred spontaneously hypertensive mice.[206] Moreover, a metformin dose of ~15 mM prolonged the mean lifespan of male mice in a transgenic mouse model of Huntington's disease by 20%,[207] while at 100 mg kg^{-1} in drinking water it extended by 8% the mean lifespan of female transgenic HER-2/neu mice, most likely by exerting an anti-tumour action.[208]



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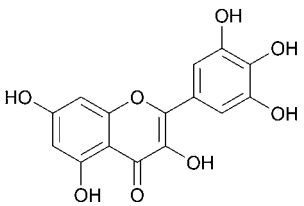
The results and experimental approaches presented in this section can be used as a useful guide for further research and for the development of screening projects aiming to identify novel compounds, structures or scaffolds with healthspan and/or lifespan increasing properties. This is particularly needed as, despite the reported beneficial effects in longevity, the aforementioned compounds may induce significant adverse-effects when administered for extensive periods in mammals (*e.g.* rapamycin[127]). For instance, in the case of resveratrol, nutraceutical companies are selling various formulations that contain the molecule[209] but despite announcements of planned clinical trials, questions about dosing and mechanism have hampered progress, and very little human data, other than short-term pharmacokinetic and safety studies are known.[210]

Notably, most compounds that have been tested by now have derived from plants and belong to various groups of phenols, whereas terpenoids, despite also being a big class of compounds haven't been tested so far in depth. Also, as most of the reported observations relate to testing of the natural compounds to the nematode *C. elegans* (apparently due to the short lifespan that allows accelerated *in vivo* screening) they should be verified in other model organisms like flies and (preferentially) mice. Furthermore, it is obvious that the assessment of the anti-ageing potential of a natural compound comprises a holistic approach, demanding the investigation of various matters, including modes of actions and molecular targets, effective dosage and bioavailability. It is also worth mentioning that, though it was believed that a natural compound's potential is based on its antioxidant ability, it seems that it could also act independently of antioxidant ability by directly modulating various signalling pathways that affect the progress of ageing. In-depth studies should also relate to structure–activity relationships, since they could further assist in the search of bioactive compounds. In this context a very interesting recent study in *C. elegans* revealed that from the structurally related flavonoids, myricetin (**45**), quercetin (**11**), kaempferol (**17**) and naringenin (**46**), solely flavonols (myricetin, quercetin and kaempferol) increased lifespan of wild type animals. In order to elucidate whether an antioxidant action was the underlying mechanism of the observed lifespan-extension, ROS-levels and lifespan were assessed in *mev-1* mutants that are short-lived due to high levels of oxidative stress.[211] While treatment with the flavonols reduced mitochondrial ROS only myricetin elongated the *mev-1* mutant lifespan, suggesting that the flavonols antioxidant action alone was not sufficient to promote longevity. Interestingly, it was also demonstrated that the structural properties of the C-ring and an increasing number of OH-groups at the B-ring of flavonols could be major prerequisites for *in vivo* lifespan extension in *C. elegans*.[157]



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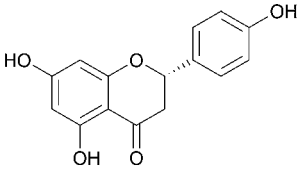

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4 Extracts with anti-ageing activity

Extracts represent valuable sources of bioactive natural compounds and thus in this section we will review those extracts that reportedly have the potential to delay cellular senescence or *in vivo* ageing in model organisms. Formulas of more than one extract that have been reported to affect ageing are only presented when their exact complete composition is known.

4.1 Herbal extracts

The perennial shrub *Rosa damascena* (Rosaceae) is cultivated mainly for rose oil that is used in perfumery, as well as for rose water used to flavour food. Rose petals are a rich source of phenolic compounds, such as flavonoid glycosides and anthocyanins and their extracts exert antioxidant, skin protective, cardiovascular disease prevention and antibacterial effects.[212–216] In support, *Drosophila* flies fed with culture medium also containing a 2 mg mL^{-1} aqueous extract of rose petals exhibited increased longevity by 27%, with no reduction in fecundity;[217] this effect was attributed to the *R. damascena* extract-mediated antioxidant action.[218]

The health promoting effects of a water alcoholic extract obtained from *Rhodiola rosea* (Crassulaceae), namely anticancer, antioxidant and cardioprotective activities[219] have been attributed to a number of secondary metabolites, including monoterpene alcohols and their glycosides, cyanogenic glycosides, phenylethanoids, phenylpropanoids, flavonoids, arylglycosides, proanthocyanidins and other phenolic acid derivatives. It was shown that these extracts increase longevity of worms and flies without negative effects on reproduction or metabolic rate.[220,221] In support, a recent study on the effects of the aqueous extract from *R. rosea* root showed a positive effect on lifespan of *S. cerevisiae*; interestingly, in this case lifespan extension was not *via* protection against oxidative stress.[219] *Eleutherococcus senticosus* (or *Acanthopanax senticosus*-Araliaceae), also termed as Siberian ginseng, is used for the treatment of various adverse conditions, including several allergies. The radix of *E. senticosus* contains several phytochemicals, including lignans, phenypropanoid glycosides, flavonoids and saponins,[222] whereas, the fruits are rich in the anthocyanin cyanidin-3-*O*-(2″-*O*-xylosyl)-glucoside, which possesses strong antioxidant activities and potent inhibitory effects on xanthine oxidase.[223] Extracts from this plant (at 250 μg mL^{-1}) were found to increase lifespan in *C. elegans* by 16% and conferred increased resistance to various stressors, including UV, heat and oxidative stress.[221] *Ginkgo biloba* (Ginkgoaceae) has been around for ~200 million years and it is thus considered as a "living fossil". Leaves and seeds of *Ginkgo* are used for therapeutic purposes in traditional Chinese medicine and contain a large number of secondary metabolites, including terpenoids, polyphenols, allyl phenols, organic acids, carbohydrates, fatty acids and lipids, inorganic salts and amino acids. From these, the flavonoid glycosides and terpene trilactones are recognized as the most active constituents of *Ginkgo* extracts, which reportedly exert antioxidant, anti-asthmatic, radical scavenging, wound healing and neuroprotective properties.[224] The extract of *G. biloba* leaves EGb-761 (which has been used for many therapeutic

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purposes) contains 24% ginko-flavone glycosides (*e.g.*, kaempferol, quercetin, and isorhamnetin derivatives) and 6% terpenoids (*e.g.*, ginkgolides and bilobalide) and at a concentration of 100 μg mL^{-1} has been found to extend nematode lifespan by ~10% and to enhance resistance to thermal and oxidative stress.[225] *Cynomorium songaricum* (Cynomoriaceae) is a parasitic plant that has been widely used in traditional Chinese medicine as tonic, and it is also consumed due to its high nutritional value.[226] Catechin and flavan-3-ol oligomers (known as procyanidins) are the most active constituents of *C. songaricum* extracts that possess remarkable antioxidant, as well as α-glucosidase and HIV-1 protease inhibitory properties.[227,228] The *C. songaricum* flavonoids act as free radical scavengers,[229] while feeding flies with *C. songaricum* extracts (20 mg mL^{-1}) enhanced cognitive behaviour, increased resistance to stress and extended female mean lifespan.[230] Finally, *n*-butanol, aqueous and alcoholic extracts of either the roots or the leaves of *Damnacanthus officinarum* (Rubiaceae) have shown *in vivo* neuroprotective and lifespan extending activity in *C. elegans* by 10–30% at doses of 0.8–1.2 mg mL^{-1}.[231] *D. officinarum* has been used in traditional Chinese medicine as a tonic agent and for the treatment of nervous system syndromes and although there are no studies on its phytochemical profile, the active ingredients are considered to be anthraquinones and their glycosides due to its close genetic relation with the more extensively studied species *Morinda officinalis* (Rubiaceae).[232] A particular area that is currently under investigation is the determination of the active ingredients in traditional medicines known to exert health-promoting effects. Specifically, a traditional Chinese herbal formula that consists of ten herbs was found to suppress oxidative stress, enhance small HSPs expression and extend mean lifespan by 11.7% in *C. elegans* when supplied at the concentration of 100 μg mL^{-1}. Subsequent studies revealed that this bioactivity was exerted by only two (out of the ten) herbs, namely the *Panax ginseng* (Araliaceae) root and the *Cinnamomum cassia* (Lauraceae) bark, which were also found to delay human amyloid beta induced toxicity in *C. elegans*.[233] Moreover, *Radix ginseng* is one of the seven components in another Chinese formula that was found to retard ageing in mice, likely by affecting mitochondria functionality.[234] Finally, a traditional medicine-related formula composed of six herbal constituents (*Paeoniae radix*, *Cnidii rhizoma*, *Carthami flos*, *Cyperi rhizoma*, *Saussureae radix and Salviae miltiorrhizae radix*) delayed senescence of HDFs under oxidative stress conditions and prevented age-related lipidosis in rats,[235,236] while another complex herbal ayurvedic formulation was found to extend flies' lifespan by 55%.[237]

4.2 Extracts derived from edible sources

As mentioned above, diet-interventions such as CR or balanced healthy diets are among the most effective approaches for extending healthspan and/or lifespan due to either sustained mild activation of stress responsive pathways and/or low levels of biomolecule damage (Fig. 3). It is thus not surprising that extracts from (among others) edible spices, legumes, vegetables or fruits have demonstrated significant anti-ageing activity when tested in model organisms. Interestingly, the so-called Mediterranean diet, which has been established by numerous epidemiological studies[238] as a dietary pattern recommended for increased healthspan and longevity, is characterized by a high intake of olive oil, fresh fruits, legumes and vegetables and conversely low intake of animal fat, meat, processed meat products and salty foods,[239,240] which may result in premature collapse (or deregulation) of the cell defensive mechanisms and/or accelerated rates of biomolecule damage (see also, Fig. 3). Particularly, olive oil (the most representative food of the traditional Mediterranean Diet) and especially extra virgin oil, has multiple beneficial effects on health and longevity in humans as it contains high levels of phenolic compounds, which possess strong antioxidant, anti-inflammatory and other health promoting properties.[241,242]

Oregano (*Origanum vulgare*, Labiatae) is an aromatic perennial herb used widely as spice. Thymol and carvacrol are the main components of oregano oil, which possess strong antimicrobial activity, while *O. vulgare* hydralcoholic extracts have a high phenolic content and are known for their anti-hyperlipidemic and antioxidant properties.[243–245] A mixture of oregano and cranberry extracts (at a concentration of 2%) increased lifespan in fruit flies even when the intervention started at middle aged flies.[246]

The combination of legumes and cereals constitutes a healthy plant protein and lipid source. Common bean (*Phaseolus vulgaris* Fabaceae; a component of the Mediterranean diet) seeds contain high levels of important nutrients, such as proteins, minerals, complex carbohydrates and vitamins, along with a number of micromolecular bioactive compounds (*e.g.* polyphenols, tannins, phytates and saponins) known for their antioxidant, anti-oncogenic or phyto-oestrogenic properties.[247] Both the acidic and basic white kidney bean hydrophilic fractions prolonged longevity of the nematodes by ~16% whereas the hydrophobic fractions reduced longevity; interestingly, these latter extracts are rich in phospholipids and glycophospholipids, which are entirely absent in the hydrophilic extracts.[248]

Spinach (*Spinacia oleracea*, Amaranthaceae) leaves contain highly oxygenated flavone glucuronides, flavonol glycosides and their acylated derivatives (*e.g.* patuletin and spinacetin) that are not common in most other vegetables and possess strong antimutagenic and antioxidant activity.[249–252] Extracts (at 100 μg mL^{-1}) from *S. oleracea* showed beneficial effects on *C. elegans* against heat and oxidative stress, as well as in extending lifespan by ~35%.[253] Similarly, the edible plant commonly known as salad rocket (*Eruca vesicaria* subsp. *sativa*, Cruciferae) is an excellent source of antioxidants like phenolic compounds, carotenoids, glucosinolates and their degradation products, such as isothiocyanates. Reportedly, *E. vesicaria* extracts (at 625 μg mL^{-1}) can inhibit the genotoxic activity of hydrogen peroxide and increase the healthspan of *Drosophila* flies.[254] Broccoli (*Brassica oleracea* var. *italica*, Brassicaceae) is a popular vegetable not only for its flavour but also for its health promoting effects, which have been mainly attributed to glucosinolates and their degradation products. Parallel supplementation of green tea catechins and broccoli extracts in flies partially reversed fat promoted mortality by up-regulating the

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antioxidant enzymes catalase and superoxide dismutase.[255] Moreover, broccoli extracts increased the lifespan of the red flour beetle (*Tribolium castaneum*) under physiological conditions or under heat stress through the activation of stress responses mediated by NRF2 and FOXO;[256] NRF2 activation was most likely mediated by sulforaphane (an isothiocyanate of cruciferous vegetables) that is found in high concentrations in broccoli preparations and was proven to be a potent inducer of NRF2.[22,36,257]

A moderate consumption of wine and/or beverages during meals is present in the Mediterranean diet pattern. *Vitis vinifera* (Vitaceae) parts, such as grape fruits, leaves, seeds and their fermentation products are widely used both as foods and in herbal medicine. Grape extracts contain large amounts of oligomers and polymers of flavan-3-ol units and are known for their potent antioxidant activity. Moreover, resveratrol (a polyphenolic ingredient of these extracts) has demonstrated longevity-promoting effects in several experimental models (see above). Similarly, as fruit extracts are the active ingredients of several dietary supplements a range of fruits (or their juices) are consumed as a vital source of a wide variety of vitamins, antioxidants and other protective natural compounds. *Prunus persica* var. *nectarine* (Rosaceae) is a subspecies of peach and is grown worldwide. Its fruits, known as "nectarines", are widely consumed because of their pleasant taste and high nutritional value, which mostly relates to the presence of important health-promoting ingredients, namely vitamin C, anthocyanins and flavonoids.[258] On a *D. melanogaster* model, food supplementation with 0–4% nectarine extract extended lifespan (by ~22% at females), increased fecundity and decreased expression of some metabolic and oxidative stress-response genes; notably, lifespan extension was observed in flies fed with either standard, CR or high-fat diets. It was proposed by the authors that nectarine promoted longevity and healthspan partly by modulating glucose metabolism and reducing oxidative damage.[259] Huckleberry (*Vaccinium corymbosum*, Ericaceae) contains a wide range of polyphenols, which have antioxidant and anti-inflammatory effects. Its proanthocyanidins-enriched extracts (at 200 $\mu g\ mL^{-1}$) increased the thermotolerance and lifespan (by 28%) of the nematodes.[260] Although berry anthocyanins display low bioavailability they exert beneficial effects on health (mostly) by their antioxidant capacity.[261] Also, it was recently demonstrated that blueberry extracts could significantly extend mean lifespan [*via* the up-regulation of antioxidant enzymes (*e.g.* superoxide dismutase and catalase)] and they also enhanced the locomotor performance of *Drosophila* flies.[262] Cranberry (*Vaccinium oxycoccos*, Ericaceae) juice exerts health benefits in humans, which have been associated with the presence of various polyphenols, including flavonols, anthocyanins and procyanidins; also cranberry phenolic compounds are known for their potent antioxidant and free radical scavenging properties.[263] Recent studies in various experimental models have shown that cranberry extracts (either alone or in combination with oregano extracts) contribute to increased healthspan and longevity[245,246,264] and these effects have been correlated in *C. elegans* with increased activity of DAF-16/FOXO.[264] The fruits of the native tropical palm tree Acai (*Euterpe oleracea*, Arecaceae) have received considerable attention as a new "super fruit" because of its potential health benefits. The high antioxidant and anti-inflammatory capacity of Acai fruits and juice are associated mainly with the presence of polyphenols, such as anthocyanins, proanthocyanidins, other flavonoids (*e.g.* orientin, homoorientin, vitexin, luteolin, chrysoeriol, quercetin and dihydrokaempferol) and lignans [*e.g.* (+)-lariciresinol, (+)-pinoresinol and (+)-syringaresinol].[265–267] In a recent study in flies, it was found that these antioxidant compounds extended lifespan through activation of stress responsive pathways.[268]

Cocoa (*Theobroma cacao*, Sterculiaceae) and chocolate are used not only as a drink because of their pleasurable taste but also as a food. There are numerous studies on the beneficial effects of *T. cacao*, most of which relate to the antioxidant properties of its phenolic ingredients. Specifically, polyphenols of the cocoa beans belong to the groups of catechins [(−)-epicatechin, (+)-catechin, (+)-gallocatechin and (−)-epigallocatechin)], anthocyanidins (cyanidin-3-α-L-arabinoside and cyanidin-3-D-galactoside) and proanthocyanidins (B1, B2, B3, B4, B5, C1, and D).[269] The antioxidant effects of cocoa and its ability to extend flies' lifespan were demonstrated under conditions of moderate oxidative stress or a heavy-metal containing diet.[270] Also, in a recent study aiming to analyse in *S. cerevisiae* and *C. elegans* by transcriptomics analyses the signalling pathways involved in cocoa polyphenols-mediated resistance to oxidative stress, it was demonstrated that these effects depend on sirtuins and DAF-16/FOXO activity.[271] The infusion of the flowering aerial parts of *Stachys lavandulifolia* subsp. *lavandulifolia* (Lamiaceae) is widely used in south Anatolia as a herbal tea and has demonstrated a remarkable radical scavenging activity.[272] As in the case of cocoa, the methanolic extract of the aerial parts is rich in phenolic compounds known for their antioxidant activity, such as the phenylethanoid glycosides (*e.g.* lavandulifolioside B, lavandulifolioside A, verbascoside and leucosceptoside A);[273] the evaluation of the water and ethanol extracts obtained from freshly picked *S. lavandulifolia* flowers and leaves on the longevity of *D. melanogaster* showed a significant positive effect on increasing longevity by ~38%.[274]

The endemic South African species *Aspalathus linearis* (Fabaceae) is cultivated to produce the herbal tea "rooibos" which is popular for its antioxidant, hypoglycaemic, anti-inflammatory and photoprotective activity.[275] The fermented rooibos (or red rooibos) is produced from the leaves and young stems by an oxidative process resulting in a colour change from green (unfermented rooibos) to red-brown. Aspalathin (a dehydrochalcone glucoside and a potent radical scavenger) is the main phenolic ingredient of the green rooibos that is converted to flavones and coloured dibenzofurans during the fermentation process.[276] It was recently reported that green rooibos (at 100 $\mu g\ mL^{-1}$) suppressed the acute oxidative damage and increased lifespan of *C. elegans* by 22.5%; it was also shown in the same study that treatment of worms with aspalathin significantly increased the expression of antioxidant enzymes and DAF-16/FOXO.[277] Similarly, green tea (*Camelia sinensis*, Theaceae; originates from China) has relatively recently become popular in western cultures because its extracts, which are rich

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in polyphenolic catechins, possess (among others) inflammatory, antioxidant, photoprotective and chemopreventative effects.[256] Green tea extracts (10 mg mL^{-1}) exert antioxidant effects in flies and prolong their mean lifespan by 16%.[278] Similarly, black tea (10 mg mL^{-1}) which is stronger in flavour and contains a large amount of antioxidant substances, including catechins and theaflavins reportedly extended flies' lifespan by 10%, likely by an upregulation of the antioxidant enzymes superoxide dismutase and catalase.[279]

Despite the promising results obtained from the aforementioned studies it is worth noting that an important issue is whether the observed biological effects can be attributed to a number of phytochemicals or are mediated by a single active component. Thus, in most (if not all) cases the ultimate goal would be to isolate and identify the bioactive ingredient(s). It is, however, not necessary to mention that a study on the action mechanism of a complex mixture of natural compounds is a very challenging and laborious task, since, in most cases, the effect may be the result of a synergistic action of many compounds without any of them exhibiting significant activity when tested individually. Furthermore, as in the case of natural compounds (see above), the healthspan and/or lifespan extending action of extracts may not necessarily relate to their *in vitro* antioxidant activity. This was elegantly demonstrated in a recent study where six aqueous extracts proved to possess significant *in vitro* antioxidant activity, either as superoxide scavengers or as xanthine oxidase inhibitors, showed negligible effects in *C. elegans* lifespan; on the contrary, the extract obtained from the fruits of *Psoralea corylifolia* elicited a fairly robust lifespan-extending effect despite being a rather modest antioxidant.[280] Thus, extensive *in vitro* screening studies for the identification of natural compounds (or extracts) with antioxidant activity should be always accompanied by cell-based and (most importantly) *in vivo* assays in model organisms when the end point is the identification of extracts with healthspan or lifespan increasing properties.

5 Conclusions and perspectives

Considering that the nematode *C. elegans* lives for few weeks while humans can live for several decades, and assuming that our common ancestor was a short-lived organism, then evolution has significantly increased lifespan. Now, after centuries of wondering and wild guessing we have started getting answers on the factors that modulate ageing. Based on the aforementioned findings it is understood that ageing is a complicated multifactorial process affecting several cellular processes and promoting the largely stochastic accumulation of stressors and damaged dysfunctional biomolecules that eventually disrupt cellular homeostasis (Fig. 1 and 2). The stochastic nature of the process is evident from both isogenic model organisms that have different lifespans in the same environment, as well as from longevity graphs which appear as curves rather than sharp corners.[281] This realization may also explain why, contrary to the defined duration of embryogenesis and early development, there is such significant variability of both healthspan and lifespan among individuals. In addition, as the doses of environmental stressors (excluding lifestyle-related stressors; *e.g.* smoking) remain relatively stable during a given lifetime, then it can be assumed that biomolecule damage and the rate of ageing are mainly affected by diet- and metabolism-derived stressors. Based on this argument natural compounds (or extracts) that could act as modulators of longevity-ensuring pathways represent promising candidates for anti-ageing (or healthspan increasing) interventions by suppressing nutrient signalling, triggering a hormetic effect that results in mild activation of the stress responsive pathways or by directly neutralizing stressors and thus reducing stochastic damage of biomolecules (Fig. 3). This concept is emphatically supported by the realization that all natural compounds (or extracts) that reportedly delay *in vivo* ageing were found to act in the very same signalling pathways found by (independent) genetic approaches to modulate longevity (Fig. 4). Therefore, we propose that most components of the pathways shown by genetic approaches to delay ageing (see above; Fig. 4) represent potential targets for screening natural compounds and/or extracts.

These targets, obviously, refer to positive regulators (*e.g.* receptors or intracellular kinases) of the nutrient signalling pathways where the identification of novel inhibitors could dampen the upstream signal resulting in CR-like effects; in this case of course whatever intervention should preferably start after maturation as physiological nutrient signalling is critical for proper development and growth (Fig. 1). Alternative strategies could relate to the identification of novel antioxidants or AGEs/ALEs crosslink breakers that could neutralize stressors, as well as the screening for natural compounds that exert a mild activation of the stress responsive pathways. In this latter category potential targets could be the sirtuins, AMPK, the FOXO, HSF1 and NRF2 transcription factors or proteolytic systems like ALS or UPS. In this approach, natural compounds (or extracts) may, at least in part, exert their beneficial effects by acting as "low-dose stressors" that pre-condition cells to resist more severe stress (*i.e.* exerting a hormetic effect). In this category, diet-derived NRF2 activators (*e.g.* from broccoli extracts;[22,36,256]) or novel HSF1 activators[282] represent promising molecules for further testing. Finally, the identification of natural compounds that can reduce the GI absorption of AGEs/ALEs could exert significant healthspan promoting effects. Considering that, compared to the rather modest estimation of $\sim 10^5$ bioactive compounds in plants,[283] the number of natural compounds with a demonstrated *in vivo* anti-ageing activity remains amazingly low (Fig. 4), carefully designed high-throughput screening studies against the aforementioned targets will certainly reveal novel compounds (or extracts) exerting potent anti-ageing (or health promoting) properties.

Since diet is central in healthspan and longevity modulation (Fig. 3) and in fact is the only feasible life-lasting applicable "intervention" in humans, edible fruits, spices, vegetables or other plant parts (*e.g.* roots) would be the first choice for isolating novel natural compounds with anti-ageing activity; bacteria and marine organisms although are not directly involved in humans' diet, also represent important sources in the level of single molecules. Extracts are another applicable option for anti-ageing purposes but in this case the analytical profile responsible for a reported phenotype (*e.g.* increase in

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healthspan and/or lifespan) should be elucidated, possibly reaching the resolution of a pure bioactive compound. Moreover, although currently used mostly for the isolation of antibiotics or anti-tumor drugs, engineering bacteria to produce products of novel gene clusters would be another promising approach for identifying natural compounds with healthspan increasing (or anti-ageing) activity.[284] Finally, in order to avoid the pitfall situations where high doses of antioxidant vitamins exerted no health benefits,[285] large-scale studies like the US National Institute on Ageing Rigorous Interventions Testing Program (aiming to screen in genetically heterogeneous mice five compounds each year[286]) are needed to test promising compounds (or extracts) and identify non-toxic doses (Fig. 5) for health- and/or life-span extension in mammals.

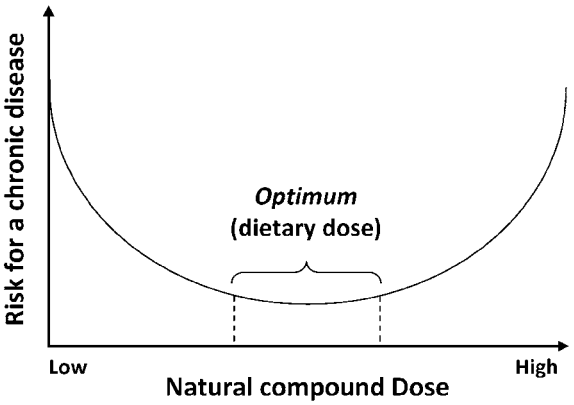

Fig. 5 The relation of responses of biological systems to the dose-related effect(s) of natural compounds (*e.g.* phytochemicals) is not linear. Deficiencies due to either low or high administered doses may lead to significant adverse effects and to chronic diseases. It seems that in most cases the beneficial effects relate to a relatively narrow (dietary relevant) optimum dose range.

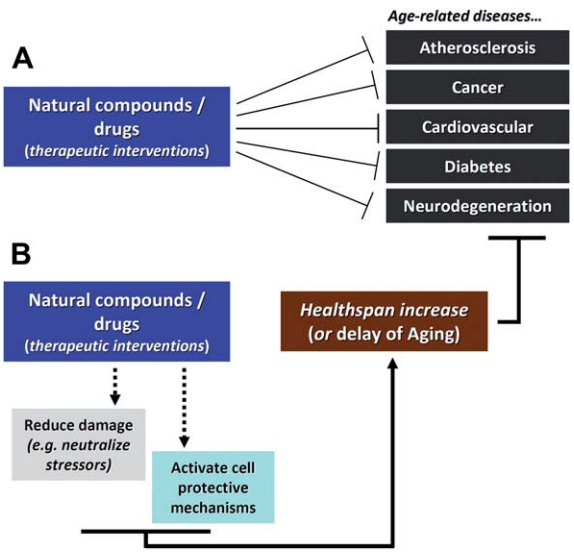

Fig. 6 Anti-ageing interventions as a systemic approach to also inhibit age-related diseases. (A) Classical therapies mostly target individual diseases in isolation when the disease has already evolved over an aged cellular landscape of high concentration of stressors and damaged biomolecules that largely correlate with imbalanced organismal homeostasis. (B) Alternatively, the identification of natural compounds (or extracts) that either neutralize stressors or trigger a mild activation of the cell protective mechanisms, apart from increasing healthspan and delaying ageing, it may also, simultaneously, suppress the appearance of most of the age-related diseases.

As ageing is the major risk factor for human diseases like metabolic syndromes, neurodegeneration and cancer,[4] we argue that natural compounds or (more safely) healthy diet mediated "interventions" can be used in the foreseeable future as an comprehensive, and surely cost-effective mean to combat these diseases (Fig. 6). In support, CR mice or primates are protected against multiple age-related diseases,[12,13,287] while longevity prolonging mutations in model organisms can suppress tumorigenesis.[15] Furthermore, various natural compounds exerting anti-ageing affects are also effective against several age-related diseases (see above). Notably, as in the case of natural compounds (Fig. 5), the "CR or gene dosage" in anti-ageing interventions is of vital importance since extreme CR can lead to several detrimental health effects, including immune deficiencies, infertility and osteoporosis.[11,288] In the same line of dose-dependent effects knocking out the insulin receptor gene causes diabetes in mice, which cannot be rescued by reconstitution of insulin action;[289] INS can be neuroprotective in Alzheimer's disease patients[290] and sustained high levels of NRF2 expression in transgenic flies decreased lifespan.[115]

In conclusion, tackling ageing and it consequences (increased disability and morbidity) is a much needed task that, evidently, requires the combined effort of scientists from distinct disciplines including chemistry, pharmacy, biology and medicine. This approach is particularly urged nowadays as the prevalence of overweight and obesity (mainly linked to calories-rich diets), along with the number of diabetic patients, has been increasing worldwide reaching (in some western countries) the levels of a pandemic phenomenon.[291] It is foreseen that the progress in understanding the genetic basis of ageing, along with the significant technological improvements in natural compounds isolation, characterization and tracking of bioactive constituents will lead to identification (*e.g.* from constituents of the Mediterranean-type diet) of novel natural compounds (or extracts) that will exert healthspan and/or lifespan increasing properties and can be thus translated into significant health benefits for humans.

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