In vivo antioxidant activity of phenolic compounds: facts and gaps

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Abstract

Background: Numerous diseases have been related with free radicals overproduction and oxidative stress. Botanical preparations possess a multitude of bioactive properties, including antioxidant potential, which has been mainly related with the presence of phenolic compounds. However, the mechanisms of action of these phytochemicals, *in vivo* effects, bioavailability and bio-efficacy still need research.

Scope and Approach: The present report aims to provide a critical review on the aspects related with the *in vivo* antioxidant activity of phenolic extracts and compounds from plant origin.

Key findings: Biological functions beyond the human metabolism were discussed, comparing *in vivo* vs. *in vitro* studies, as also focusing the conditioning factors for phenolic compounds bioavailability and bio-efficacy. Furthermore, an upcoming perspective about the use of phytochemicals as life expectancy promoters and anti-aging factors in human individuals was provided.

Conclusions: Overall, and despite all of those advances, the study of the biological potential of numerous natural matrices still remains a hot topic among the scientific community. In fact, the available knowledge about the responsible phytochemicals for the biological potential, their mechanisms of action, the establishment of therapeutic and prophylactic doses, and even the occurrence of biochemical inter-relations, is considerable scarce.

Keywords: Aging-related diseases; Antioxidant activity; *In vivo* studies; Phenolic extracts/compounds; Bioavailability.

1. Introduction

In the last years, oxidative stress-related diseases/disorders have gained a special attention. Metabolic, neurodegenerative, cardiovascular, mitochondrial diseases and even cancer, are among the most frequent (Chaturvedi & Beal, 2013; Halliwell, 2012; Singh, Sharad, & Kapur, 2004). Numerous studies have been investigating the underlying triggering factors, in order to understand the mechanisms of action of free radicals, as well as to discover effective substances towards preventing and even reversing the occurrence of oxidative damages (Espín, García-Conesa, & Tomás-Barberán, 2007; Fernandez-Panchon, Villano, Troncoso, & Garcia-Parrilla, 2008).

Antioxidants, both from natural and synthetic sources, have proved to be highly effective to control the magnitude of free radicals production, to prevent its undesirable effects, as well as to support the organism antioxidant and detoxifying mechanisms (Holst & Williamson, 2008; Kapravelou et al., 2015; Valko et al., 2007; Yeh & Yen, 2006).

Phenolic compounds have shown promising antioxidant properties, with its potential being directly related with the type of solvent used in the extraction, but also with plant origin, growing conditions, harvesting time, and storage conditions (Avello, Pastene, Bustos, Bittner, & Becerra, 2013; Taârit, Msaada, Hosni, & Marzouk, 2012; Trabelsi et al., 2012). The study of the antioxidant potential of phenolic extracts derived from plant species is one of the hot topics among the scientific community; however, *in vitro* studies are the most common (Dai & Mumper, 2010; Larrosa, García-Conesa, Espín, & Tomás-Barberán, 2010; Rubió, Motilva, & Romero, 2013). Nevertheless, these studies do not consider biochemical, metabolic and other physiological parameters (Devasagayam et al., 2004; Espín et al., 2007; Fernandez-Panchon et al., 2008). *In vivo* studies have been mainly performed in eukaryotic cells, mice, fishes, guinea-pigs and

rabbits, but studies involving human clinical trials remain scarce (Fernandez-Panchon et al., 2008; Goodman, Bostick, Kucuk, & Jones, 2011; Rubió et al., 2013). Several biological and physiological variables can be accessed and measured by those studies, including the metabolism of the tested matrices, as well as their bioavailability (Espín et al., 2007; Holst & Williamson, 2008).

Not only natural but also synthetic antioxidants suffer numerous biochemical reactions along ingestion, digestion and absorption by the organisms. Therefore, and despite the current advances, the effective bioavailability of the different antioxidants is not clearly defined: while many of them are ingested on its active form, others need to be metabolized to be biologically active, or even become inactive; furthermore, the co-ingestion of other nutrients, as also several endogenous factors, and the inter and intra-individual variations, affect their bioavailability in relation to the ingested dose (Espín et al., 2007; Heim, Tagliaferro, & Bobilya, 2002; Holst & Williamson, 2008). This fact explains why some plant species and even isolated compounds do not evidence positive effect through *in vitro* studies, but a strong antioxidant potential is observed when *in vivo* studies are carried out, and *vice-versa*.

In this sense, the objective of the present report was to review data related with *in vivo* antioxidant activity of phenolic extracts and compounds, highlighting parameters involved in their metabolism, bioavailability and capacity to prevent/avert aging-related diseases.

2. Human nutrition: nutrients and phytochemicals

2.1. Critical perspective

Dietary practices play an important and unquestionable role at health level. Increasing evidences have shown that certain foods (currently known as functional foods) possess

numerous health benefits, being able not only to prevent a wide variety of human disorders, but also to revert and even block degenerative processes (Devasagayam et al., 2004; Espín et al., 2007; Murray & Pizzorno, 2005). In fact, some of those foods and even dietary practices confer immediate therapeutic actions.

In the last years, numerous changes have been observed in daily diets, in part due to the modern lifestyle and globalization (Goodman et al., 2011; Kaushik, Satya, Khandelwal, & Naik, 2010). Sensorial gratification is one of the most important points in food choices by individuals, but it does not mean that provides correct nourishment (Murray & Pizzorno, 2012). Increasing evidences have shown that diets rich in plant foods is protective towards a wide variety of health conditions; and in contrary, diets' providing a low percentage of plant foods are considered a triggering factor to the development of human organisms disorders (Murray & Pizzorno, 2005, 2012). In fact, a substantial prevalence of chronic and degenerative disorders has been observed, which curiously becomes more frequent with the implementation of globalization and changes in dietary patterns. Metabolic (obesity, gout, diabetes), cardiovascular (high blood pressure, varicose veins, embolisms, strokes, arteriosclerosis, hearth dysfunctions), gastrointestinal (constipation, diverticulitis, bowel inflammatory syndromes, ulcers, haemorrhoids), and auto-immune disorders, and even cancer, are among the most frequent, with the highest rates of incapacity and dependence in worldwide population (Espín et al., 2007; Fernandez-Panchon et al., 2008; Murray & Pizzorno, 2012; Rubió et al., 2013).

2.2. From nutritional attributes to effective biological functions

Considering that enriched-diets in fruits and vegetables have been suggested to provide a dose-dependent protective effect against chronic disorders, numerous studies have

been carried out towards to support those evidences (Rubió et al., 2013). Among the numerous publications, researchers have highlighted that fruits and vegetables act as barrier against cancer, branding them as "chemopreventers", due to the presence of bioactive "phytochemicals" (Murray & Pizzorno, 2012). These plant-derived molecules comprise pigments, such as chlorophyll, carotenes, flavonoids and other phenolic compounds; vitamins; dietary fibers, enzymes, minerals, among other minor constituents (Devasagayam et al., 2004; Grotewold, 2006; Murray & Pizzorno, 2005). In addition, they establish relations with other plant constituents including those through synergistic mechanisms (Ndhlala, Stafford, & Staden, 2013). This explains why in many cases whole matrices give a higher protection than isolated/single nutrients. Despite being vestigial components, phytochemicals play a crucial role in preventing and treating illnesses.

Regarding phenolic compounds, increasing evidences prove their protective activity against a multitude of human conditions (Carocho & Ferreira, 2013; Grotewold, 2006; Mukherjee & Houghton, 2009). Mainly responsible for colour, smell and even protection of fruits and flowers, these phytochemicals possess really important bioactive attributes. Despite being each class of phenolic compounds mainly responsible for a specific bioactivity, they commonly evidence polyvalence reactions, being even able to strengthen the potential of other compounds, to block side effects of some constituents and also to acquire other biological properties (when combined in whole matrices) (Mukherjee & Houghton, 2009). Antioxidant activity is one of the most studied bioactive potential of phenolic compounds, in spite of the difficulty to quantify in an accurate mode, mainly through *in vitro* studies. Numerous variables act as conditioning factors; so, false-positive or negative results could be achieved. For example, phenolic matrices with pronounced *in vitro* antioxidant activity, could not have the same efficacy

when *in vivo* studies are carried out; but, the opposite effect can also occur (Halliwell, 2012; Heim et al., 2002; Mukherjee & Houghton, 2009). This fact could be explained mainly due to two main reasons: firstly, *in vitro* studies do not consider whole human metabolism, biological barriers and the entire chemical reactions of the human organism; on the other hand, some compounds, ingested on their active form lack the bioactive potential after metabolism, while others acquire their bioactive form only after being metabolized (Espín et al., 2007; Heim et al., 2002; Holst & Williamson, 2008). In addition, it is also important to highlight that biochemical interactions (between phytochemicals and foods/drugs) could eliminate, reduce and even improve their bioactivity, making these compounds harmful or beneficial to the organisms (Fernandez-Panchon et al., 2008; Holst & Williamson, 2008).

2.3. Benefits and constraints beyond human metabolism

Human organisms need a correct metabolic function in order to ensure a good health and well-being. Through human metabolism, numerous harmful substances (commonly known as free radicals) are produced and, therefore, effective endogenous detoxifying and neutralizing processes need to function properly in order to prevent damages (Devasagayam et al., 2004; Valko et al., 2007). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are the three most important enzymes (considered primary enzymes) involved in the direct elimination of reactive oxygen species- ROS (hydroxyl radical, superoxide radical, hydrogen peroxide, peroxyl radical, hydroperoxide, singlet oxygen), through metabolism of those toxic oxidative intermediates (Devasagayam et al., 2004; Singh et al., 2004; Valko et al., 2007). On the other hand, the secondary enzymes glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G-6-PDH), and glutathione-S-transferase (GST) are involved in the

detoxification of ROS by decreasing peroxide levels or maintaining a correct supply of metabolic intermediates (such as glutathione and NADPH), crucial for an optimum functioning of the primary antioxidant enzymes (Singh et al., 2004). Notwithstanding, endogenous metabolism is also a source of harmful substances; stress, underlying diseases/disorders, ingested chemicals and drugs, pesticides and other food contaminants are able to attack organisms. In addition, pollution, modern lifestyle, radiations, inadequate behaviours, dependencies, among other factors comprise important aggressors, which also improve free radicals production (Devasagayam et al., 2004; Singh et al., 2004; Valko et al., 2007).

One of the most important and basic functions of cells is to maintain homeostasis; however, by itself, the proper human metabolism is a triggering factor to cellular unbalance and, consequently, dysfunction. Furthermore, in face of a poor nutritional status and weak ingestion of antioxidant phytochemicals, associated with a continuous exposure to toxic substances (mainly by oral or dermal routes and inhalation), a higher risk to develop disease/dysfunction will be doubtless installed (Valko et al., 2007). In addition, and considering the modern lifestyle, the human endogenous detoxifying and antioxidant processes of the organisms are not able to neutralize the free radicals produced daily. Still more, endogenous antioxidants require an appropriate supplying of micronutrients and other functional biomolecules, which act mainly as cofactors, in order to ensure an optimal functioning (Fardet, Rock, & Rémésy, 2008; Singh et al., 2004).

In this sense, it is of the utmost importance to provide enriched-food sources of antioxidants. As referred above, phenolic matrices comprise an extremely rich source of phytochemicals, which present a multitude of health benefits, including their ability to act as free radicals scavengers. Currently, several controversies still exist and no definite

consensus has been established on the antioxidant potential of natural matrices (Carocho & Ferreira, 2013). It is obvious that not all the studied variables can be controlled; itself, this is a sufficient fact, which leads to different conclusions. On the other hand, when *in vivo* studies are carried out, additional confusing factors are present which cannot be fully controlled. For example, the interference of some foods and even nutrients on the bioavailability and bioactivity of phenolic matrices is doubtless established, with milk being one of the most studied. Several reports have evidenced that milk interferes with the antioxidant potential of enriched-phenolic matrices, including blueberries (Serafini et al., 2009), tea (Langley-Evans, 2000a; Lorenz et al., 2007) and chocolate (Serafini et al., 2003). However, other authors have also reported contradictory results for the same phenolic matrices (Hof, Kivits, Weststrate, & Tijburg, 1998; Hollman, Hof, Tijburg, & Katan, 2001; Leenen, Roodenburg, Tijburg, & Wiseman, 2000). Thus, as *in vivo* studies are poorly developed, those contradictions remain unclear.

3. *In vitro* vs. *in vivo* studies: challenges and current perspectives

Medicinal plants have deserved an increasing interest in the last years. In fact, they possess a multitude of health benefits, widely known since pre-historic era. Notwithstanding, due to the absence of scientific validation, they were poorly recommended and even used by worldwide population; but, currently, significant changes have been achieved (Halberstein, 2005). Among the bioactive properties, the antioxidant potential has been one of the most studied, for numerous plant species, different plant parts, extraction procedures and evaluation assays. But even other bioactive properties have been increasingly studied (Dai & Mumper, 2010; Rubió et al., 2013). Obviously studying the *in vitro* properties of natural matrices should be the first objective to carry out; but, numerous studies in which promising antioxidant effects

were achieved, lack of *in vivo* validation (Fernandez-Panchon et al., 2008; Rubió et al., 2013). For example, by comparing the number of publications, from 2000 up to 2014, a pronounced increase on the magnitude of studies in which the *in vitro* and *in vivo* antioxidant potential of phenolic matrices have been observed. However, still remains existing a significant gap related with the *in vivo* validation of the antioxidant potential of phenolic extracts. Further, numerous studies report effective *in vitro* antioxidant potential of natural matrices, in an exponential manner; but *in vivo* studies are still scarce. Another interesting fact is that, contrarily to *in vitro* studies, the *in vivo* experiments do not follow the previously mentioned exponential growth. Normally, *in vivo* studies are developed by using several animal models; however, to the majority of the tested phenolic matrices, in which significant free radicals scavenging activity was observed, no *in vivo* studies were carried out to confirm this potential. The study of new phenolic matrices is undoubtedly important, but to deepen its potential, namely through chemical characterization of the bioactive constituents, followed by testing the *in vivo* potential and related mechanisms of action, if applicable, carrying out clinical trials, is of the utmost importance. It is important to highlight that *in vitro* methods possess numerous constraints, and for that reason, a significant variation is observed when different methods are used to evaluate the same biochemical parameter. In addition, it is very important not forget that the whole human metabolism gives a significant contribution to the antioxidant potential. Through the entire human metabolism numerous compounds are converted on their active forms, while others are inactivated or even linked to several biomolecules that can change the original effect. None of those aspects could be achieved by using *in vitro* studies, and apart from that, false-negative and false-positive results could be achieved. Therefore, *in vivo* studies should be carried out to confirm the *in vitro* results.

Besides phenolic matrices, the *in vitro* and also *in vivo* antioxidant potential of individual phenolic compounds, derived from natural matrices, has been also carried out. By observing the number of publications, from 2000 up to 2014, in which the *in vitro* and *in vivo* antioxidant potential of phenolic compounds was accessed, similarly to phenolic extracts, *in vitro* studies have increased exponentially, while *in vivo* experiments remain poorly investigated. Furthermore, despite the evaluation of specific compounds, not least important is to evaluate synergisms, antagonisms, polyvalence reactions and even lack of biological effect in mixtures of those compounds. Individual phenolic constituents exist in different proportions in whole natural matrices; so, the final observed potential is not always the sum of each one of the individual phenolic compounds present.

Currently, numerous phytochemical formulations are available, not only including plant crude extracts but also isolated phenolic compounds, mixtures of them or even with other biological constituents (vitamins, minerals, trace elements, among others). In this sense, apart from the elucidation of the effects of isolated and even mixtures of phenolic compounds derived from natural matrices, through *in vitro* studies, the development of *in vivo* experiments is of the major importance. Clinical trials should be also carried out, as well as the validation of numerous phytochemical formulations used for antioxidant purposes.

4. *In vivo* antioxidant activity of phenolic extracts/compounds

4.1. Plant phenolic extracts with in vivo antioxidant activity

Animal models comprise the main focus of the studies towards evaluating *in vivo* antioxidant activity of phenolic matrices (**Table 1**). Rats (Wistar and albino Wistar, Sprague Dawley and Wistar-Hannover), followed by mice (Swiss, Kunming and CF1)

and Swiss albino inbred mouse, are the most frequently used laboratory animals. Those animals are submitted to a broad spectrum of stress-inducing agents, such as ethanol, D-galactose, iron, alloxan, streptozotocin (STZ), carbon tetrachloride (CCl_4), bromobenzene, radiation, noise exposure, methylmercuric chloride (MeHgCl), tert-butyl hydroperoxide (t-BOOH) and hydrogen peroxide (H_20_2).

Acetone, chloroform, ethanol, ethyl acetate, methanol, water, ethanol:water, acetone:water:HCl, petroleum ether, and methanol:1N HCl were used as solvents to obtain the different phenolic extracts tested. Plant species belonging to Leguminoseae and Rhamnaceae, followed by Fabaceae, Poaceae, Rubiaceae, Nymphaeaceae, Rosaceae and Moringaceae have been mostly studied as sources of phenolic extracts. However, Anacardiaceae, Annoneaeceae, Apiaceae, Asteraceae, Cannabaceae, Chenopodiaceae, Ericaceae, Lamiaceae, Liliaceae, Moraceae, Myrtaceae, Passifloraceae, Pinaceae, Schisandraceae, Solanaceae, Symplococaceae, Umbeliferae, Verbenaceae and Vitaceae have also been used.

The evaluation of the *in vivo* antioxidant potential is mainly assessed by the effects on different biochemical parameters directly involved in the maintenance of a balanced/correct antioxidant status, such as: malondialdehyde (MDA), CAT, SOD, glutathione (GSH), GSH-Px, GST, GR, xanthine oxidase (XOD), peroxidase (Px), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), c-Glutamyl transpeptidase (c-GT), ferric reducing antioxidant power (FRAP), 2,2′-azobis(2-amidino-propane) dihydrochloride (APPH)-hemolysis, protein carbonylation (PC), diene conjugates, collagen glycation, total antioxidant capacity (TAOC), monoamine oxidase (MAO), oxidative hemolysis, oxygen radical absorbance capacity (ORAC), gamma glutamyl

transferase (GGT), glutamate oxaloacetate transaminases (GOT), glutamate pyruvate transaminases (GPT) and 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulphonate (ABTS).

Table 2 shows the *in vivo* antioxidant potential of phenolic extracts, evaluated through clinical trials, using healthy human volunteers or groups of individuals in special physiological conditions, namely basketball players during training (Chang et al., 2007), postmenopausal women (Lorenz et al., 2007) and human pre-diabetic Mauritians (Somanah, Bourdon, Rondeau, Bahorun, & Aruoma, 2014). Among of the studied plant families only five of them were also previously studied in animal models: Asteraceae, Ericaceae, Myrtaceae, Rosaceae and Vitaceae, but not the same extract types.

Lactuca sativa L. and *Helichrysum plicatum* ssp. *plicatum* capitulums were the studied Asteraceae plant extracts tested in human volunteers and Wistar-albino rats, respectively. Among to the Ericaceae family, *Vaccinium angustifolium* Ait. and *Vaccinium corymbosum* L. were studied in healthy human volunteers, while *Vaccinium ashei* (Mau and Centurion varieties) was tested in Sprague Dawley rats. In the Myrtaceae family, *Ugni molinae* Turcz. and *Myrciaria dubia* McVaugh were studied in human healthy volunteers and STZ-induced diabetic Wistar rats, respectively. In relation to Rosaceae family, while *Fragaria ananassa* Duch was tested in human healthy volunteers, *Cydonia oblonga* Mill. cv. Smyrna, *Malus domestica* Borkh. cv. Fugi and *Pseudocydonia sinenses* Schneid. var. Toukarin were evaluated in healthy Wistar rats. Lastly, among the Vitaceae family, *Vitis vinifera* L. extract was tested in human healthy volunteers, and different *V. vinifera* varieties were also studied in healthy Swiss mice. Other plant families tested in human volunteers include Caricaceae, Convolvulaceae, Malvaceae and Theaceae. Total radical trapping parameter (TRAP), FRAP, total antioxidant status (TAS), ORAC, luminal-enhanced chemiluminescence measurement (LCL), PC, GSH, MDA, 8-hydroxy-2-deoxyguanosine (8-OHdG), 4-

hydroxynonenal (4-HNE), trolox equivalent antioxidant capacity-cupric ion reducing antioxidant capacity (TEAC-CUPRAC), endothelial function, lipid hydroperoxides and inhibition of hemolysis were the most commonly methods to evaluate human biochemical parameters (as shown in the **Table 2**).

As previously mentioned, for the majority of the plant extracts tested in animal models, in which pronounced antioxidant properties were observed, no clinical trials involving human volunteers were carried out. Nevertheless, it is important to highlight that the achievement of a significant antioxidant potential in animal models (rats, mice, mouse, etc.) does not mean that the same potential will be found in human individuals. It is important to clearly understand the real effects of plant phenolic extracts, their bioactive individual compounds and mechanisms of action, and even to establish prophylactic and therapeutic doses, not only in animal models but also in human individuals.

4.2. Phenolic compounds with in vivo antioxidant activity

Table 3 shows the *in vivo* antioxidant potential of individual phenolic compounds, by using animal models. Anthocyanins, followed by hydroxybenzoic and hydroxycinnamic acids comprise the most studied phenolic classes. Flavones, flavonols, coumestans, diferuloylmethanes and simple phenols, were also studied. Similarly to the phenolic extracts, rats (Wistar, Sprague-Dawley, Rowett Hooded Lister strain) and mice (ICR, Kunming) are the most commonly used animal models, followed by Hartley albino guinea-pigs and the marine fish *Dicentrarchus labrax*. The measured biochemical parameters include MDA, CAT, SOD, GSH, GSH-Px, Px, ALT, AST, AAPH, TAOC, ORAC, 4-HNE, heme oxygenase-1 (HO-1), lipid hydroperoxides, 8-OHdG, learning and memory abilities, auditory brain response, immunohistochemical and western blot

analysis (Nrf2, NQO1 and HO-1), as well as the recovery levels of ferulic (FA) and *p*-coumaric (PCA) acids, and related metabolites.

Since 2010, a significant improvement of the *in vitro* studies was observed, in which the antioxidant potential of phenolic compounds has been studied; however, a small portion evaluated its *in vivo* potential. The most tested phenolic compounds are purchased from commercial sources, while those isolated from natural matrices are considerably scarce. Leaves of *Apium graveolens* L. var. *dulce* (apiin), corn bran (FA and PCA), *Alpinia oxyphylla* Miq. (protocatechuic acid), *Abies koreana* E.H. Wilson (cyanidin 3-glucoside, delphinidin 3-glucoside, malvidin 3-glucoside, peonidin 3-glucoside and petunidin 3-glucoside) and *Ipomoea batatas* (L.) Lam. (anthocyanin) seems to be the main phenolic matrices from which phenolic compounds were isolated, towards the evaluation of *in vivo* antioxidant effects. It is a fact that numerous phenolic compounds are common in several plant species, but the existence of tenuous differences on their relative abundance is, normally, predictor of the bioactive potential (Avello et al., 2013; Naghdi Badi, Yazdani, Ali, & Nazari, 2004; Parker, Wang, Pazmiño, & Engeseth, 2007; Wojdylo, Oszmianski, & Czemerys, 2007).

Numerous and significant advances have been reported, highlighting the antioxidant potential of individual phenolic compounds. Among them, apigenin, quercetin, kaempferol, myricetin, luteolin, isorhamnetin, neochlorogenic, caffeic, ferulic and *p*-coumaric acids, and their derivatives are the most reported (Heim et al., 2002; Sulaiman, Sajak, Ooi, & Seow, 2011; Wojdylo et al., 2007). So, and despite the evidences of promising antioxidant potential, mainly through *in vitro* experiments, it is urgent to validate the *in vivo* activity of these and other phenolic constituents of plant species. In addition, apart from the effect of slight differences on the phenolic composition, geographical and environmental variations, harvesting time, storage and

even extraction procedures are also able to produce considerable variations in phenolic plant contents, and consequently on their total antioxidant potential. Several studies have also reported that different culture conditions are able to produce significant changes on the phenolic composition of the same plant (Avello et al., 2013; Farhat, Landoulsi, Chaouch-Hamada, Sotomayor, & Jordán, 2013; Sulaiman et al., 2011).

By analysing **Figure 1** reporting studies in which phenolic compounds were evaluated for *in vivo* antioxidant potential in human volunteers, other significant constraints could be observed. Firstly, from the *in vivo* experiments in animal models, only three of the studied phenolic compounds were further studied in human volunteers: gallic acid, ferulic acid and rutin. The relative antioxidant efficiency (RAE) of those compounds varied between 17.2 and 27.0%, while for the others, RAE was lower than that. Only ellagic acid (RAE=109.9%), caffeic acid (RAE=40.2%), epigallocatechin gallate (RAE=43.1%), eriodictyol (RAE=40.8%), taxifolin (30.7%), apigenin (RAE=35.3%), kaempferol (RAE=58.8%), quercetin (RAE=59.1%), sesamin (RAE=109.9%) and sesaminol (RAE=56.4%) evidenced a higher potential. However, as previously mentioned, it is important to highlight that those compounds are widely recognized for their antioxidant properties, being even used as food preservatives. Additionally, some researchers also use them as positive controls, in order to assess the bioactive properties of other compounds/substances. Interestingly, Wang and Goodman (1999) only used commercial compounds on their experiment, remaining unclear the real antioxidant effect of these phenolic compounds, isolated from natural sources, as well as their bioavailability and bio-efficacy. In fact, several reports have shown that the recovery of phenolic compounds in plasma, urine and faeces, after ingestion of enriched-natural matrices in phenolic compounds, is poor (Serafini et al., 2002; Z. Zhao et al., 2009; Z. Zhao, Egashira, & Sanada, 2005).

In relation to the *in vivo* antioxidant potential of anthocyanins from *Vaccinium angustifolium* Ait., tested in human volunteers (**Figure 2**), only four of them were previously studied in animal models, but isolated from a different natural source (*Abies koreana* E.H. Wilson). Cyanidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, malvidin 3-*O*-glucoside and petunidin 3-*O*-glucoside were the isolated anthocyanins from natural sources in which *in vivo* experiments, both using animal models and human healthy volunteers, were carried out. Curiously, *Abies koreana* E.H. Wilson presented a higher relative abundance of anthocyanins than *Vaccinium angustifolium* Ait.: 1.3625 g total antocyanins/100 g (Ramirez-Tortosa et al., 2001) and 1.20 g total anthocyanins/100 g (Mazza, Kay, Cottrell, & Holub, 2002), respectively. Considering these results, it is important to highlight that numerous matrices with *in vitro* and *in vivo* significant antioxidant potential, could be highly effective and bioavailable, in human individuals. But, as no clinical trials are available for the majority of them, their effect remains questionable.

4. Bioavailability and bio-efficacy: conditioning factors

Plant phytochemicals possess numerous health benefits, and despite the chemical composition of each species determines/predicts the final bioactive potential, geographical, environmental and culture conditions, harvesting time, storage, extraction procedures, type of solvents and plant parts used, also have a pronounced impact (Naghdi Badi et al., 2004; Taârit et al., 2012). In fact, several studies have described the effect of each one of the previously described triggering factors, being *in vitro* studies the most frequently used for this purpose. Besides, it has also been described that several phenolic matrices with *in vitro* bioactive potential, particularly anti-oxidative effects, do not present the same potential throughout *in vivo* studies, and *vice-versa*.

Furthermore, the presence of these phenolic compounds in free or bound forms directly influences their potential. For example, Germano et al. (2006) evaluated the *in vivo* antioxidant potential of phenolic acids, isolated from *Trichilia emetica* Vahl., in male Wistar rats, and observed significant differences on the phenolic contents, directly dependent from the type of applied treatment (alkaline hydrolysis vs. no treatment). Then, using the free and bound phenolic extract forms, pronounced variations on their ability to inhibit methyl linoleate (MeLo) autoxidation were observed: phenolic extracts submitted to alkaline hydrolysis presented a higher antioxidant potential. Similar results were achieved in the ascorbate/Fe^{2+} - induced lipid peroxidation assay. Afterwards, the phenolic acids content was measured in plasma, before and after β-glucoronidase treatment; the authors observed that the most abundant phenolic acids (caffeic, *p*-coumaric, protocathecuic, vanillic and galic acids), appeared in higher concentration in plasma in comparison with hydrolyzed phenolic extracts (Germanò et al., 2006). Taking into account the obtained results, it is evident that some phenolic extracts and, therefore, phenolic compounds, need to be metabolized to be biologically active. Currently, there are no doubts about the importance of the assessment of the *in vivo* biological potential in phenolic matrices with significant *in vitro* potential. Fardet et al. (2008), reported similar results, namely the effects of metabolism on the bioavailability of whole-grain cereals and cereal products. In contrast, other authors have reported that some phenolic extracts and even phenolic compounds display a lower bioavailability after ingestion (Z. Zhao et al., 2005).

There are increasing evidences that important food-phenolic compounds interactions affect their bioavailability, and consequently biological potential. One of the most studied are milk-phenolic interactions (Fernandez-Panchon et al., 2008). For example, Serafini et al. (2009) observed that the ingestion of *Vaccinium corymbosum* L.

(blueberries) fruit results in a significant increase of the endogenous plasma antioxidant defences, and also caffeic and ferulic acids level. In addition, they observed that, *post*-ingestion, caffeic acid was found in plasma, but not in the food matrix. It means that metabolism favours its absorption and appearance in the circulatory system. Otherwise, the ingestion of blueberries in association with milk impaired the *in vivo* antioxidant properties of blueberries, and reduced the absorption of caffeic acid (Serafini et al., 2009). But, numerous controversies still remain unclear; while some authors described that milk impairs the bioavailability of phenolic extracts/compounds (Langley-Evans, 2000b; Serafini et al., 2003, 2009), other concluded that no significant interactions were observed (Hof et al., 1998; Hollman et al., 2001; Leenen et al., 2000).

Bioavailability is commonly defined as the amount of a food constituent that is present in the gut, as a consequence of the release of this constituent from the solid food matrix, and may be able to pass through the intestinal barrier (Saura-Calixto, Serrano, & Goñi, 2007). Different steps directly contribute to this process, namely liberation, absorption, distribution, metabolism and elimination. As previously cited, and despite several variables each one determining the previous steps, inter-individual variations possess a large influence on these processes (M. J. Rein et al., 2013). One of the first and most important factors in the bioavailability is the bioaccessibility: fraction of a compound, which is released from the food matrix in the gastrointestinal lumen and that becomes available for intestinal absorption (M. J. Rein et al., 2013), or the amount of compound reaching the enterocyte in a form suitable for absorption (Scholz & Williamson, 2007). From a nutritional perspective, the composition of the digested food matrix, the existence of synergic and antagonistic reactions between the different components, and also physicochemical properties (pH, temperature, texture, etc.) influence this process, apart from the mastication (M. J. Rein et al., 2013). Particularly to the plant foods,

another special consideration needs to be highlighted. The composition of plant cell wall is markedly resistant to degradation in the upper gut that, therefore, makes difficult the liberation of the active constituents, such as phenolic compounds. Those facts were previously discussed and widely confirmed by some authors. In fact, different reports have stated the benefits of hydrolysis, fermentation, dissolution and even mixture of different phenolic matrices in the improvement of polyphenols bioavailability and its biological potential (Dey & Kuhad, 2014; Fardet et al., 2008; Kountouri, Mylona, Kaliora, & Andrikopoulos, 2007; Pinelo, Landbo, Vikbjerg, & Meyer, 2006; Scalbert et al., 2000). But, extensive studies, which aim to understand the real interference of those methodologies on the improvement of phenolic matrices bioavailability, are significantly scarce. However, it is important to refer that this is a key step to ensure the bio-efficacy of plant bioactive compounds (M. J. Rein et al., 2013). So, it is crucial to enhance the knowledge in this area.

Until now, apart from the bioacessibility, the absorption of these compounds can be also influenced by solubility, interaction with some dietary constituents, molecular changes, protein transporters, human organisms metabolism and, lastly, effects of gut microbiota (M. J. Rein et al., 2013). Those factors, considered the most important determinants to the bioavailability, and then bio-efficacy of bioactive compounds, are schematically described in **Figure 3**. Plant bioactive compounds need to be bioavailable to exert beneficial effects in order to provide a significant improvement of health and well-being of the worldwide population, mainly by reducing the frequency of oxidative-stress related diseases/disorders.

5. Aging-related diseases: evidences of the *in vivo* role of phenolic antioxidants

The improvement of the life expectancy and longevity comprises one of the most important purposes of human individuals. Over decades, aging was considered a natural process, i.e. a normal phase, in which a progressive decrease in function, degeneration and malfunction of cells is observed; therefore, cells start to die at a faster rate than new ones are generated. In fact, birth, growth and death markedly represent the three most significant phases of the organisms' life cycle (Murray & Pizzorno, 2012). However, with the advances of science and technology, new achievements have been stated.

The modern definition of aging comprises a progressive post-maturation decline in physiological capacity, accompanied by an increased susceptibility to disease and mortality risk (Ergin, Hariry, & Karasu, 2013). In fact, the common aging process is associated with a progressive cell failure and, consequently, improves the probability of occurrence of dysfunction/disease/disorders. Notwithstanding, numerous causes of premature aging and cell death have been pointed out during the past decades. It is convenient to highlight that formerly the poor health and hygiene conditions, and medical assistance impaired significantly the life expectancy and increased infant mortality. Currently, in parallel with the improvement of life expectancy, increasing rates of premature aging and death have also been observed (Chaturvedi & Beal, 2013; Murray & Pizzorno, 2012). However, cardiovascular, neurodegenerative, gastrointestinal and metabolic diseases, and even cancer comprise the main causes of morbidity and mortality (Valko et al., 2007). More recently, auto-immune, respiratory and osteoarticular disorders have increased their frequency (Musumeci, Szychlinska, & Mobasheri, 2015; Rubió et al., 2013; Valko et al., 2007).

Pollution, smoking, obesity, sedentary lifestyle, poor nutrition and nutritional deficiencies are pointed as the main contributor factors. It appears a paradox, because in association with greater dietary availabilities and food choices, higher rates of

nutritional deficiencies and human disorders/diseases are observed (Murray & Pizzorno, 2012). *Per se*, the physical exercise is a triggering factor that accelerates the aging process, while rich source of free radicals; but physical inactivity too, besides to be more dangerous. Apart from the importance of stress management, physical activity, health-promoting lifestyle, and dietary choices are of utmost importance.

All of the most important causes of death are directly related with dietary practices. Dairy products, meat and cereals comprise the base of nutrition to the majority of individuals, while fruits and vegetables/plant products are consumed in a lower frequency than expected. Extremely rich in vitamins, minerals, trace elements, fibers, and also numerous phytochemicals, among them phenolic compounds, those natural matrices have proved to be promising prophylactic and therapeutic agents, widely known in traditional medicine since ancient times (Vanaclocha & Cañigueral, 2003). Particularly in relation to their antioxidant activity, those plant phytochemicals act not only as free radical scavengers, metal chelators, hydrogen donators and oxygen quenchers, but also they recycle other antioxidant compounds, promote an adequate homeostasis and proper enzymatic function, among other benefits (some of them are not yet discovered) (Carocho & Ferreira, 2013; Dai & Mumper, 2010; A. Li et al., 2014).

Apart from the extensive knowledge about the antioxidant activity of natural matrices, mainly obtained from *in vitro* studies, recently, new advances regarding *in vivo* studies have been shared. A significant part of them, have focused on the study of phenolic matrices and not so much on isolated/individual compounds. From the *in vivo* studies, a great part of phenolic matrices exerts its effects by inhibition of the ROS formation and reduction of oxidative stress, as also by preventing tissue damages (mainly when oxidative stress-inducers are administered) (Shi, An, Jiang, Guan, & Bao, 2006; Silva et al., 2013; Su, Wang, & Liu, 2009). The effects on gut microbiota are also being

increasingly studied; in fact, not only several phenolic matrices are converted on their active forms but also they exert directional modulatory activities on the gut microbiota, by inhibition of opportunistic flora without interfering with the beneficial bacteria. However, the mechanisms of action are not completely understood (Cueva et al., 2010; Silva et al., 2013; Velderrain-Rodríguez et al., 2014). On the other hand, several isolated phytochemicals, for example, curcumin, exert their effects by causing arsenic methylation and improvement of urinary elimination, apart from the Nrf2 nuclear translocation, activation of Nrf2 regulation proteins, and promote detoxification of antioxidant enzymes (Gao et al., 2013).

6. Concluding remarks and future perspectives

The study of biological effects of natural matrices, including the discovering of their active constituents, is a hot topic that focuses the attention of numerous scientists. In fact, the use of botanical preparations dates since pre-historic era, but due to the lack of scientific evidences, including modes of action, no solid recognition or assertion was achieved. Prior to the use of synthetic drugs, botanical preparations were used for a multitude of health conditions, as also for averting the aging process. Then, with the increasing use of synthetic molecules, natural therapies passed to a second stage. But, currently, the focus on natural matrices (namely, rich in phenolic compounds) properties has gained attention. *In vitro* studies increased in an exponential manner, and numerous bioactive properties were confirmed; then, *in vivo* studies mainly in animal models have also increased, but in smaller proportions.

Despite those advances, to the majority of plant species no extensive knowledge is available, namely the responsible phytochemicals for the observed bioactivity, their mechanisms of action, therapeutic and prophylactic doses, synergism, antagonisms and

other inter-relations between them. Clinical trials are very important, in order to develop future and effective alternatives to improve the health and well-being of individuals. In addition, it is of the major importance to prove the effective therapeutic potential of phytochemical preparations, together with the increasing number of publications related with the discovery of plant species with bioactive potential, as well as to evaluate other variables, such as bioavailability and bio-efficacy, nutrient-phytochemicals and drug-phytochemicals interactions.

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Table 1. Plant species used to obtain phenolic extracts with *in vivo* antioxidant activity evaluated in animal models.

Plant	References
Afzelia africana SM	(Atawodi, Iliemene, & Onyike, 2014)
Astragalus sinicus L.	(Lim et al., 2011)
Beta vulgaris L.	(Vulić et al., 2014)
Capsicum baccatum L. var. *pendulum*	(Kappel et al., 2008)
Cassia fistula Linn.	(Maheep, Sunil, Yogesh, Durgesh, & Kanika, 2010)
Ceratonia siliqua	(Hsouna et al., 2011)
Choerospondias axillaris (Roxb.) B.L. Burtt and A.W. Hill	(H. Wang, Gao, Zhou, Cai, & Yao, 2008)
Clerodendrum infortunatum L.	(Gouthamchandra, Mahmood, & Manjunatha, 2010)
Coffea arabica (60%) and *Coffea canefora* var. *robusta* (40%) mixture	(Contini, Baccelloni, Frangipane, Merendino, & Massantini, 2012)
Ocimum sanctum L.	(Samson, Sheeladevi, & Ravindran, 2007)
Parkia biglobosa	(Ademiluyi & Oboh, 2012)
Paspalum scrobiculatum L.	(Hegde, Rajasekaran, & Chandra, 2005)
Passiflora edulis Sims	(Silva et al., 2013)
Pinus koraiensis L.	(Su et al., 2009)
Piper guineense Schumach. et Thonn	(Adefegha & Oboh, 2012)
Prosopis juliflora	(Almaraz-Abarca et al., 2007)
Pseudocydonia sinensis Schneid. var. Toukarin	(Hamauzu, Inno, Kume, Irie, & Hiramatsu, 2006)
Pueraria lobata (Willd.) Ohwi	(Bebrevska et al., 2010)
	(Duarte-Almeida,

Dipteryx alata Vog.	(Siqueira et al., 2012)
Eleusine coracana (L.) Gaertn.	(Hegde et al., 2005)
Euryale ferox Salisb.	(Wu et al., 2013)
Ferula szovitsiana DC	(Dehghan, Shafiee, Ghahremani, Ardestani, & Abdollahi, 2007)
Ficus glomerata Roxb.	(Verma, Vijayakumar, Rao, & Mathela, 2010)
Glycine max L. Merrill	(Ademiluyi & Oboh, 2012)
Helichrysum plicatum ssp. *plicatum*	(Aslan, Orhan, Orhan, Sezik, & Yesilada, 2007)
Hemerocallis fulva Linn.	(Que, Mao, & Zheng, 2007)
Hordeum vulgare L.	(Qingming et al., 2010)
Humulus lupulus L.	(X. Wang, Yang, Yang, & Tian, 2014)
Malus domestica Borkh. cv. Fuji	(Hamauzu et al., 2006)
Meyna spinosa Roxb.	(Sen, De, Devanna, & Chakraborty, 2013)
Moringa oleifera Lam.	(Jaiswal et al., 2013; Verma, Vijayakumar, Mathela, & Rao, 2009)
Myrciaria dubia McVaugh	(Gonçalves, Lellis-Santos,
Saraca indica L.	(Sen et al., 2014)
Schisandra chinensis	(Cheng et al., 2013)
Symplocos cochinchnensis var. *laurina*	(Sunil & Ignacimuthu, 2011)
Torilis leptophylla L.	(Saeed, Khan, & Shabbir, 2012)
Vaccinium ashei Reade (Maru and Centurion varieties)	(Molan, Lila, & Mawson, 2008)
Vigna subterranea L. Verdc	(Ademiluyi & Oboh, 2012)
Vigna unguiculata	(Kapravelou et al., 2015)
Vitis vinifera varieties	(Gris et al., 2013)
Xylopia aethiopica (Dun.) A. Rich	(Adefegha & Oboh, 2012)
Zizyphus jujuba cv. *Banzao*	
Zizyphus jujuba cv. *Goutouzao*	
Zizyphus jujuba cv. Jinsizao	(H. Zhao, Zhang, & Yang, 2014)
Zizyphus jujuba cv. Junzao	
Zizyphus jujuba cv. Pozao	

Nelumbo nucifera Gaertn.	Curi, Lajolo, & Genovese, 2014) (Huang et al., 2010; Rai, Wahile, Mukherjee, Saha, & Mukherjee, 2006)	*Zizyphus jujuba cv. Yuzao* *Zizyphus jujuba cv. Xiaozao*

Table 2. Plant phenolic extracts with *in vivo* antioxidant activity evaluated in clinical trials with human healthy volunteers.

Family	Plant	Preparation	Concentration	Dosage	Period	Assays	References
Asteraceae	*Lactuca sativa* L.	Fresh lettuce Stored lettuce	250 g/day	Single dose	1 day	TRAP	(Serafini et al., 2002)
Caricaceae	*Carica papaya* L.	Fermented ripe pulp	30 mg/mL	1 dose (200 mL)/day	14 weeks [a)]	TAS, inhibition of hemolysis and level of PC	(Somanah et al., 2014)
Convolvulaceae	*Ipomoea batatas* (L.) Lam.	Cooked leaves	200 g/day	2 doses (each 100 g)	7 weeks	TAS, GSH, 8-OHdG, MDA, 4-HNE	(Chen et al., 2008)
				Single dose	2 periods (each lasting 2 weeks) [b)]	TAS, lipid hydroperoxides, 8-OHdG, MDA	(Chang et al., 2007)
Ericaceae	*Vaccinium angustifolium* Ait.	Freeze-dried wild strawberry dispersed in water	200 mg/mL	1 dose/day	7 days	ORAC and TAS	(Kay & Holub, 2002)
	Vaccinium corymbosum L.	Fresh fruits	200 g/day	1 dose/week	2 weeks	TAS, FRAP and TRAP	(Serafini et al., 2009)
Malvaceae	*Theobroma cacao* L.	M&M's Semi-Sweet Chocolate Mini Baking Bits	105 g (80 g of procyanidin-rich chocolate)	Single dose	1 day	TBARS, TRAP and LCL	(D. Rein et al., 2000)
		Commercial product	100 g	Single dose	1 day	FRAP	(Serafini et al., 2003)

Myrtaceae	*Ugni molinae* Turcz.	Infusion of leaves	10 mg/mL	2 doses (100 mL)/day	3 days	TBARS, and TEAC-CUPRAC	(Avello et al., 2013)
Oleaceae	*Olea europaea*	Fresh fruits	100 g/day	Single dose	24 h	TAS, plasma total polyphenol assay	(Kountouri et al., 2007)
Rosaceae	*Fragaria x ananassa,* Duch.	Fresh fruit	500 g	2 doses (250 g)/day	16 days	FRAP and TEAC, erythrocytes hemolysis	(Tulipani et al., 2011)
Theaceae	*Camelia sinensis* (L.) Kuntze	Black tea	16.25 mg/mL	Six doses (200 mL)	1 day	FRAP	(Langley-Evans, 2000b)
			6.67 mg/mL	Single dose (300 mL)	1 day	FRAP	(Leenen et al., 2000)
			20 mg/mL	Single dose (300 mL)	1 day	TRAP	(Serafini, Laranjinha, Almeida, & Maiani, 2000)
			3.33 mg/mL	Eight doses (150 mL)/ day, every 2h	3 days	Plasma concentration-time curve of quercetin or kaempferol	(Hollman et al., 2001)
			10 mg/mL	Single dose (500 mL)	1 day [c)]	Endothelial function (FMD and NMD)	(Lorenz et al., 2007)
		Green tea	6.67 mg/mL	Single dose (300	1 day	FRAP	(Leenen et

				mL)			al., 2000)
			20 mg/mL	Single dose (300 mL)	1 day	TRAP	(Serafini et al., 2000)
			16.67 mg/mL	1st week (150 mL), 2nd week (300 mL), 3rd week (450mL)/day	3 weeks	TAS	(Sung et al., 2000)
			3.33 mg/mL	Eight doses (150 mL)/ day, every 2h	3 days	Plasma concentration-time curve of quercetin or kaempferol	(Hollman et al., 2001)
Vitaceae	*Vitis vinifera* L.	White wine dealcoholized	20 mg/mL	Single dose (300 mL)	1 day	TRAP	(Serafini et al., 2000)
		Red wine dealcoholized	20 mg/mL	Single dose (300 mL)	1 day	TRAP	(Serafini et al., 2000)
		Whole grapes	250 g	1 dose/day	4 weeks	ORAC-PCA	(Parker et al., 2007)
		Whole raisins	50 g of golden raisins 50 g sun dried-raisins	1 dose/day	4 weeks		(Parker et al., 2007)

[a)] Human pre-diabetic Mauritians; [b)] Basketball players during training; [c)] Postmenopausal women; FMD, flow-mediated dilatation; NMD, nitro-mediated dilatation.

Table 3. Phenolic compounds with *in vivo* antioxidant activity evaluated in animal models.

Tested compound	Origin	Time of exposure	References
Flavan-3 -ols			
(-)-Epicatechin	Commercial	90 min	(Hamauzu et al., 2006)
Flavones			
Apiin	*Apium graveolens* L. var. *dulce* (leaves)	28 days	(P. Li et al., 2013)
Flavonols			
Rutin	Commercial	2 h	(Sen et al., 2013)
		28 days	(P. Li et al., 2013)
		2 h	(Sen et al., 2014)
Hydroxycinnamic acids			
5-caffeoylquinic acid	Commercial	90 min	(Hamauzu et al., 2006)
Ferulic acid	Corn bran	10 days	(Z. Zhao et al., 2005)
	Commercial	14 days	(Yeh & Yen, 2006)
		21 days	(Fetoni et al., 2010)
p-coumaric acid	Corn bran	14 days	(Yeh & Yen, 2006)
		10 days	(Z. Zhao et al., 2005)
Hydroxybenzoic acids			
Gallic acid	Commercial	14 days	(Yeh & Yen, 2006)
Gentisic acid		14 days	
p-hydroxy-benzoic acid		14 days	
Protocatechuic acid	*Alpinia oxyphylla* Miq.	9 days	(Shi et al., 2006)
Coumestans			
Pyrocatechol	Commercial	1 day	(Roche & Bogé, 2000)
Anthocyanins			
Cyanidin 3-glucoside	*Abies koreana* E.H.Wilson	2 weeks	(Ramirez-Tortosa et al., 2001)
Delphinidin 3-glucoside			
Malvidin 3-glucoside			

Peonidin 3-glucoside Petunidin 3-glucoside Anthocyanin	*Ipomoea batatas* (L.) Lam.	30 days	(J. G. Zhao, Yan, Lu, & Zhang, 2013)
Diferuloylmethanes			
Curcumin	Commercial	6 weeks 31 days	(Gao et al., 2013) (Xie et al., 2014)
Simple phenols			
Hydroquinone Phenol Resorcinol	Commercial	1 day	(Roche & Bogé, 2000)

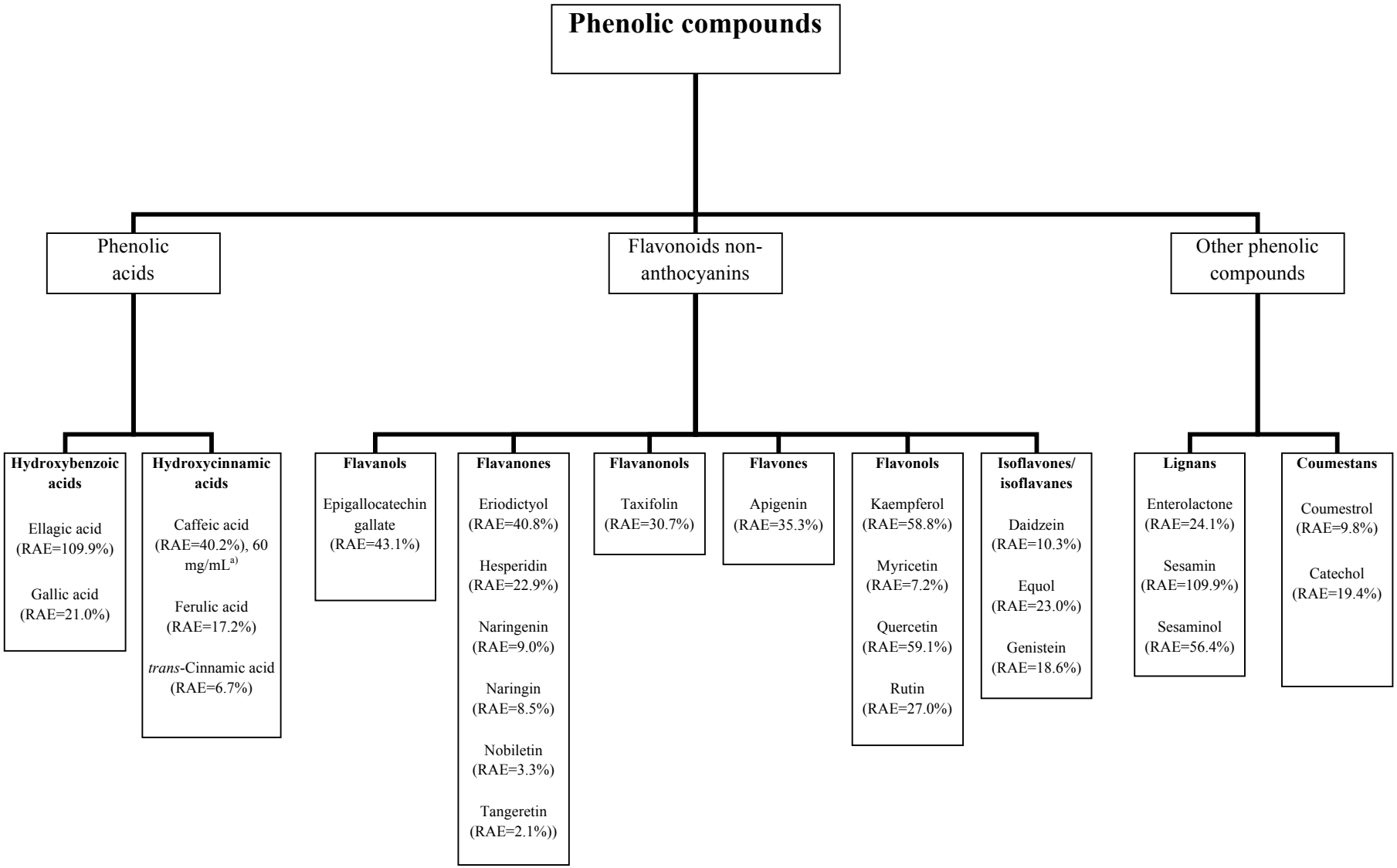
Phenolic compounds
Phenolic acids
Flavonoids non-anthocyanins
Other phenolic compounds
Hydroxybenzoic acids
Ellagic acid (RAE=109.9%)
Gallic acid (RAE=21.0%)
Hydroxycinnamic acids
Caffeic acid (RAE=40.2%), 60 mg/mL a)
Ferulic acid (RAE=17.2%)
trans-Cinnamic acid (RAE=6.7%)
Flavanols
Epigallocatechin gallate (RAE=43.1%)
Flavanones
Eriodictyol (RAE=40.8%)
Hesperidin (RAE=22.9%)
Naringenin (RAE=9.0%)
Naringin (RAE=8.5%)
Nobiletin (RAE=3.3%)
Tangeretin (RAE=2.1%))
Flavanonols
Taxifolin (RAE=30.7%)
Flavones
Apigenin (RAE=35.3%)
Flavonols
Kaempferol (RAE=58.8%)
Myricetin (RAE=7.2%)
Quercetin (RAE=59.1%)
Rutin (RAE=27.0%)
Isoflavones/ isoflavanes
Daidzein (RAE=10.3%)
Equol (RAE=23.0%)
Genistein (RAE=18.6%)
Lignans
Enterolactone (RAE=24.1%)
Sesamin (RAE=109.9%)
Sesaminol (RAE=56.4%)
Coumestans
Coumestrol (RAE=9.8%)
Catechol (RAE=19.4%)

Figure 1. Phenolic compounds with *in vivo* antioxidant activity assessed in human healthy volunteers, during 1 h of exposure, expressed as RAE, carried out by Wang and Goodman (1999). [a)]Caffeic acid was isolated from *Coffea* spp. and tested during 2h in healthy human volunteers to evaluate its free and total phenolic content, through β-glucuronidase and alkaline hydrolysis procedures.

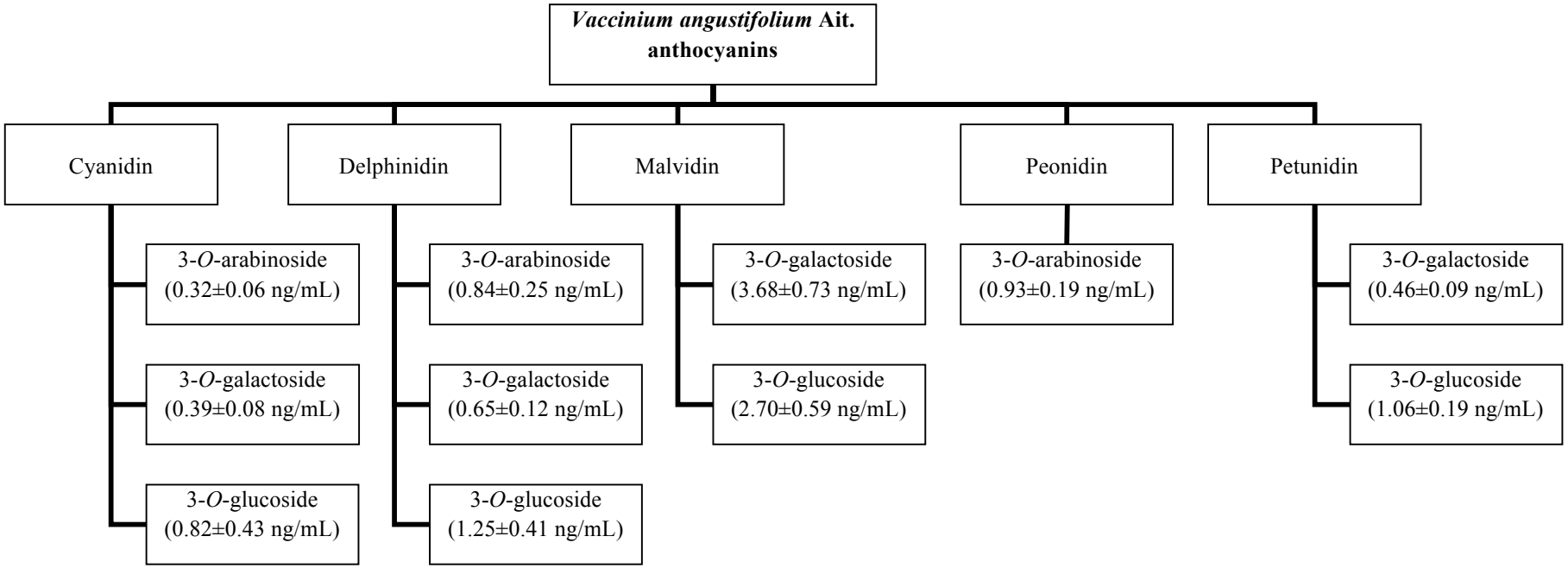

Figure 2. *In vivo* antioxidant activity of *Vaccinium angustifolium* Ait. (100 g of freeze-dried blueberries - equivalent to a 1.20 g of total anthocyanins) ingested by human healthy individuals. TEAC and ORAC assays were used to assess serum antioxidant status after 1 to 4h. The rate of absorption to the blood serum was also evaluated (Mazza et al., 2002). Results are expressed as mean values of the serum anthocyanins concentrations, 4h after ingestion of *V. angustifolium*.

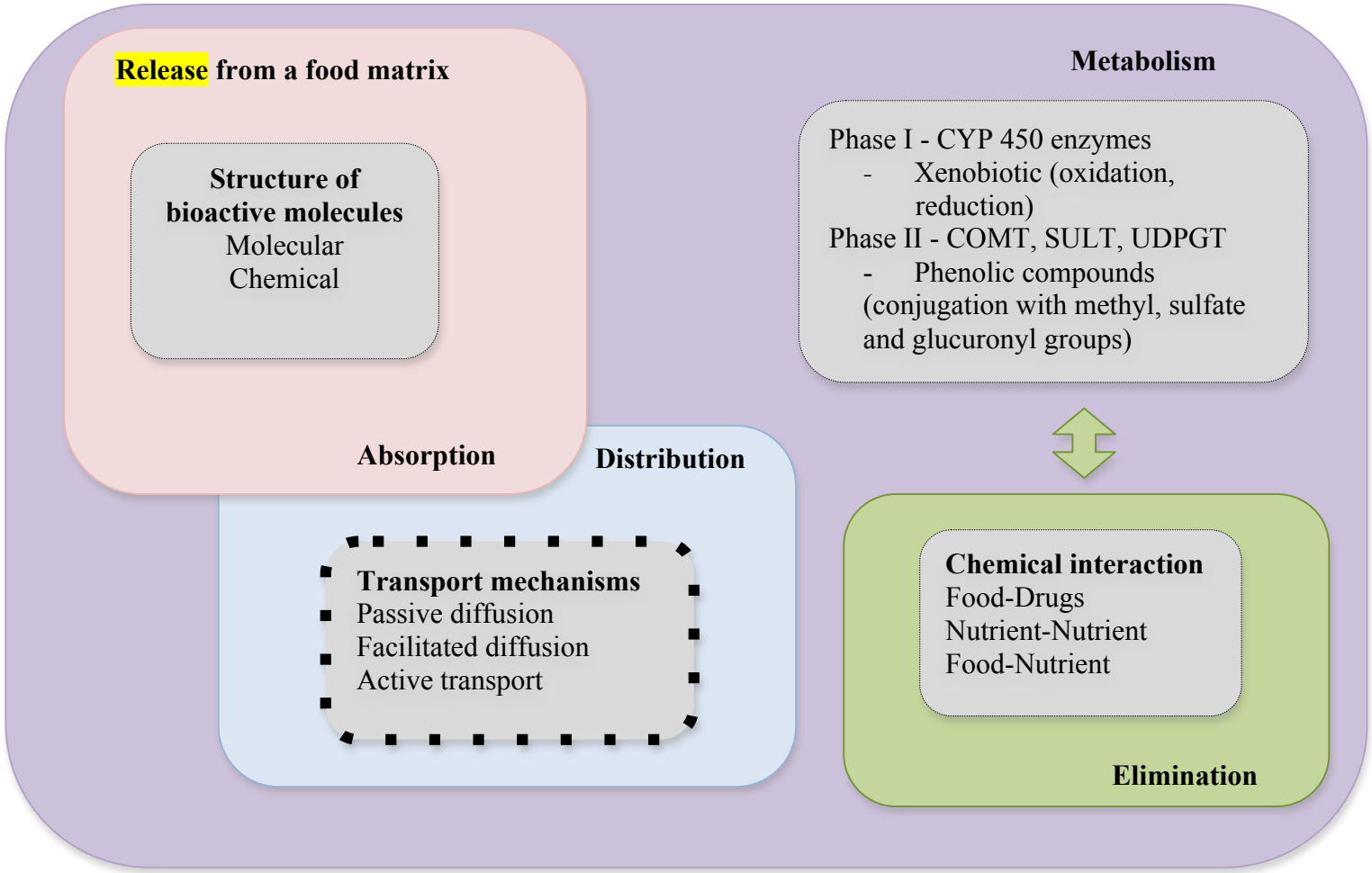

Figure 3. The most important factors in the bioavailability and bio-efficacy of bioactive compounds. CYP450, Cytochrome 450 enzymes; COMT, Catechol-*O*-methyltransferases; SULT, Sulphotransferases; UDPGT, Uridine-5'-diphosphate glucuronosyl-transferases.