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Antioxidants in Natural Matrices: The Case of Phenolics

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ABSTRACT

Small quantities of reactive species of oxygen and nitrogen are produced in physiological processes occurring in all organisms. However, when they are formed in excess or when the antioxidant defence system is depleted, homeostasis is disrupted favouring pro-oxidants. Loss of oxidative status has been linked to several diseases. Thus, antioxidants compounds may assist the prevention and therapeutics of diseases in which oxidative phenomena are involved, e.g. cancer, chronic inflammatory disease, cardiovascular disorders and aging. There are several synthetic compounds with antioxidant properties, although their use has been restricted due to their toxicity. So, there is a growing interest on the search of natural compounds with antioxidant potential, which may exhibit improved tolerability. Natural matrices have proven antioxidant activity, which is due to their chemical composition. These matrices present a wide range of low molecular weight phytochemicals, like alkaloids, carotenoids and organic acids, which constitute the basis of their antioxidant capacity. Phenolic compounds are among those with more interest, because they are largely distributed in nature and can exert their antioxidant activity at several levels. As different natural matrices have distinct compounds contents, with several structures, they offer different protective mechanisms. In this chapter we describe different vegetable matrices, like *Catharanthus roseus*, *Passiflora edulis*, *Rumex induratus* and insect-plant system considerations, involving *Pieris brassicae*. All refered matrices display antioxidant activity, with special attention being given to their phenolic compounds. Because no single method is able to provide exact information about antioxidant potential, some methods currently used to assess this capacity will be referred.

Keywords: antioxidants, *Catharanthus roseus*, phenolic compounds, *Pieris brassicae*, *Passiflora edulis*, *Rumex induratus*

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INTRODUCTION

Life with oxygen has led to the evolution of biochemical adaptations that exploit the reactivity of oxygen species (Noctor and Foyer 1998). Although oxygen is an essential molecule of aerobic organisms, several reactive oxygen species (ROS), like superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) or hydroxyl radical ($HO^{\bullet}$), are produced as a byproduct of normal metabolism (Sies 1993). $O_2^{\bullet-}$ and H_2O_2 are synthesized at very high rates even under optimal conditions by respiratory electron transport chain, oxidation of xanthine oxidase and glucose and by activity of several enzymes (Noctor and Foyer 1998), e.g. NADPH oxidase and cytocrome P450 (Sun *et al.* 2008). In addition to ROS, other reactive species, such as reactive nitrogen species (RNS), like nitric oxide and peroxynitrite, come from normal intracellular biological functions, inflammatory processes and exposure to xenobiotics (Darley-Usmar and Halliwell 1996).

To maintain homeostasis, oxidative agents are inactivated by an array of intra and extracellular antioxidants (Sies 1993), which interrupt cascades of uncontrolled oxidation (Noctor and Foyer 1998). Antioxidants are compounds that inhibit or delay the oxidation of other mole-

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cules, by inhibiting initiation or propagation of oxidizing chain reactions (Velioglu *et al.* 1998; Halliwell 2011). So, for a compound to be defined as an antioxidant, it must satisfy two basic conditions: when present in low concentration relative to the substrate to be oxidized it can delay, retard, or prevent the autoxidation or free radical-mediated oxidation, and the resulting radical formed after scavenging must be stable through intramolecular hydrogen bonding on further oxidation (Rice-Evans *et al.* 1996).

Antioxidant defenses include enzymatic and non-enzymatic molecules. Glutathione is one of the most important non-enzymatic antioxidant molecules, being an effective ROS detoxifier (Halliwell 2011). The reduced form of glutathione, GSH, is a tripeptide that exists interchangeably with the oxidized form, GSSG (Noctor and Foyer 1998). Glutathione provides the cell with multiple defenses, not only against reactive species, but also against other toxic insults, like xenobiotics (Hayes *et al*. 1999). α-Tocopherol is another important molecule, which prevents lipid peroxidation (Bartoli *et al.* 1999). Other non-enzymatic defenses include histidine-peptides, the iron-binding proteins (transferrin and ferritin), dihydrolipoic acid, melatonin, and plasma protein thiols (Pietta 2000). The enzymatic antioxidant defense system includes superoxide dismutase, catalase, glutathione peroxidase, glutathione *S*-transferase and glutathione reductase (Noctor and Foyer 1998). Superoxide dismutase is a major scavenger of $O_2^{\bullet-}$, which catalyses its dismutations with great efficiency, resulting in the production of H_2O_2 and molecular oxygen (O_2) (Chakrabarty *et al.* 2007). On the other hand, catalase converts H_2O_2 to water and O_2 (Noctor and Foyer 1998). The various defenses complement each other, acting against different species in different cellular compartments. Despite these defense antioxidants, some reactive species still escape causing damage (Pietta 2000). Thus, the antioxidant system is also provided with antioxidants able to repair injury, which includes proteases, lipases, transferases and DNA repair enzymes (Varma *et al.* 1995).

Nevertheless, a disequilibrium in the oxidant/antioxidant status can still occur when there is an incomplete efficiency of our endogenous defense systems and/or when the organism is exposed to aggressions (cigarette smoke, air pollutants, UV radiation, inflammation, etc.) in which reactive species are produced in excess, causing oxidative stress in cells (Sies 1993; Pietta 2000). When some reactive species persist in the cells, other reactive species can be formed and attack biological molecules, such as lipids, proteins, enzymes, DNA and RNA, being involved in a wide variety of processes, like membrane damage, lipid peroxidation, protein oxidation and fragmentation, carbohydrate damage, mutagenesis, carcinogenesis and signal transduction pathways alteration (Sies 1993; Pulido *et al.* 2000). The accumulation of unrepaired products may be critical for causing an extensive damage to cells and tissues, leading to several diseases, such as diabetes, cardiovascular disease, chronic inflammation, aging, mutations, cancer and neurodegenerative diseases, like Alzheimer and Parkinson (Halliwell 1994; Finkel and Holbrook 2000). Reactive species have already been implicated in over 100 diseases (Parr and Bolwell 2000). In face of this, antioxidants have deserved a great interest nowadays, to protect the human body against reactive species that may cause pathological conditions (Halliwell *et al.* 1995; Polterait 1997), by lowering this concentration in the organism and, consequently, reducing likelihood that one of those species will cause an initiation event (Parr and Bolwell 2000).

Currently, there is a large number of phenolic synthetic antioxidants, which are compounds with phenolic structures with various degrees of alkyl substitution, such as butylated hydroxytoluene and butylated hydroxyanisole. These compounds have been widely used as food antioxidants (Velioglu *et al.* 1998). However, their applications have been restricted, due to their metabolism, which originates potential reactive intermediates (Branen 1975; Ito *et al*. 1983). So, the search for new natural products with antioxidant properties is a very active domain.

This review addresses phenolics as antioxidants and describes natural matrices with important phenolics content.

PHENOLICS

Phenolics represent the most abundant and widely spread class of plant metabolites, playing important functions in the plant, including support of the plant body, protection against biotic and abiotic stresses, herbivore deterrence, and signaling in plant-plant and plant-microbe interactions. For humans, phenolics are the basis of several plant-derived drugs and recently they have attracted much attention due to their implication in protection against cancer, cardiovascular and neurodegenerative diseases, associated to their antioxidant activity (Ferreres *et al.* 2008a).

Plant phenolics cover several groups of compounds, such as simple phenolics, phenolic acids, flavonoids, isoflavonoids, proanthocyanidins, coumarins, stilbenes, lignans, tannins and lignins. They are defined as compounds having, at least, one aromatic ring substituted by, at least, one hydroxyl group (Bruneton 1999; Andrade *et al.* 2008). The hydroxyl group(s) can be free or engaged in another function, as ether, ester or glycoside (Bruneton 1999).

There are many evidences that phenolic compounds provide a major contribution to the antioxidant activity of plant extracts (Velioglu *et al*. 1999; Couto *et al*. 2011). They are multifunctional and can act by several processes as reducing agents, hydrogen donating antioxidants, and singlet oxygen quenchers. Metal chelation properties and interaction with biomembranes have also been proposed. However, when phenolics form complexes with metal ions, they can behave as antioxidants or pro-oxidants, depending on the reaction conditions, concentration and free radical source (Cao *et al.* 1997; Croft 1998). The specific mode of inhibition of oxidation in lipophilic phase by individual polyphenols is not clear, but they may act by chelating copper ions, scavenging lipid alkoxyl and peroxyl radicals, acting as chain breaking antioxidants, and by regenerating α-tocopherol through reduction of the α-tocopheroxyl radical (Rice-Evans *et al.* 1996).

Phenolic structures often display the capacity to strongly interact with proteins and cross biomembranes, which is essential for their bioavailability. These capacities are due to their hydrophobic benzoic rings and the hydrogen-binding potential of the phenolic hydroxyl groups. Phenolics have also the ability to act as antioxidants by inhibiting some enzymes involved in radical generation (cytochrome P450, lipoxygenases, cyclo-oxygenase and xanthine oxidase) (Laughton *et al*. 1991; Parr and Bolwell 2000) and by upregulating endogenous antioxidant enzymes (superoxide dismutase, catalase and gluthatione peroxidase) (Zhan and Yang 2006). The regulation of some enzymes by phenolics can be, in part, due to their regulatory effects on signalling pathway, changing the phosphorylation state of important regulatory proteins (Lee *et al*. 2010). This mechanism also explains several actions of phenolics: in inflammation, phenolics induce a down-regulation of NF-κB (Huang *et al*. 2006) and, in cancer, inhibit cell proliferation, modulating a signal transduction pathway, as mitogen-activated protein kinase (Kim *et al*. 2006).

Synergistic effects of phenolic compounds with other antioxidant molecules, like ascorbic acid, β-carotene and α-tocopherol (Croft 1998), and their involvement in the regulation of intracellular glutathione levels are known (Myhrstad *et al.* 2002). So, phenolics have potential benefits by regulation of cellular processes. Some particularities of the two main classes of phenolics, flavonoids and phenolic acids, will be referred below.

Flavonoids

Flavonoids are compounds with a diphenylpropane (C_6-C_3-C_6) skeleton (**Fig. 1**), which includes monomeric flavanols, flavanones, anthocyanidins, flavones, flavonols, isoflavones,

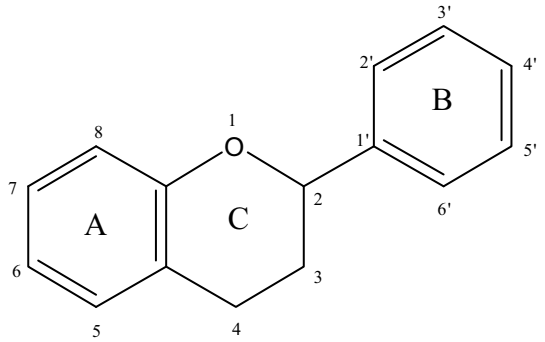

Fig. 1 Basic structure of flavonoids.

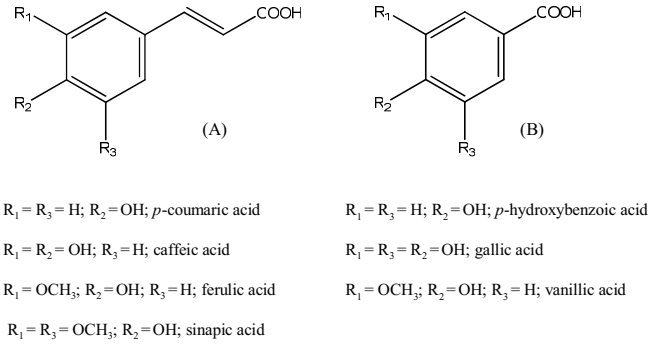

Fig. 2 Main phenolic acids. Hydroxycinnamic acids (**A**) and benzoic acids (**B**).

aurones and chalcones (Rice-Evans *et al.* 1996). They constitute a large and one of the most relevant classes of phenolic compounds. More than 4000 flavonoids have been found, being frequent components of the human diet (Cao *et al*. 1997).

Flavonoids play important biological activities, exhibiting antitumoral (Deschner *et al.* 1991; Elangovan *et al*. 1994), antiplatelet (Tzeng *et al.* 1991), anti-ischemia (Rump *et al*. 1995), anti-allergic and anti-inflammatory (Ferrándie and Alcaraz 1991; Middleton and Kandaswami 1992) properties. Most of the beneficial health effects of flavonoids are attributed to their antioxidant ability (Heim *et al.* 2002). Like other polyphenols, there are several mechanisms for the antioxidant activity of flavonoids and one of the most important is the interaction with biomembranes. Flavonoids also interact with and permeate the lipid bilayer, inhibiting lipid peroxidation (Saija *et al.* 1995). The incorporation of flavonoids in cellular membranes is affected by electrostatic interactions, formation of hydrogen bonds with polar groups of the phospholipids, hydrophobic interactions with fatty acyl chains, and by the molecular geometry of the phospholipids (Rego and Oliveira 1995). Thus, the interaction of an agent with biomembranes or its uptake into the membranes is strongly dependent on its lipophilicity, expressed as the partition coefficient (Brown *et al.* 1998). Another contributory mechanism to antioxidant activity of flavonoids is their ability to stabilize membranes, by decreasing membrane fluidity (Arora *et al.* 1998).

The antioxidant activity depends on the flavonoid structure, being ascribed to the number of substituted hydroxyl or methoxyl groups and glycosylation around the flavonoid skeleton (Heim *et al.* 2002). The unsaturation in the C ring appears to be important, because it allows electron delocalization across the molecule for stabilization of aryloxyl radical (Rice-Evans *et al.* 1996). The presence of hydroxyl groups in B ring also contributes for the antioxidant activity and molecules bearing *ortho*-dihydroxyls are more active than those not possessing such functionalities. It is known that the *ortho*-dihydroxyl substitution on phenol renders the oxidated intermediate, *ortho*-hydroxyphenoxyl radical, more stable due to intramolecular hydrogen bonding interaction. The *ortho*-hydroxyphenoxyl radical and/or *ortho*-semiquinone radical anion can easily be further oxidized to form the final *ortho*-quinone (Seabra *et al.* 2006). In addition, phenolic hydroxyl groups are good hydrogen donors, being able to react with reactive species in a termination reaction that breaks the cycle of radicals' generation (Andrade *et al*. 2008). So, when a flavonoid is glycosylated, there is a reduction of its activity when compared to the corresponding aglycones (Rice-Evans *et al*. 1996). The same occurs with *O*-methylation due to steric effect that perturbs the planarity (Heim *et al.* 2002). In addition, the presence of a hydroxyl group in positions 5 and/or 3 of the flavonoid and carbonyl group at C-4 contribute to the scavenging potential by increasing resonance (Das and Pereira 1990; Seabra *et al.* 2006). In summary, the position and degree of hydroxylation is fundamental to the antioxidant activity of flavonoids, particularly in terms of the *o*-dihydroxylation of the B ring, the carbonyl group at position 4, and a free hydroxyl group at positions 3 and/or 5 in the C and A rings, respectively (Rice-Evans *et al.* 1996; Leopoldini *et al*. 2011).

Phenolic acids

Two groups of phenolic acids can be distinguished: derivatives of benzoic acid and derivatives of cinnamic acid (**Fig. 2**). They consist in phenyl, as a basic structure, linked to a carboxylic group (benzoic acids) or to a propenoic acid (cinnamic acids) (Lafay and Gil-Izquierdo 2008).

In general, phenolic acids display more antioxidant activity than other acids due to stabilization of radicals by electron delocalization. As example, cinnamic acids are more active than phenylacetic acid. By the same reason, compounds derived from cinnamic acid are better antioxidants than benzoic acids. The insertion of an ethylenic group between a phenyl ring carrying a *p*-hydroxyl group and the carboxyl group, as in *p*-coumaric acid, has a highly favorable effect on the reducing properties of the hydroxyl group compared with cinnamic acid (Rice-Evans *et al.* 1996). Incorporation of a hydroxyl group into *p*-coumaric acid adjacent to that in the *para* position, as in caffeic acid, increases the antioxidant activity (Rice-Evans *et al.* 1996). So, the antioxidant activity of phenolic acids and their esters depends on the number of hydroxyl groups and of several other groups, which can cause steric hindrance or electron donating effects on the ring (Rice-Evans *et al.* 1996).

CONSIDERATIONS ON PHENOLICS COMPOSITION AND ANTIOXIDANT POTENTIAL

There are many works concerning antioxidant activity of polyphenols in natural matrices. Sample preparation is fundamental for the analysis of phenolics in those materials. To accomplish the complete identification of phenolics, especially flavonoids, the ideal solution would be high-performance liquid chromatography coupled to mass spectroscopy and nuclear magnetic resonance (HPLC-MS-NMR) (Molnár-Perl and Füzfai 2005). However, this system is present in only few laboratories and simpler techniques, such as HPLC, capillary electrophoresis (CE), capillary electrochromatography (CEC) or gas chromatography (GC), provide useful information for the identification and quantification of phenolics in natural matrices (Molnár-Perl and Füzfai 2005). Thus, for the analysis of phenolic compounds, the HPLC coupled with photodiode array detector (PAD) is the more widely used system, because it is less expensive equipment and it allows UV spectrum determination (Andrade *et al.* 2008).

With respect to antioxidant activity, there are many ways to assess the potential of a compound or an extract (Parr and Bolwell 2000). The antioxidant activity of a matrix can depend upon the reactive species generated and/or the oxidants used in a given system (Cao *et al.* 1997).

Furthermore, antioxidants may respond differently to distinct radical or oxidant sources (Prior *et al*. 2005). There is no simple universal method to accurately and quantitatively measure the antioxidant capacity (Andrade *et al*. 2008). The comparison and general interpretation of the results are almost impossible due to the diversity of experimental conditions and differences in physicochemical characteristics of oxidizable substrates (Stratil *et al*. 2006). Due to the enormous diversity of methods to assess the antioxidant potential, only some methods, used with the matrices involved in this chapter, will be approached: DPPH$^{\bullet}$ (1,1'-diphenyl-2-picrylhydrazyl radical) assay, which is a method of general screening, and diverse assays involving reactive species of biological importance (superoxide radical, nitric oxide, hydroxyl radical and hypochlorous acid).

DPPH$^{\bullet}$ is a free radical, commonly used to screen antiradical capacity. The reduction of DPPH$^{\bullet}$ by an antioxidant results in the loss of its aborbance at 515 nm, which can be measured spectophotometrically (Fukumoto and Mazza 2000).

The biological significance of superoxide radical is linked to its capacity to generate other reactive species, like hydroxyl radical and peroxynitrite (Ternay and Sorokin 1997). Superoxide radical may be generated both in an enzymatic system, containing xanthine and xanthine oxidase (X/XO), or in a chemical one, with NADH/phenazine methosulfate (NADH/PMS) (Andrade *et al*. 2008). This free radical induces the reduction of nitroblue tetrazolium (NBT) to formazan, which can be measured spectophotometrically at 560 nm.

Nitric oxide ($^{\bullet}$NO) is a short-lived free radical, which has important biological functions, including vasodilation, neurotransmission and inflammation (Nathan 1992). However, $^{\bullet}$NO can damage cells and originate other reactive species, as peroxynitrite. $^{\bullet}$NO is spontaneously released from sodium nitroprusside, in aqueous solution at physiological pH and under light irradiation. The $^{\bullet}$NO amount present in the test solution is measured by reaction with Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine). The absorbance of the chromophore formed is determined at 562 nm (Baliga *et al*. 2003).

Hydroxyl radical is the most reactive radical known in chemistry (Halliwell 1991) and it can be formed from H_2O_2 and $O_2^{\bullet-}$ in the presence of certain transition metals. Reducing agents at low concentrations, as ascorbic acid, favours this reaction (Aruoma *et al.* 1989). Hydroxyl radical originated by the Fenton system reacts with deoxyribose, forming malonyldialdehyde. The scavenging effect can be assessed spectophotometrically at 532 nm, after addition of thiobarbituric acid (TBA). This assay can be performed without ascorbic acid in order to check for pro-oxidant activity, or without ethylenediamine tetraacetic acid (EDTA) to evaluate metal chelation capacity (Valentão *et al*. 2003).

Hypochlorous acid (HOCl) has an important role in inflammatory process. Nevertheless, it can easily oxidize thiol groups, causing sulfhydryl oxidation (Aruoma *et al*. 1989). This constitutes the basis of a method to assess the activity against HOCl, in which the transformation of 5-thio-2-nitrobenzoic acid (TNB) to 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) is measured spectophotometrically at 412 nm (Valentão *et al*. 2003).

The complexity of the chemical composition of plants renders the assessment of the individual antioxidant potential of their constituents rather difficult, not withstanding the possible synergistic effect between the several antioxidant compounds present (Huang *et al.* 2005). Therefore, it seems important and most realistic to evaluate the activity of whole extracts, because interactions may occur among their different constituents (Andrade *et al.* 2008). Besides this, it should be kept in mind that herbal water extracts, namely infusions, are an important source of antioxidant phenolic compounds in the human diet (Ferreres *et al.* 2008a).

PHENOLIC ANTIOXIDANTS IN NATURAL MATRICES: SOME CASE STUDIES

Catharanthus roseus

Catharanthus roseus (L.) G. Don (formerly *Vinca rosea* L., Apocynaceae), commonly known as the Madagascar periwinkle, was originally an endemic subshrub species of Madagascar, having now acquired a pantropical distribution. Besides being a popular ornamental plant (Filippini *et al*. 2003), *C. roseus* has become one of the best-studied medicinal plants (Ferreres *et al.* 2008a). This species has been used in traditional medicine in several situations, such as oral hypoglycemic and antipyretic agent (Ross 2003; Sottomayor and Ros Barceló 2005), and to treat menorrhagia due to its bleeding arresting properties (Ross 2003; Jaleel *et al.* 2008).

C. roseus is very rich in alkaloids, which are found in all parts of the plant (Jaleel *et al.* 2008), being mainly known for two terpenoid indole alkaloids, vinblastine and vincristine (Sottomayor and Ros Barceló 2005). These last were the first natural anticancer agents to be clinically used, although their enormous complexity renders them unfeasible to be synthesized in the laboratory (Costa *et al.* 2008). As the leaves of this species are, currently, the only known source of these alkaloids (Costa *et al.* 2008), tons of plant material wastes are created only by their extraction (Pereira *et al*. 2010). However, these wastes can be used as an antioxidants source (Jaleel *et al.* 2006).

The great antioxidant activity is mainly due to the phenolic diversity of *C. roseus*. Leaves and stems are particularly rich in phenolic acids, namely caffeoylquinic acids, which are present in higher amounts (**Fig. 3**) (Ferreres *et al.* 2008a; Pereira *et al*. 2009a). On the other hand, seeds and petals have a great variety of flavonoids, namely quercetin, kaempferol and isorhamenetin derivatives (**Fig. 3**) (Ferreres *et al*. 2008a; Pereira *et al*. 2009a). Among the four vegetal materials, petals present the highest phenolics content (Pereira *et al*. 2009a). No phenolic compound was identified in the roots (Pereira *et al*. 2010).

The different phenolics distribution in the vegetal tissues explains, at least in part, their distinct antioxidant activity (Ferreres *et al*. 2008a; Pereira *et al*. 2010). All aqueous extracts from different parts of *C. roseus* revealed a concentration-dependent antioxidant capacity against DPPH$^{\bullet}$, superoxide and nitric oxide radical (**Table 1**). In the DPPH$^{\bullet}$ assay, with the roots presenting the strongest effects (Pereira *et al*. 2010), and stems the weakest ones (Ferreres *et al*. 2008a). Concerning superoxide radical, the seeds and roots were the most effective materials, while petals showed the lowest activity (Ferreres *et al.* 2008a; Pereira *et al*. 2010). The analyzed extracts displayed protective activity against nitric oxide, being the roots more active than the other plant parts (Ferreres *et al*. 2008a; Pereira *et al.* 2010). Thus, besides the scavenging capacity observed against both superoxide radical and nitric oxide, *C. roseus* may also prevent the formation of other biologically important oxidative species resultant from the reaction of those two, like peroxynitrite and hydroxyl radical. Some of the compounds found in *C. roseus* have already revealed antioxidant capacity, in several systems, like 5-*O*-caffeoylquinic (Heo *et al.* 2007), quercetin, kaempferol and isorhamnetin glycosides (Han *et al.* 2004; Hyun *et al*. 2006).

As referred above, roots revealed great antioxidant activity, but no phenolics were found (Pereira *et al*. 2010), which means that other compounds are responsible for this activity. Following roots, petals were the plant part most active against nitric oxide and DPPH$^{\bullet}$ radical. In this case, phenolics probably had an important contribution for the activity, since petals were the plant material containing the highest proportion of phenolics. Stems and leaves were generally less active than petals and seeds, suggesting that flavonoids are probably more effective as antioxidants than phenolic acids.

C. roseus, mainly roots, petals and seeds, seems to be a

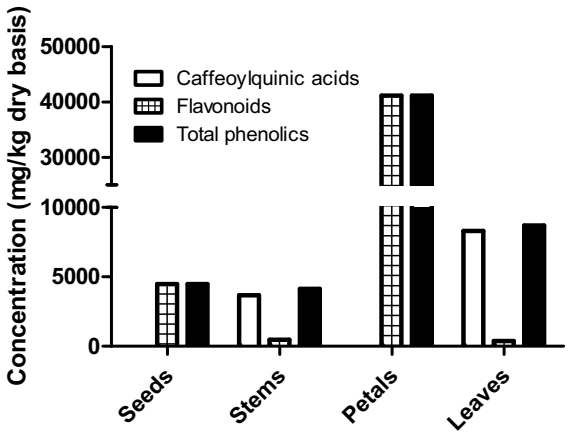

Fig. 3 Quantification of phenolics compounds in *C. roseus* plant parts aqueous extract. Adapted from Pereira *et al*. (2009a).

Table 1 Comparison between antioxidant activities of *C. roseus* aqueous extracts. Adapted from Pereira *et al*. 2010 and Ferreres *et al*. 2008a.

Assay	Vegetal material				
	Seeds	**Roots**	**Stems**	**Leaves**	**Petals**
DPPH$^{\bullet}$[a]	256	153	476	447	197
$O_2^{\bullet-}$[a]	74	74.6	202	90	260
$^{\bullet}NO$[b]	320	189	546	505	232

[a] IC_{50} (μg/mL); [b] IC_{25} (μg/mL)

good source of natural antioxidants, which could be used in a number of applications, as in food, pharmaceutical or cosmetic industries (Ferreres *et al.* 2008a; Pereira *et al.* 2010). Thus, *C. roseus* extracts constitute a reliable and inexpensive source of antioxidants, being an economic opportunity for *C. roseus* producers worldwide, as this application could occur in concomitance with the current alkaloid exploitation (Pereira *et al*. 2010).

Passiflora edulis

The genus *Passiflora* comprises approximately 450 species, but only a few are exploited, like *Passiflora edulis*. Two types of *Passiflora edulis* are grown commercially, a purple form (*Passiflora edulis* Sims) and a yellow form (*Passiflora edulis* var. *flavicarpa* Degener) (Carvalho-Okano and Vieira 2001). *P. edulis* Sims, usually called passion fruit, is the best known among them. It is originated in Brazil and is now being cultivated in many other countries for its edible fruits and pharmacologic properties (Ferreres *et al*. 2007a). In fact, *P. edulis* is very popular, not only because of its pleasant fruits, but also for its leaves, which infusion has been largely used as sedative, tranquilizer (Corrêa 1978), antihypertensive, antimalarial and to treat heart problems (Jamir *et al*. 1999). Leaves extracts are also used in many pharmaceutical preparations and in food industries (Petry *et al.* 2001).

Alkaloids, phenols, as glycosyl flavonoids, and cyanogenic compounds are known to occur in the *Passiflora* genus (Dhawan *et al.* 2004). *P. edulis* is very rich in flavonoids and their hidroalchoolic extract is reported to have almost twice the flavonoid amount than that of *P. alata* (Petry *et al.* 2001).

There are many studies about the phenolic composition and antioxidant activity of *P. edulis* fruits (Murcia *et al*. 2001; Talcott *et al*. 2003; Bendini *et al*. 2006; Kuskoski *et al*. 2006; Genovese *et al*. 2008), but much less on their leaves. *Passiflora* species, including *P. edulis*, are rich in *C*-glycosyl flavones (Ferreres *et al*. 2007a) an unusual feature in flavonoid derivatives. *P. edulis* leaf extracts present mono-*C*-glycosyl, *O*-glycosyl-*C*-glycosyl and *O*-glycosyl flavones, being luteolin and apigenin derivatives (Ferreres *et al*. 2007a). Like other *Passiflora* species, flavonoids in *P. edulis* leaves have deoxyhexose moieties, as rhamnose (Ayanoglu *et al*. 1982; Escobar *et al.* 1983; McCormick and Mabry 1983).

Some compounds found in *P. edulis*, like luteolin (Es-Safi *et al*. 2005; Du *et al*. 2006) and apigenin (Du *et al.* 2006) glycosides, have well-known antioxidant properties. The aqueous lyophilized extract of *P. edulis* leaves exhibited a strong concentration dependent antioxidant potential in DPPH$^{\bullet}$ assay, with an IC_{50} value of 128 μg/mL (Ferreres *et al*. 2007a). Regarding superoxide radical, an IC_{25} at 59 μg/mL was found when using NADH/PMS system and an IC_{25} at 99 μg/ml, when superoxide radical was generated in the enzymatic X/XO system (Ferreres *et al*. 2007a). *P. edulis* leaves extract also revealed an effective xanthine oxidase inhibitory activity, which was concentration-dependent (IC_{25} at 121 μg/mL). According to these results, it may be inferred that *P. edulis* leaves lyophilized extract has antioxidant activity achieved by both the scavenging of superoxide radical and xanthine oxidase inhibition (Ferreres *et al*. 2007a).

This extract also appeared to have some capacity as scavenger of hydroxyl radical, in a concentration-dependent manner (IC_{10} at 5.8 μg/mL) (Ferreres *et al*. 2007a). However, it seems that *P. edulis* leaves have both antioxidant and pro-oxidant effects, the first being more pronounced than the latter, because the extract revealed to be an effective substitute for ascorbic acid in Fenton system (Ferreres *et al.* 2007a). In addition, *P. edulis* leaves extract did not exhibit capacity for chelation of metals as iron ions (Ferreres *et al.* 2007a).

P. edulis displayed protective effects against damage by hypochlorous acid, in a concentration-dependent manner, with an IC_{25} of 932 μg/mL (Ferreres *et al.* 2007a). These results are interesting, considering the use of *P. edulis* leaves as an anti-inflammatory and the fact that hypochlorous acid is produced in the organism at sites of inflammation, by the oxidation of Cl^- ions, catalyzed by neutrophil-derived myeloperoxidase, in the presence of H_2O_2 (Aruoma *et al.* 1989). In addition, Shimoi *et al*. (2000) have described the deconjugation of luteolin glucuronides to luteolin by neutrophils glucuronidase at sites of inflammation in rats (Shimoi *et al.* 2000).

Other study demonstrated that *P. edulis* leaf extracts have significant protective effects against carbonyl protein formation (Rudnicki *et al.* 2007). This is of particular importance, since oxidized proteins (enzymes, receptors, membrane transporters, among other) are often functionally inactive (Stadtman 2001), playing a toxic role in the pathogenesis of several diseases, especially in neurodegenerative diseases (Dean *et al*. 1997). So, the intake of *P. edulis* aqueous extract might exert a protective role in the organism.

Rumex induratus

The *Rumex* (Polygonaceae) genus comprises several species, with known biological activities. For example, *Rumex nepalensis* is known for the psychopharmacological (Ghosh *et al.* 2002) and purgative (Ghosh *et al*. 2003) effects, while *Rumex patientia* containes antioxidant and cytotoxic agents (Demirezer *et al*. 2001). *Rumex acetosa* has effects in body weight and in serum levels of aminoacids and minerals (Ladeji *et al*. 1997). Many of these activities are due to the occurrence of several phenolic compounds, namely phenolic acids and flavonoids (Saleh *et al.* 1993; Hasan *et al*. 1995; Trichopoulou *et al.* 2000; Tolrà *et al*. 2005), and organic acids (Tolrà *et al.* 2005). In addition, *Rumex* species are known for the presence of anthraquinones, which may also act as antioxidants (Midiwo and Rukunga 1985; Saleh *et al.* 1993; Hasan *et al*. 1995; Tolrà *et al*. 2005).

Rumex induratus Boiss and Reuter is an endemic Iberian herb, which fundamentally occurs in rocky habitats of the thermo-Mediterranean region (Ferreres *et al.* 2006a; Guerra *et al.* 2008). This species is usually consumed in salads (Ferreres *et al.* 2006a). However, its high oxalic acid content has been implicated in oxalic intoxication, mainly in children (DerMarderosian and Beutler 2002). Despite its

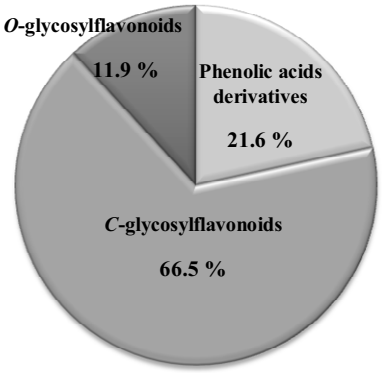

Fig. 4 Distribution of different phenolic compounds classes in *R. induratus* leaves. Adapted from Ferreres *et al*. (2006a).

high consumption, there are few studies concerning the nutritional value and composition or biological potential of *R. induratus*.

R. induratus leaves have several compounds derived from hydroxycinnamic acids and polyhydroxyflavones (Ferreres *et al*. 2006a; Guerra *et al*. 2008). The existence of *C*-glycosylflavones presenting a hydroxyl in the 3 position (flavonols), like 6-*C*-hexosyl-quercetin, is not common in nature, but occurs in *R. induratus* leaves (**Fig. 4**) (Ferreres *et al*. 2006a). *O*-Glycosylflavonoids present in *R. induratus* leaves are hexosides or rutinosides of quercetin, isorhamnetin, and diosmetin (Ferreres *et al.* 2006a). Aqueous extract of *R. induratus* leaves demonstrated activity against superoxide radical in a concentration dependent manner (Ferreres *et al.* 2006a), being formed an IC_{50} at 67.5 μg/mL, when it was generated by the X/XO system, and an IC_{50} of 336.9 μg/mL, when radical was generated by NADH/PMS system (Ferreres *et al.* 2006a). An inhibitory effect on xanthine oxidase (IC_{25} at 708.8 μg/mL) was also noticed (Ferreres *et al.* 2006a). *R. induratus* leaves also exhibited a strong concentration-dependent antioxidant potential against nitric oxide (IC_{50} at 92.7 μg/mL) and $DPPH^{\bullet}$ radical (IC_{50} at 106.5 μg/mL), and a lower activity against hypochlorous acid (IC_{20} at 171.3 μg/mL) (Guerra *et al*. 2008).

The antioxidant properties exhibited by *R. induratus* extract are extremely valuable, since by simultaneously scavenging superoxide radical and nitric oxide, it can avoid the generation of other oxidative agents, namely peroxynitrite and, ultimately, hydroxyl radical (Halliwell *et al*. 1995). Obviously, the chemical composition of *R. induratus* leaves determines the observed activity. In fact, antioxidant properties have been observed for hydroxycinnamic acids derivatives (Fukomoto and Mazza 2000; Hamerski *et al*. 2005), luteolin, apigenin, quercetin, and diosmetin glycosides (Miyake *et al*. 1997; Materska and Perucka 2005; Nilsson *et al*. 2005; Wu *et al*. 2005).

In general, the intake of *R. induratus* may provide nutritional and health benefits associated with the consumption of fruits and vegetables, because it is an interesting source of bioactive compounds, like phenolics. Thus, due to its agreeable taste and high phenolic content, it can be a good alternative in the preparation of salads, which, nowadays, are mainly composed of tomatoes and/or lettuce.

Ecological system with *Pieris brassicae*

Several plants are the source of flavonoids and other secondary metabolites during feeding stages of herbivores, including insects (Burghardt *et al*. 1997). Larvae of *Pieris brassicae* L. (Lepidoptera: Pieridae) are specialists on crucifers (Ferreres *et al.* 2007b) and constitute a frequent pest of some *Brassica* species (Muriel and Grez 2002). In the following text, it is approached the phenolic composition and antioxidant activity of three ecological systems involving *P. brassicae* and distinct *Brassica* host plants.

1. *Brassica* species

Brassica vegetables belong to the Cruciferous family, which includes a variety of economically significant horticultural crops (Sousa *et al*. 2008). They are consumed in enormous quantities throughout the world, being very important in human nutrition, and are reported to possess cancer preventive properties (Beecher 1994) that have been attributed to the glucosinolates and their derived products (Stoewsand 1995), and to flavonoids and other phenolics (Le Marchand 2002).

Brassica oleracea is native of the Mediterranean region and southwestern Europe (Vallejo *et al.* 2004; Cartea *et al.* 2008). Nevertheless, *B. oleracea* forms are now grown in other regions all over the world (Ferreres *et al*. 2009a). Tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) and kale (*Brassica oleracea* L. var. *acephala*) are two *B. oleracea* varieties, which will be considered. Turnip (*Brassica rapa* var. *rapa* L.), one of the oldest cultivated vegetables, being used for human consumption all over the world (Sasaki and Takajashi 2002), will also be addressed.

Tronchuda cabbage, turnip and kale have, in general, the same kind of compounds. Flavonoids glycosides and acylated flavonoids glycosides were described in all above mentioned *Brassica* species (Ferreres *et al*. 2005, 2006b, 2008a, 2008b, 2009b). All matrices present kaempferol and quercetin derivatives. Turnip and kale have also isorhamnetin derivatives (Ferreres *et al.* 2006a, 2008b, 2009b). Glycosilation is located in 3 and/or 7 position of flavonoids and 3,7-di-*O*-glucosides derivatives, as isorhamnetin-3,7-di-*O*-glucoside, are characteristic compounds of the *B. rapa* var. *rapa* (Ferreres *et al*. 2008b).

Flavonoids with more than three sugar residues are not usual in plants (Ferreres *et al.* 2005), although the production of flavonoids with an unusually high degree of glycolsylation by *B. oleracea* varieties is known (Llorach *et al.* 2003; Vallejo *et al*. 2004). Kaempferol-3-*O*-sophorotrioside-7-*O*-glucoside, kaempferol-3-*O*-sophorotrioside-7-*O*-sophoroside and kaempferol-3-*O*-tetraglucoside-7-*O*-sophoroside were detected in tronchuda cabbage (Ferreres *et al.* 2005). In kale, the degree of glycosylation of flavonoids ranges from two to five hexose residues (Ferreres *et al.* 2009b). In turnip, kaempferol-3-*O*-sophorotrioside-7-*O*-glucoside was the only compound with more than two sugars (Ferreres *et al.* 2008b).

In turnip, the acids present in acylated compounds are methoxycaffeic, caffeic, sinapic, ferulic and *p*-coumaric (Ferreres *et al*. 2008b). Thonchuda cabbage leaves have monoacylated and diacylated compounds (Ferreres *et al.* 2005). The degree of acylation of kale is lower, with a high number of non-acylated glycosides being noted and absence of diacylated derivatives (Ferreres *et al*. 2009b).

In addition, kale and tronchuda cabbage have hydroxycinnamic acyl gentiobiosides (Ferreres *et al.* 2006b, 2009b).

2. *Brassica* species: Influences in *Pieris brassicae* materials phenolics

Insect–plant associations have long been a pivotal subject of interest for many entomologists and biologists because of their economic importance in agriculture and in ecological systems (Honda *et al*. 1998). The role of plant chemistry in shaping plant-insect relationships is well recognized, with a close association of certain oligophagous insects with specific chemicals of their host plants (Renwick 2002). The phenolic profile of the host plant has a determinant role in insect's uptake and metabolism (Burghardt *et al.* 1997).

P. brassicae has a life cycle that lasts about 45 days from egg to adult (Muriel and Grez 2002). It can feed on various species of Brassicaceae in larvae form (Ferreres *et al.* 2007b, 2009a). *P. brassicae* has been thoroughly studied for the association with its host plants as a model for oviposition and feeding modulation, as a consequence of the plant's chemical composition (Renwick 2002).

Lepidoptera, in particular in butterfly families like the

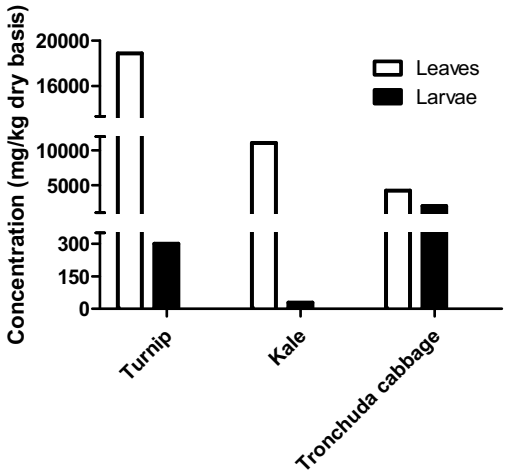

Fig. 5 Phenolics compounds amounts in *Brassica* species and in *P. brassicae* having them as host plant. Adapted from Ferreres *et al*. (2009b), Pereira *et al*. (2009b) and Sousa *et al*. (2009).

Table 2 Main metabolic pathways of *P. brassicae* and more important alterations in materials' insect composition in relationship to host plant (Ferreres *et al*. 2007c, 2008b, 2009b, 2009c; Pereira *et al*. 2009b).

Metabolic reactions	Main alterations in insect materials' composition comparing with host plant
Deacylation	↑ non acylated derivatives
	↑ free acids
	↓ acylated derivatives
Demethoxylation	Appearance or ↑ *p*-coumaroyl derivatives
Deglycosylation	↓ 3,7-di-*O*-glucosides
	↑3-*O*-glucosides
Sulfation	Appearance sulfated compounds

Papilionidae, Nymphalidae, and Lycaenidae, where they form part of the wing pigmentation (Burghardt *et al.* 1997; Schittko *et al*. 1999; Burghardt *et al*. 2001), since insects are unable to synthesize flavonoids or their precursors *de novo* (Kayser 1985). There is evidence that the flavonoid content of butterflies from the same species may drastically vary according to the host plant used during the larval stages (Burghardt *et al.* 1997; Schitto *et al*. 1999).

Comparing the phenolic composition of *Brassica* species with that of *P. brassicae* feed with them, it was verified a distinct composition, with compounds from the host plant being biotransformed or accumulated by the insect (**Table 2; Fig. 5**).

Deacylation is one of the main metabolic reactions, occurring in *P. brassicae* organism (Ferreres *et al.* 2009c). In larvae extract of *P. brassicae*, feruloyl and sinapoyl acids were found among the major compounds. As these free acids exist in lower concentration in turnip and are absent in tronchuda cabbage and kale, their high concentration in *P. brassicae* is a consequence of deacylation of acylated flavonols present in host plant (Ferreres *et al.* 2009c; Pereira *et al.* 2009b).

Demethoxylation is another reaction, which occurs in *P. brassicae.* Demethoxylation of the sinapoyl and/or feruloyl acylated flavonoids leads to the appearance of compounds acylated with *p*-coumaroyl moiety, which are not present in turnip and in tronchuda cabbage (Ferreres *et al*. 2007b).

Deglycosylation is another common reaction *in P. brassicae*. In larvae fed with tronchuda cabbage, flavonol-3-*O*-glycosides represent more than ca. 50% of their food plant (Ferreres *et al*. 2007b). This can be ascribed to the deglycosylation of C-7 of the flavonols glycosylated at 3 and 7 positions, present in tronchuda cabbage. Another explanation is a higher efficiency of sequestration of flavonol-3-*O*-glycosides (Ferreres *et al.* 2007b). A similar case is the presence of quercetin-3-*O*-sophoroside in all periods of starvation in larvae fed with tronchuda cabbage, when this compound is absent in host plant (Ferreres *et al.* 2009c). Analysing *P. brassicae* fed with other *Brassica* species, it is clear that the most frequent loss of the sugar by insect is in 7-position (Ferreres *et al*. 2008b, 2009b).

Another curious finding is the preference of *P. brassicae* for quercetin derivatives (Ferreres *et al*. 2007b; Pereira *et al.* 2009b), as larvae present high amounts of these compounds, than *Brassica* species. So, *P. brassicae* seems to selectively sequester these flavonoids or kaempferol glycosides are metabolized into quercetin glycosides by the larvae (Ferreres *et al*. 2007b). This is in accordance with other studies defending that larvae selectively sequester and metabolize quercetin and kaempferol derivatives (Burghardt *et al.* 2001), while myricetin derivatives, flavones and isoflavonoids are mostly excreted (Schittko *et al.* 1999). Besides the aglycone of these flavonoids, the sugar moiety also seems to play a role in the profile of the compounds sequestered by the insect (Pereira *et al.* 2009b). In turnip leaves, different glycosides, which include 3-*O*-glucosides, 3,7-di-*O*-glucosides and 3-*O*-sophorosides exist, although only 3-*O*-sophoroside derivatives, such kaempferol-3-*O*-sophoroside, were sequestered and accumulated in the larvae's body fed with turnip, while the remaining two were excreted (Ferreres *et al.* 2008b; Pereira *et al.* 2009b).

Comparing phenolic composition of *P. brassicae* larvae, butterfly, exuviae and larvae's excrements, it is clear that exuviae and butterflies are much poor than larvae or excrements (Ferreres *et al.* 2008b). Butterfly and exuviae revealed only trace amounts of phenolic compounds (Ferreres *et al.* 2008b), because they are mainly excreted by the larvae, not being transferred to wings (Pereira *et al.* 2009b). These results constitute an exception, because it is known that in Lepidoptera order, there is flavonoid uptake and part of them are responsible for wing pigmentation (Burghardt *et al.* 1997; Schittko *et al*. 1999; Burghardt *et al*. 2001). Moreover, *P. brassicae* butterflies are white and not colored. In *P. brassicae* fed with kale, also, larvae revealed phenolics in very low amounts, while excrements have high phenolics amounts (Ferreres *et al.* 2009b).

Excrements are produced only at the larval stage (Ferreres *et al.* 2008b), having compounds similar to those of larvae. Excrements are the insect's material containing higher phenolics content, in amounts and in diversity (Ferreres *et al.* 2008b). These results are not surprising, considering that phenolic compounds are sequestered and undergo metabolization, regarding their detoxification and excretion. Excrements are, usually, rich in sulfated compounds, some of them also found in *P. brassicae*. Sulfated derivatives are not present in *Brassica* species. So, the possibility that these compounds come from the diet is rejected (Ferreres *et al.* 2008b, 2009c). This kind of compounds is very usual in animals' metabolic process (Ferreres *et al.* 2008b). In fact, the sulfated compounds have enhanced hydrophilicity, which may contribute to easier excretion, as it yields passage through the cell membrane more difficult (Pereira *et al.* 2009b). The interest in these sulfated conjugates arises from the biological activities observed before, like antioxidants or anticoagulants (Op de Beck *et al.* 2003; Guglielmone *et al.* 2005).

As an example of the complex relationship between insect and host plant composition, in *P. brassicae* fed with tronchuda cabbage kaempferol-3-*O*-sophoroside-7-*O*-glucoside decreased between 1 and 8h of starvation (Ferreres *et al.* 2009c). Most probably, this compound underwent a deglycosilation at 7-position, a process characteristic of *P. brassicae*, thus originating kaempferol-3-*O*-sophoroside, which are after consumed in sulfation process that yields kaempferol-3-*O*-sophoroside-sulfate (Ferreres *et al.* 2009c).

Different composition of the host plants lead to distinct *P. brassicae* phenolics profile. So the composition of the plant material in shaping the metabolic profile upon feeding is very important.

3. Antioxidant activity of *Pieris brassicae*

There are several studies concerning the antioxidant activity of *P. brassicae* and its relation with the host plant. *P. brassicae* fed with tronchuda cabbage extract exhibited a strong antiradical activity against DPPH$^{\bullet}$, in a concentration dependent way, being much more effective than the host plant (**Table 3**) (Sousa *et al.* 2009). *P. brassicae* also revealed scavenging activity for hydroxyl radical, in a concentration dependent way, being superior to that exhibited by tronchuda cabbage (**Table 3**) (Sousa *et al.* 2009). However, pro-oxidant effect was observed for higher concentrations of larvae extract (≥ 7.8 μg/mL), while tronchuda cabbage displayed pro-oxidant activity at all tested concentrations (Sousa *et al.* 2009). For hypochlorous acid, *P. brassicaee* extract showed some concentration-dependent protective activity, although it was less effective than tronchuda cabbage (Sousa *et al.* 2009). When using X/XO system, tronchuda cabbage revealed higher activity against superoxide radical in comparison to *P. brassicae*. However when this radical was generated by PMS/NADH system, the opposite was observed (**Table 3**) (Sousa *et al.* 2009). Nevertheless, it should be highlighted that *P. brassicae* fed with tronchuda cabbage displayed a potent inhibitory effect on xanthine oxidase (IC_{25} at 358 μg/mL), in a concentration dependent manner, and the host plant did not. Thus, *P. brassicae* exerts an effective protective role against superoxide radical by acting as both scavenger and xanthine oxidase inhibitor (Sousa *et al.* 2009). Both *P. brassicae* materials and tronchuda cabbage extracts showed no metal chelating activity (Sousa *et al*. 2009). In a general way, *P. brassicae* fed with tronchuda cabbage revealed to be more effective antioxidant than its host plant, although the last presented higher phenolics content (**Fig. 5**) (Sousa *et al.* 2009). So, the phenolic qualitative composition seems to be determinant for the antioxidant potential exhibited (Sousa *et al.* 2009). Flavonol-3-*O*-glucosides seem to contribute to a greater extent to the strongest protective effects displayed by *P. brassicae* fed with tronchuda cabbage, because they are formed at higher relative amounts in the larvae (Sousa *et al.* 2009). In fact, it is known that the addiction of a second glycoside residue decreases the activity due to steric hindrance (Fukumoto and Mazza 2000). Additionally, the presence of higher amounts of quercetin derivatives in *P. brassicae* can also explain its highest antioxidant properties (Sousa *et al.* 2009), once it is well established that quercetin is a more potent antioxidant than kaempferol, due to the presence of a cathecol group in the B ring of the former (Pietta 2000).

For *P. brassicae* fed with *B. rapa* var. *rapa*, butterfly was the most active material in DPPH$^{\bullet}$ and nitric oxide scavenging assays (**Table 4**). However, concerning superoxide radical scavenging, excrements are the most active matrix. *B. rapa* var. *rapa* leaves are again less active (**Table 4**) (Pereira *et al.* 2009b), although they are the matrix with more phenolics. Excrements are the second richer in phenolics content and their activity against superoxide radical is, probably, due to 3-*O*-sophorosides derivatives. As butterflies are, in general, the most active material, other compounds (non phenolic) are probably responsible for its activity.

As happened for *P. brassicae* fed with turnip, butterflies of *P. brassicae* fed with kale exhibited the strongest capacity against DPPH$^{\bullet}$ and nitric oxide, with an IC_{25} value of 19 μg/mL and IC_{20} at 47 μg/ml, respectively, being kale less active in both assays (IC_{25} at 257 μg/mL and IC_{20} at 261 μg/ml, respectively) (Ferreres *et al.* 2009b). Kale and *P. brassicae* excrements also exhibited a concentration-dependent superoxide radical scavenging capacity, with the excrements being the most active (IC_{25} at 51 μg/mL) (Ferreres *et al*. 2009b). Against this reactive species, larvae and butterflies showed antioxidant activity until 130 and 260 μg/mL, respectively, above which a decrease of scavenging effect was noticed (Ferreres *et al.* 2009b). Analyzing compounds that could contribute for antioxidant activity, kaempferol derivatives seem to contribute to some extension to the superoxide radical scavenging ability of excrements of *P. brassicae* fed with kale, because kaempferol derivatives represent more than 80% of compounds of this matrix (Ferreres *et al.* 2009b).

Table 3 Antioxidant activity of *P. brassicae* larvae fed with *Brassica oleracea* var. *costata* and the host plant. Adapted from Sousa *et al*. 2009.

Assay	Tronchuda cabbage leaves	*P. brassicae* fed with tronchuda cabbage
DPPH$^{\bullet}$[a]	678	97
HO$^{\bullet}$[b]	9.2	6.1
HOCl[c]	257	453
$O_2^{-\bullet}$		
X/XO system[a]	186	251
PMS/NADH system[b]	358	7.4

[a] IC_{50} (μg/mL); [b] IC_{25} (μg/mL); [c] IC_{10} (μg/mL)

Table 4 Comparison between antioxidants activities of different materials of *P. brassicae* fed with *Brassica rapa* var. *rapa* and the host plant extracts. Adapted from Pereira *et al*. 2009b.

Assay	Turnip leaves	*P. brassicae* fed with turnip		
		Larvae	Excrements	Butterfly
DPPH$^{\bullet}$[a]	557	178	217	46
$O_2^{-\bullet}$[b]	100	35	17	26
NO$^{\bullet}$[c]	53	20	16	5
Phenolic total [d]	18881.3	300.5	10226.7	-

[a] IC_{50} (μg/mL); [b] IC_{25} (μg/mL); [c] IC_{10} (μg/mL); [d] mg/kg, dry basis

In general, *P. brassicae* material always exhibited stronger antioxidant capacity than host plants, which may be a consequence of selective sequester and metabolism of antioxidant compounds. Due to the potent activity exhibited by *P. brassicae* materials, their applications as antioxidants would allow to take some profit from this frequent pest in *Brassica* productions, which means great losses to producers. In addition, *P. brassicae* and host *Brassica* species may constitute a good source of health promoting compounds, namely, flavonoids (Ferreres *et al.* 2005).

CONCLUSION

Plants and other natural matrices are a rich source of phenolic compounds, which have proven antioxidant activity. All matrices described have different compositions and also different activities. The same occurs with composition and activity of an insect's materials, which is dependent of the insect host plant composition, such as described for *P. brassicae*.

Total antioxidant capacity in plants and their derived products results from the interactions between the several constituents, phenolics and non phenolics, which may include synergistic or additive effects. Therefore, it is important to consider the extracts as a whole, because their effects are not due to a single compound, but to a large number of structurally related and unrelated compounds contributing to that of overall effect.

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