Bioactivity of phenolic acids: metabolites versus parent compounds

Sandrina A. Heleno\textsuperscript{a,b}, Anabela Martins\textsuperscript{a}, Maria João R.P. Queiroz\textsuperscript{b}, Isabel C.F.R. Ferreira\textsuperscript{a,*}

\textsuperscript{a}Centro de Investigação de Montanha, Escola Superior Agrária, Campus de Santa Apolónia, apartado 1172, 5301-854 Bragança, Portugal.
\textsuperscript{b}Centro de Química, Universidade do Minho, Campus de Gualtar 4710-057 Braga, Portugal.

* Author to whom correspondence should be addressed (e-mail: iferreira@ipb.pt telephone +351-273-303219; fax +351-273-325405).
ABSTRACT

Phenolic acids are present in our diet in different foods. In particular, mushrooms are a good source of these molecules. Due to their bioactive properties, phenolic acids are extensively studied and there is evidence of their role in disease prevention. Nevertheless, in vivo, these compounds are metabolized and circulate in the organism as glucuronated, sulfated and methylated metabolites, displaying higher or lower bioactivity. To clarify the importance of the metabolism of phenolic acids, the knowledge about the bioactivity of the metabolites is extremely important.

In this review, chemical features, biosynthesis and bioavailability of phenolic acids are discussed as well as the chemical and enzymatic synthesis of their metabolites. Finally, the metabolites bioactive properties are compared with that of the corresponding parental compounds.

Keywords: Mushrooms; phenolic acids; biosynthesis/bioavailability; metabolites; chemical/enzymatic synthesis; bioactivity
1. Introduction

Mushrooms are rich sources of bioactive compounds with an enormous variety of chemical structures (Ferreira, Barros & Abreu, 2009). In particular, different bioactive properties have been attributed to phenolic acids from mushrooms, namely antitumor (Vaz, Almeida, Ferreira, Martins & Vasconcelos, 2012; Heleno, Ferreira, Calhelha, Esteves & Queiroz, 2014a), antimicrobial (Alves, Ferreira, Froufe, Abreu, Martins & Pintado, 2013), and antioxidant (Piazzon, Vrhovsek, Masuero, Mattivi, Mandoj & Nardini, 2012).

However, very little is known about phenolic acids bioactive forms in vivo and the mechanisms by which they may contribute toward disease prevention. Moreover, several studies dealing with the biological effects of phenolic acids have ignored the question of their achievable concentrations in the circulation after ingestion as well as the possibility of metabolism (Rechner, Kuhnle, Bremner, Hubbard, Moore & Rice-Evans, 2002). There is accumulating evidence suggesting that phenolic acids are rapidly metabolized in the human organism (Scalbert & Williamson, 2000; Rechner et al., 2002; Nardini, Forte, Vrhovsek, Mattivi, Viola & Scaccini, 2009).

After absorption from the gastrointestinal tract, these molecules suffer conjugation reactions and several changes in their initial structure and circulate in human plasma as conjugated forms, glucuronide, methylated and sulfated derivatives. These changes in their structures may increase or decrease the bioactivity of the initial phenolic acids (Rechner et al., 2002; Piazzon et al., 2012).

Therefore, detailed knowledge concerning the conjugative and metabolic events and resulting plasma levels following the ingestion of a polyphenol-rich diet is crucial for the understanding of their bioactivity (Rechner et al., 2002). Despite the large data concerning the bioactivity of phenolic acids, only a few studies deal with the bioactive
properties of their metabolites, especially as most of those molecules are not commercially available (Piazzon et al., 2012).

In this review, several features of the phenolic acids found in mushrooms will be discussed, namely the chemical characterization, biosynthesis pathways, the bioavailability and metabolism, as well as the chemical and enzymatic synthesis of glucuronated, sulphated and methylated metabolites of different phenolic acids. The antioxidant, antimicrobial and antitumor properties of the metabolites will be discussed and compared with the bioactivities of the corresponding parental phenolic acid.

2. Chemical features and biosynthesis of phenolic acids usually found in mushrooms

Mushrooms have been extensively studied during the last decades due to their bioactive potential (Ferreira et al., 2009) attributed to different molecules including phenolic acids. These compounds (Figure 1) have been identified in different mushroom species around the world (Valentão et al., 2005; Puttaraju, Venkateshaiah, Dharmesh, Urs & Somasundaram, 2006; Ribeiro, Valentão, Baptista, Seabra & Andrade, 2007; Kim et al., 2008).

Phenolic acids can be divided into two major groups, hydroxybenzoic acids and hydroxycinnamic acids, which are derived from non-phenolic molecules of benzoic and cinnamic acid, respectively. Chemically, these compounds have at least one aromatic ring in which at least one hydrogen is substituted by a hydroxyl group (Figure 1).

Phenolic compounds, where phenolic acids are included, are secondary metabolites from plants and fungi. These compounds are produced for protection against lightning UV, insects, viruses and bacteria. There are even certain plant species that develop phenolic compounds to inhibit the growth of other plant competitors (allelopathy).
Examples of phenolic acids with allelopathic action are caffeic and ferulic acids. It is believed that phenolic compounds were fundamental for plants in the conquer of their terrestrial environment. This is the case of lignin which stimulates the development of the vascular system, giving stiffness to the vessels (Gross, 1985).

Phenolic acids are synthesized from the shikimate pathway from L-phenylalanine or L-tyrosine (Rice-Evans, Miller & Paganga, 1996) (Figure 2, Table 1). Phenylalanine and tyrosine are very important amino acids in this pathway since these amino acids are the common precursors of the majority of the natural phenolic products (Figure 2, Table 1).

Firstly, it occurs a deamination of the phenylalanine and/or the tyrosine giving cinnamic and/or p-coumaric acids as results of these steps, respectively. Cinnamic and p-coumaric acid aromatic rings are then hydroxylated and methylated to form its derivatives e.g., ferulic and caffeic acids, being deamination, hydroxylation and methylation the main three reactions involved in the formation of phenolic acids (Figure 2, Table 1) (Gross, 1985). Benzoic acid formation can result from the degradation of the side chain of cinnamic acid. As mentioned for cinnamic and p-coumaric acids, the same reactions of hydroxilation and methylation can occur in the aromatic ring of benzoic acid giving the correspondent derivatives e.g., protocatechuic and p-hydroxybenzoic acids (Gross, 1985).

3. In vivo human metabolism of phenolic acids

3.1. Bioavailability of phenolic acids

Despite the extensive literature describing the biological effects of phenolic acids, little is known about how they are absorbed from diet.
Phenolic acids are present in almost all the plant-derived foods, representing a significant portion of the human diet. The average phenolic acid intake in humans has been reported to be in the order of 200 mg/day depending on the diet habits and preferences (Clifford & Scalbert, 2000). The most frequently encountered and studied phenolic acids are caffeic and ferulic acids. Caffeic acid is also found in the form of esters being chlorogenic acid the most frequently encountered (Clifford & Scalbert, 2000). Coffee is normally the matrix studied for the absorption of these molecules since this matrix is a good source of bound phenolic acids such as caffeic, ferulic and p-coumaric acids (Nardini, Cirillo, Natella & Scaccini, 2002). In patients that ingested a specific quantity of coffee, Marmet, Actis-Gorettta, Renouf & Giuffrida, (2014) several methylated, glucuronated and sulfated metabolites of phenolic acids circulating in plasma, were identified. In another study, Fumeaux et al. (2010) described that after a specific dose of coffee consumption, several hydroxycinnamic acid sulphate and glucuronide conjugates were present in human biological fluids. Nardini et al. (2009) studied the absorption of phenolic acids from white wine and reported that after the consumption of a single wine drink, hydroxycinnamic acids are absorbed from the gastrointestinal tract and circulate in the blood after being largely metabolized to glucuronide and sulfate conjugates.

In the past decades, mushrooms have received special attention because they are described as being rich sources of phenolic acids that are amongst the major contributors to their medicinal properties (Ferreira et al., 2009). The extensive knowledge of the phenolic acids bioavailability is essential to understand their conjugations and bioactivities in the organism. Phenolic acids are a considerable group inside the polyphenols family and there is evidence that when they are absorbed in the free form as they are mostly found in mushrooms, their absorption and conjugation,
specially glucuronation, follow the same pathways that the absorption and conjugation of flavonoids and other polyphenols (Cremin, Kasim-Karakas & Waterhouse, 2001). Thus, during the absorption, polyphenols are conjugated in the small intestine and later in the liver, occurring methylation, sulfation and glucuronidation as the main conjugation reactions (Manach, Scalbert, Morand, Rémésy & Jiménez, 2004). This is a very important process of detoxication to avoid potential toxic effects and also because increasing their hydrophilicity they can easily be eliminated by the biliary or the urinary route. These conjugation mechanisms are very efficient and aglycones are present in the blood in very low concentrations after the consumption of polyphenols.

Circulating polyphenols are conjugated derivatives that are extensively bound to albumin. Generally polyphenols are secreted by the biliary pathway into the duodenum, in the distal segments of the intestine where they are under the action of bacterial enzymes, particularly β-glucuronidase and, after this step, they can be reabsorbed what may lead to a longer presence of polyphenols within the body (Manach et al., 2004).

The bioavailability of polyphenols is crucial to their biological properties. To study the bioavailability of these molecules, different authors measured their concentrations in the plasma and urine after the ingestion of a known content as pure compounds or incorporated in food (Pérez-Jiménez et al., 2010).

When ingested in the free form phenolic acids are rapidly absorbed by the small intestine and are later conjugated (Scalbert & Williamson, 2000). Nevertheless, the chemical structures of the compounds can also influence the conjugation reactions as well as the amounts of metabolites formed by the gut microflora in the colon (Scalbert & Williamson, 2000). For example, in chlorogenic acid (a caffeic acid ester linked to quinic acid), the ester bound can change its biological properties. In human tissues there
are no esterases able to release caffeic acid from chlorogenic acid (Plumb et al., 1999). Thus, the only local possible for chlorogenic acid metabolism is the colon.

In agreement with this, ferulic or other hydroxycinnamic acids bound to cell walls are also not released from human endogenous enzymes and require release by enzymes such as xylanases and esterases of the colonic microflora (Kroon, Faulds, Ryden, Robertson & Williamson, 1997). When these molecules are hydrolyzed by the colonic flora, the efficiency of their absorption is reduced because flora can degrade the aglycones releasing simple aromatic acids (Scalbert & Williamson, 2000), meaning that the efficiency of absorption of phenolic acids is markedly reduced when they are present in the esterified form rather than in the free forms (Azuma et al., 2000; Olthof, Hollman & Katan, 2001). Olthof et al. (2001) reported that in patients with colonic ablation, caffeic acid was better absorbed than chlorogenic acid. In another report, where chlorogenic acid was given to rats, no intact compound was detected in plasma in the following 6h, and the maximum concentrations of metabolites obtained after administration of caffeic acid in the same conditions, were much higher than those of the metabolites obtained after chlorogenic acid administration (glucuronated and sulfated derivatives of caffeic and ferulic acids) (Azuma et al., 2000). Glucuronated metabolites, for example acyl glucuronides can react with sulphydryl and hydroxyl groups and can be hydrolyzed back to the aglycone (Spahn-Langguth & Benet, 1992). These molecules can go through covalent binding to plasma proteins (Zhou et al., 2001), and react with glutathione and transacylate cellular macromolecules (Faed, 1984). Moreover, acyl glucuronides go through rearrangements due to intramolecular acyl migration from –OH to the adjacent –OH, giving different positional isomers.

The rearrangement of glucuronides through the biosynthetic C-1 isomers to other positional isomers is very important because only 1-O-substituted acyl glucuronides are
substrates of \( \beta \)-glucuronidase, an enzyme commonly used to identify these conjugates that are very important (Sinclair & Cardwell, 1982) when measuring the concentrations of phenolic acids present in biological fluids as glucuronic acid esters. The migration of acyl groups occurs in plasma, bile and urine (Faed, 1984). Additionally, if \( \beta \)-glucuronidase hydrolysis is used to liberate the aglycones in the sample containing the rearranged isomers, the concentrations of glucuronic acid conjugates of carboxylic acid might be underestimated (Faed, 1984).

3.2. Conjugation reactions for metabolites formation

After the ingestion and once absorbed, phenolic acids are conjugated by methylation, sulfation and glucuronidation reactions that are controlled by specific enzymes that catalyze these steps (Figure 3).

Catechol-\( O \)-methyl transferase catalyses the transfer of a methyl group from \( S \)-adenosyl-L-methionine to polyphenols that have an \( o \)-diphenolic moiety (Wu, Cao & Prior, 2002). The methylation generally occurs in the 3´position of the polyphenol with a minor proportion of 4´-\( O \)-methylated product also formed. These enzymes showed the highest activity in the liver and kidneys (Piskuta & Terrao, 1998).

Sulfotransferases catalyze the transfer of a sulfate moiety from 3´-phosphoadenosine-5´-phosphosulfate to a hydroxyl group on polyphenols and this conjugation reaction occurs in the liver (Piskuta & Terrao, 1998).

The membrane-bound enzymes UDP-glucuronosyltransferases that are located in the endoplasmatic reticulum in many tissues catalyze the transfer of a glucuronic acid from UDP-glucuronic acid to polyphenols. The presence of glucuronidated metabolites in the mesenteric or portal blood after perfusion of polyphenols in the small intestine of rats
shows that glucuronidation of polyphenols first occurs in the enterocytes before conjugation in the liver (Crespy et al., 2001).

Conjugation reactions appear to vary according to the nature of the polyphenol and the dose ingested. Sulfation has generally a higher-affinity, lower-capacity pathway than glucuronidation, so that when the ingested dose is increased, a rise from sulfation toward glucuronidation occurs (Koster, Halsema, Scholtens, Knippers & Mulder, 1981). Identification of circulating metabolites has been undertaken for only a few polyphenols. Further investigation is needed to know not only the nature and number of the conjugating groups but also the positions of these groups on the polyphenol structure, because these positions can affect the biological properties of the conjugates (Day, Bao, Morgan & Williamson, 2000). There are only a few studies describing the proportions of the various conjugates and the percentages of free forms in plasma but the main circulating compounds are generally glucuronides (Zhang et al., 2003).

To be eliminated these metabolites can follow two pathways: the biliary or the urinary pathway. Large extensively conjugated metabolites are more likely to be eliminated in the bile, whereas small conjugates such as monosulfates are preferentially excreted in the urine (Crespy et al., 2003).

4. Bioactive properties of phenolic acids

Phenolic acids are often included in human diet and have been largely studied due to their bioactivities such as antioxidant (Rice-Evans et al., 1996, Ferreira et al., 2009), antitumor (Carocho & Ferreira., 2013; Heleno et al., 2014a) and antimicrobial (Alves et al., 2013) properties, among others. In particular, mushrooms are a source of these molecules in diet (Table 2).
Gallic acid, besides having astringent and styptic uses has also several bioactivities reported such as antineoplastic, bacteriostatic, antimelanogenic and antioxidant properties (Kim, 2007). This molecule showed anticancer properties in prostate carcinoma cells (Kaur, Velmurugan, Rajamanickam, Agarwal & Agarwal, 2009). Grupta, Grupta &Mahmood (2007), reported gallic acid as a potent inhibitor of brush border sucrose and other disaccharides in the mammalian intestine, while Kratz et al. (2008) described its promising activity as an anti-HSV-2 (Herpes simplex virus) agent. Furthermore, Lu et al. (2010) proposed gallic acid as a candidate for treatment of brain tumors due to its ability to suppress cell viability, proliferation, invasion and angiogenesis in human glioma cells. In another study, this phenolic acid proved to induce HeLa cervical cancer cells death by apoptosis and necrosis (You, Moon, Han & Park, 2010).

*p*-Hydroxybenzoic acid, has been reported to have antioxidant activity against free radicals (Rice-Evans et al., 1996), antimicrobial activity against pathogenic bacteria and fungi (Heleno et al., 2013a) among other bioactivities such as estrogenic and antimutagenic properties (Pugazhendhi, Pope & Darbre, 2005).

Protocatechuic acid possesses several bioactivities such as antioxidant (Ferreira et al., 2009), antimicrobial (Alves et al., 2013), cytotoxic (Yip, Chan, Pang, Tam & Wong, 2006), chemopreventive, apoptotic (Yin, Lin, Wu, Tsao, & Hsu, 2009), and neuroprotective (An, Guan, Shi, Bao, Duan & Jiang, 2006) properties, being also a LDL oxidation inhibitor (Hur et al., 2003).

Another phenolic acid with beneficial properties is vanillic acid that showed antisicking and anthelmintic activities, being also able to suppress hepatic fibrosis in chronic liver injury (Itoh et al., 2010). Furthermore, this compound proved to be able to inhibit snake venom 5’-nucleotidase (Dhananjaya, Nataraju, Gowda, Sharath & D'Souza, 2009).
Syringic acid revealed antioxidant, antibacterial and hepatoprotective activities (Kong, Zhao, Shan, Xiao & Guo, 2008; Itoh et al., 2010).

Gentisic acid has been described as having anti-inflammatory, antirheumatic and analgesic activities, being also a cytostatic agent and capable to inhibit low density lipoprotein oxidation in human plasma (Ashidate et al., 2005).

Lin & Nakatsui (1998) described that salicylic acid has analgesic, antipyretic, anti-inflammatory, antiseptic and antifungal properties.

Besides being antioxidant (Rice-Evans et al., 1996; Ferreira et al., 2009), cinnamic acid was also reported as an antitumor agent by Vaz et al. (2012) that verified its capacity to inhibit cell growth in a non-small lung cancer cell line (NCI-H460). Heleno et al. (2014a) in agreement with the mentioned authors, referred the cytotoxicity of cinnamic acid against the same cell line, also revealing activity against colon (HCT15) and cervical (HeLa) carcinoma cell lines. Ekmekcioglu, Feyertag & Marktl (1998), reported that this compound was able to inhibit proliferation and modulate brush border membrane enzyme activities in human colon adenocarcinoma cells (CaCo-2).

Different authors (Alves et al., 2013; Heleno et al., 2013a) described cinnamic acid as an antimicrobial agent, showing activity against Gram positive and Gram negative bacteria (either clinical isolates or collection microorganisms), and fungi.

*p*-Coumaric acid also revealed antioxidant activity against free radicals (Rice-Evans et al., 1996), and antitumor activity against breast (MCF7), NCI-H460 and HCT15 carcinoma cell lines (Heleno et al., 2014a). In a study about antimicrobial activity performed by Lou, Wang, Rao, Sun, Ma & Li (2012), the authors reported that *p*-coumaric acid had dual mechanisms of bactericidal activity: it was able to disrupt bacterial cell membranes and binding to bacterial genomic DNA to inhibit cellular
functions, leading to cell death. Heleno et al. (2014b) also described a promising antimicrobial activity of \( \rho \)-coumaric acid against several pathogenic bacteria and fungi. Caffeic and ferulic acids have also been accounted for their bioactive properties. These phenolic acids showed antioxidant activities measured by common assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl radical), ABTS (2,2’-azino-bis(3-thylbenzothiazoline-6-sulphonic acid)) and ORAC (oxygen radical absorbance capacity) methods (Vilãno, Fernández-Pachón, Troncoso & García-Parrilla., 2005; Piazzon et al., 2012). These compounds have been also reported as antimicrobial agents against pathogenic bacteria and fungi (Alves et al., 2013). Yagasaki, Miura, Okauchi & Furuse (2000) that studied the activity of chlorogenic acid and its related compounds such as caffeic acid, proved that both chlorogenic acid as well as the released compound caffeic acid were able to significantly reduce the invasion of a rat ascites hepatoma cell line (AH109A), without altering the cell proliferation.

The diverse biological functions of these phenolic acids suggest potential pharmacological activities (Khadem & Marles, 2010). Thus, looking at all the promising bioactivities and knowing that mushrooms are a rich source of these molecules we can conclude that mushrooms are a good option to include in our daily diet.

4. Controversy on \textit{in vivo} bioactivity of polyphenols

As mentioned above, phenolic acids represent a significant portion of polyphenols family in our diet. Their bioactivity, specially antioxidant properties are related with the phenolic hydroxyl groups attached to ring structures and these molecules can act as reducing agents, hydrogen donators, singlet oxygen quenchers, superoxide radical
scavengers and as metal chelators over hydroxyl and peroxyl radicals, superoxide anions and peroxynitrites (Terpinc et al., 2011).

Nevertheless, there has been some controversy about the bioactivity of polyphenols after metabolism. Once ingested, these molecules are metabolized and transformed in methylated, glucuronated and sulfated metabolites (Manach et al., 2004).

Glucuronidation and sulfation conjugation reactions are described to have a significant impact on the bioactivity of polyphenols. These conjugation reactions significantly reduce polyphenols antioxidant activity, since both sulfation and glucuronidation occur at the reducing hydroxyl groups of the phenolic structure, which are the functions mainly responsible for the antioxidant properties of polyphenols (Piazzon et al., 2012). There are only a few studies about the biological properties of the conjugated derivatives of polyphenols, where phenolic acids are included, present in plasma or tissues because of the lack of precise identification and commercial standards.

Polyphenols are expected to act at water-lipid interfaces and may also be involved in oxidation regeneration pathways with vitamins C and E, since the hydrophobicity of these compounds is intermediate between that of vitamin C (highly hydrophilic) and that of vitamin E (highly hydrophobic) (Manach et al., 2004).

Glucuronidation and sulfation render polyphenols more hydrophilic, which can affect their site of action and interactions with other antioxidants (Manach et al., 2004).

For phenolic acids, bearing a carboxyl function in addition to the hydroxyl groups, glucuronidation can occur at the reducing hydroxyl group (phenyl-O-glucuronides) and at the carboxylic group (acyl glucuronides). Therefore, acyl glucuronides retain all the free reducing hydroxyl functions of the parent compound while in the case of phenyl-O-glucuronides, at least one hydroxyl group of the phenolic acid is bound to the glucuronate moiety (Piazzon et al., 2012).
Cren-Olive, Teissier, Duriez & Rolando (2003), described that methylated polyphenols present lower ability to protect low density lipoproteins (LDL) from \textit{in vitro} oxidation than the respective parental compound. On the other hand, several authors reported an increase on the antioxidant capacity of plasma after the consumption of various polyphenol-rich foods, meaning that some of the polyphenol metabolites retain antioxidant activity (Serafini, Laranjinha, Almeida & Maiani., 2000).

Nevertheless, conjugation reactions might enhance certain specific bioactivities. For example, Koga & Meydani (2001), described that plasma metabolites of catechin have inhibitory effect on monocyte adhesion to interleukin 1 β-stimulated human aortic endothelial cells, while catechin had no effect. In another study, quercetin 3-\textit{O-}glucuronide presented vascular smooth muscle cell hypertrophy by angiotensin II (Yoshizumi et al., 2002). In contrast, Spencer et al. (2001), reported that two flavonoid metabolites (5- and 7-\textit{O-}glucuronides of epicatechin) were unable to protect fibroblasts and neuronal cells from oxidative stress \textit{in vitro}, while epicatechin and methylepicatechin were protective. The bioactivity of phenolic acids and metabolites will be compared and discussed in section 6.

Further investigation on the bioactivities of these molecules is needed to better understand the effects of the type and position of conjugation on the various potential activities of polyphenols.

5. \textit{Chemical and enzymatic synthesis of phenolic acid metabolites}

There are only a few reports in the literature describing the synthesis of metabolites from phenolic acids to evaluate their bioactivities in comparison with the corresponding parental compounds.
Piazzon et al. (2012) as well as our research group (Heleno et al., 2013a; Heleno et al., 2014a), described the chemical or enzymatic synthesis of glucuronated, sulfated and methylated derivatives of several phenolic acids, obtaining structures similar to those formed in vivo after phenolic acids metabolism (Figure 4A-E).

For the glucuronidation reactions, the authors reacted the phenolic acids with acetobromo-α-D-glucuronic acid methyl ester, potassium carbonate and dimethyl sulfoxide (Heleno et al., 2013a) or with sodium hydroxide and acetonitrile (ACN) (Piazzon et al., 2012) at room temperature, to obtain the glucuronide protected forms of the parental acids (Figure 4A-D). Piazzon et al. (2012) continued the reaction to obtain the final deprotected glucuronide using potassium hydroxide, water and chloridric acid as reaction conditions to obtain the final deprotected glucuronide (Figure 4A).

The same authors also synthesized glucuronides from FA using mouse liver microsomes. After extraction procedures and HPLC analysis with electrochemical or UV detection, FA, FAG and another compound, the ferulic acid 4′-O-glucuronide (FFG) were identified and quantified, being the ratio between FFG and FAG of 2.4:1, residual FA was also quantified (Piazzon et al., 2012).

For the methylation reactions, the authors performed several different steps in order to obtain the complete series of methylated compounds derived from each phenolic acid. CAM, CoAM1 and HAM1 were obtained by reacting the parental compound with sulphuric acid in methanol at room temperature, affording the desired derivatives that have the carboxylic group from the parental compound replaced by a methoxy group, HAM1 and CoAM1 have a free hydroxyl group in the p-positions (Figure 4B-D) (Heleno et al., 2014a).

CoAM2 and HAM2 were achieved by reacting the parental compounds with dimethyl sulphate and potassium carbonate in acetone at 45-50°C, affording the desired
compounds that have both carboxylic and hydroxyl groups replaced by methoxyl groups (Figure 4C,D) (Heleno et al., 2014a).

To afford CoAM3 and HAM3, the compounds HAM2 and HAM3 were reacted with NaOH and ethanol at 65ºC affording the desired compounds in which the carboxylic group remains but the hydroxyl group from the p-position is replaced with a methoxyl group, completing the series (Figure 4C,D) (Heleno et al., 2014a).

Piazzon et al. (2012) synthesized ferulic acid sulphate ester and caffeic acid monosulfate esters according to procedures previously described by Todd, Zimmerman, Crews & Alberte (1993). Briefly, the sulfurochloridic acid (ClOS₃H) was added dropwise to ferulic and caffeic acids in pyridine and the reaction stayed on stirring at 20ºC. The authors obtained ferulic acid 4´-O-sulfate, caffeic acid 4´-O-sulfate and caffeic acid 3´-O-sulfate (Figure 4E).

6. Bioactivity of phenolic acids versus their metabolites

All the glucuronated, methylated and sulphated compounds mentioned above and the respective parental phenolic acids were studied for their bioactivities namely, antioxidant (Piazzon et al., 2012), antimicrobial (Heleno et al., 2013a; Heleno et al., 2014b) and antitumor (Heleno et al., 2014a) properties, in order to compare the biological activity of the parental compounds before and after metabolism in vivo.

Piazzon et al. (2012) also evaluated the antioxidant activity of some commercial glucuronides derived from ferulic and caffeic acids namely, ferulic acid-4´-O-glucuronide (FFG), caffeic acid-3´-O-glucuronide and caffeic acid-4´-O-glucuronide.

The antioxidant activity of the glucuronated and sulphated compounds was measured by both the ferric reducing antioxidant power (FRAP, ferric reducing ability) and the ABTS radical scavenging assays.
Concerning the glucuronide derivatives, the results showed that FAG, the acyl glucuronide of ferulic acid was able to retain antioxidant activity, similar to the activity of the parental compound in the FRAP assay, while in the ABTS assay its activity decreased almost 50% in comparison with ferulic acid antioxidant activity for this assay.

However, the phenyl-\(O\)-glucuronide of ferulic acid, the ferulic acid 4\(^{\prime}\)-\(O\)-glucuronide, showed much lower antioxidant activity than ferulic acid in both antioxidant activity assays. Caffeic acid 3\(^{\prime}\)-\(O\)-glucuronide also retained a strong antioxidant activity in comparison with the parental phenolic acid activity for the FRAP assay, while for the ABTS its activity was also a half of the activity of caffeic acid.

Caffeic acid 4\(^{\prime}\)-\(O\)-glucuronide displayed a good antioxidant activity with ABTS assay, which is about a third with respect to that of caffeic acid, while with the FRAP assay, it was about 20-fold lower than the one of caffeic acid.

Regarding the sulfate derivatives of ferulic and caffeic acids, Piazzon et al. (2012) verified that their activity is lower when compared with the antioxidant activities of the parental phenolic acids for both assays. These results showed the importance of the reducing hydroxyl groups in the antioxidant activity of phenolic acids and their metabolites.

Considering all the metabolites tested the acyl glucuronide of ferulic acid and the phenyl 3\(^{\prime}\)-\(O\)-glucuronide of caffeic acid retained a strong antioxidant capacity. These compounds remained with the 4\(^{\prime}\)-hydroxyl groups in the aromatic ring that is determinant for the antioxidant activity (Hodnick, Milosevljevic, Nelson & Pardini., 1988; Pulido, Bravo & Saura-Calixto., 2000).

Heleno et al. (2013a) and Heleno et al. (2014b) evaluated the antimicrobial activity of \(p\)-hydroxybenzoic, cinnamic and \(p\)-coumaric glucuronated protected forms and
methylated metabolites, and compared their antimicrobial activities with those of their parental compounds. The authors measured the antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, and *Micrococcus flavus*), Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *Enterobacter cloacae*), and fungi (*Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus ochraceus*, *Aspergillus niger*, *Trichoderma viride*, *Penicillium funiculosum*, *Penicillium ochrochloron*, *Penicillium verrucosum* var. *cyclopium*), and also the demelanizing activity against the mentioned fungi.

Regarding the antibacterial activity, the glucuronide protected forms of *p*-hydroxybenzoic, cinnamic and *p*-coumaric acids (HAGP, CAGP and CoAGP) revealed lower activity than the parental compounds, with the exception of CoAGP that maintained the antibacterial activity of *p*-coumaric acid.

Methylated derivatives of *p*-hydroxybenzoic acid (HAM1, HAM2 and HAM3) presented lower antibacterial activity than the parental compound HA. Cinnamic acid presented an excellent antibacterial activity against all the tested bacteria, while its methylated derivative revealed a much lower activity. Methylated derivatives of *p*-coumaric acid (CoAM1, CoAM2 and CoAM3) revealed higher activity than the parental compound.

Concerning the antifungal activity, glucuronated protected forms, HAGP, CAGP and CoAGP, revealed higher activity than the respective parental compounds against almost all the fungi tested.

Regarding the methylated derivatives, all the *p*-hydroxybenzoic acid derivatives showed lower activity than the parental compound as well as the methylated derivative of cinnamic acid, CAM. Nevertheless, all the *p*-coumaric acid derivatives, CoAM1, CoAM2 and CoAM3 showed higher activity than the parental compound.
Compounds HAM1 and HAM3 showed demelanizing effect on *A. fumigatus*, lowering the amount of conidia and giving nude vesicle without conidia, as well as compounds CoAM1, CoAM2, HAM3 and CAM on *P. verrucosum*.

Heleno et al. (2014a), reported the cytotoxicity of *p*-hidroxybenzoic, cinnamic and *p*-coumaric acid glucuronated protected forms (HAGP, CAGP and CoAGP) and methylated derivatives (HAM1, HAM2, HAM3, CAM, CoAM1, CoAM2 and CoAM3), against five human tumor cell lines namely, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung carcinoma), HCT15 (colon carcinoma), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma).

The authors reported that the glucuronated protected forms presented higher cytotoxicity than the parental molecules against all the tested cell lines. Regarding methylated derivatives of *p*-hydroxybenzoic acid, HAM1 and HAM2, showed higher cytotoxicity than the parental phenolic acid, as well as *p*-coumaric acid methylated derivatives. The presence of an ester group increased the growth inhibitory activity of the compounds; HAM1 and CoAM1 have the carboxylic group replaced by an ester group and an hydroxyl group in the *para* position, while HAM2 and CoAM2 have also a carboxylic group replaced by an ester group and a methoxy group in the *para* position. However, HAM3 and CoAM3 that remains the carboxylic group of CoA but with a methoxyl group in the *para* positions showed none or very weak activity. Cinnamic acid methylated derivative, CAM, that has also the carboxylic group replaced by an ester and no groups in *para* position presented lower activity than cinnamic acid.

All the tested compounds revealed no toxicity on non-tumor porcine liver cells.
7. Concluding remarks

Overall, the results from antioxidant activity revealed that, although ferulic and caffeic acids are extensively metabolized after absorption, their glucuronated metabolites can retain a strong antioxidant activity and might still exert a significant antioxidant action in vivo. These two phenolic acids are the most representative in human diet and, after absorption, they are metabolized and circulate in the human plasma as conjugated forms. Thus, the strong antioxidant activity exhibited by some of these metabolites might contribute to the increase of plasma antioxidant activity measured after the intake of phenolic acids rich-foods (e.g., mushrooms) as described before.

Regarding the antimicrobial activity, the methylation reactions in the parental molecules have considerably increased the activity of CoA. However, the inclusion of acetyl groups increased the antifungal activity but maintained the antibacterial effects. In the case of HA and CA, despite the inclusion of methyl groups did not increase the antimicrobial activity, the demelanizing activity of the parental compounds increased.

Concerning the antitumor potential, in most of the cases the substitution of the carboxylic group (in parental organic acids) for an ester (in methylated derivatives) increased the cytotoxicity of the parental compounds. Glucuronated protected derivatives had considerably increase the cytotoxicity of the respective parental compounds due to the inclusion of acetyl molecules in the parental compound.

Those reports allow a comparison between parental molecules and derived metabolites. It is extremely important to understand the behavior of organic acids (including phenolic acids) regarding their bioactivity after metabolism. Furthermore, future studies are needed in order to clarify specific mechanistic pathways of these molecules.
Acknowledgements

The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT, Portugal) and FEDER-COMPETE/QREN/EU for the financial support through the research centres (PEst-C/QUI/UI0686/2011 and PEst-OE/AGR/UI0690/2011). S.A. Heleno (BD/70304/2010) also thanks FCT, POPH-QREN and FSE for her grant.

References


synthesized glucuronated and methylated derivatives as antibacterial and antifungal agents. *Food & Function*, in press.


Table 1. Main enzymes involved in the biosynthesis of phenolic acids through shikimate pathway from L-phenylalanine or L-tyrosine.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting molecule</th>
<th>Enzyme</th>
<th>Final compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phenylalanine</td>
<td>Phenylalanine ammonia lyase (PAL)</td>
<td>Cinnamic acid</td>
</tr>
<tr>
<td>2</td>
<td>Cinnamic acid</td>
<td>Oxidase (Presumed β-oxidation)</td>
<td>Benzoic acid</td>
</tr>
<tr>
<td>3</td>
<td>Benzoic acid</td>
<td>Benzoic acid 4-hydroxylase</td>
<td>p-Hydroxybenzoic acid</td>
</tr>
<tr>
<td>4</td>
<td>p-Hydroxybenzoic acid</td>
<td>p-Hydroxybenzoic acid 3-hydroxylase</td>
<td>Protocatechuic, Gentisic acid</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Protocatechuic acid</td>
<td>Protocatechuic acid 5-hydroxylase</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>6</td>
<td>Protocatechuic acid</td>
<td>Protocatechuic acid</td>
<td>Protocatechuic acid 3-O-methyltransferase</td>
</tr>
<tr>
<td>7</td>
<td>Vanillic acid</td>
<td>Vanillic acid 5 hydroxylase and Vanillic acid 5-O-methyltransferase</td>
<td>Veratric acid</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
<td>Enzyme Name</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>--------------------</td>
<td>----------------------------------</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="Cinnamic acid" /></td>
<td>Cinnamic acid 4-hydroxylase</td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="p-Coumaric acid" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="L-Tyrosine" /></td>
<td>Tyrosine ammonia lyase (TAL)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="p-Coumaric acid" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td><img src="image" alt="p-Coumaric acid" /></td>
<td>p-Coumaric acid 3-hydroxylase</td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Caffeic acid" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td><img src="image" alt="Caffeic acid" /></td>
<td>Caffeic acid 3-O-methyltransferase</td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Ferulic acid" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ferulic acid 5-hydroxylase and Caffeic/5-hydroxyferulic acid $O$-methyltransferase (COMT)

Caffeic + quinic acid

4-Caffeate CoA ligase and Quinate $O$-hydroxycinnamoyltransferase

3,4 or 5-$O$-caffeoylquinic acid
Table 2. Phenolic acids identified in mushroom species. Adapted from (Ferreira et al., 2009).

<table>
<thead>
<tr>
<th>Phenolic acid</th>
<th>Mushroom species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td><em>Termitomyces heimii, Termitomyces mummiformis, Lactarius deliciosos, Pleurotus sajor-caju, Hydnum repandum, Lentinus squarrulosus, Sparassis crispa, Morchella conica, Russula brevipes, Geastrum arinarius, Cantharellus cibarius, Lactarius sanguifluus, Macrolepiota procera, Cantharellus clavatus, Auricularia polytricha, Pleurotus djamor, Lentinus sajor-caju, Termitomyces tylerance, Morchella anguisticeps, Termitomyces microcarpus, Helvella crispa, Termitomyces shimperi, Pleurotus ostreatus, Agaricus bisporus, Flammulina velutipes, Pleurotus eryngii, Lentinus edodes, Agaricus blazei, Phellinus linteus, Ganoderma lucidum, Inonotus obliquus</em></td>
<td>Puttaraju et al., 2006; Kim et al., 2008</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td><em>Agaricus bisporus (white), Agaricus bisporus (brown), Lentinus edodes, Russula cyanoxantha, Tricholoma equestre, Amanita rubescens, Suillus granulatus, Agaricus arvensis, Agaricus silvicola, Agaricus romagnesii, Lactarius deliciosos, Lepista nuda, Lycoperdon molle, Sarcodon imbricatus, Ramarya botrytis, Tricholoma acerbum, Sparassis crispa, Phellinus linteus, Inonotus obliquus, Ganoderma lucidum, Coprinopsis atramentaria, Lactarius bertillonii, Lactarius vellereus, Rhodotus palmatus, Xerocomus chrysenteron, Morchella esculenta</em></td>
<td>Mattila et al., 2001; Ribeiro, Rangel, Valentão, Baptista, Seabra &amp; Andrade, 2006; Ribeiro et al., 2007; Heleno et al., 2012; Heleno et al., 2012b; Heleno et al., 2013</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td><em>Agaricus bisporus (white), Agaricus bisporus (brown), Lentinus edodes, Termitomyces mummiformis, Boletus edulis, Lactarius deliciosos, Pleurotus sajor-caju, Lentinus squarrulosus, Hydnum repandum, Sparassis crispa, Morchella conica, Russula brevipes, Lentinus sajor-caju, Lactarius sanguifluus, Macrolepiota procera, Cantharellus clavatus, Auricularia polytricha, Pleurotus djamor,</em></td>
<td>Mattila et al., 2001; Puttaraju et al., 2006; Kim et al., 2008; Heleno et al., 2012b; Heleno, et al., 2013</td>
</tr>
<tr>
<td>Termitomyces tylerance, Morchella anguiceps, Termitomyces microcarpus, Helvella crispa, Termitomyces shimperi, Termitomyces heimii, Lepista nuda, Ramarya botrytis, Pleurotus ostreatus, Flammulina velutipes, Pleurotus eryngii, Agaricus blaezi, Inonotus obliquus, Phellinus linteus, Ganoderma lucidum, Lactarius bertillonii, Lactarius vellereus, Rhodotus palmatus, Xerocomus chrysenteron, Morchella esculenta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vanillic acid</strong></td>
<td>Pleurotus sajor-caju, Hydnum repandum, Lentinus squarrulosus, Morchella conica, Russula brevipes, Lactarius sanguifluus, Macrolepiota procera, Cantharellus clavatus, Auricularia polytricha, Pleurotus djamor, Helvella crispa, Termitomyces microcarpus, Termitomyces shimperi, Lentinus sajor-caju, Termitomyces heimii, Lycoperdon molle, Tricholoma acerbum</td>
<td></td>
</tr>
<tr>
<td><strong>Syringic acid</strong></td>
<td>Termitomyces mummiformis, Hydnum repandum, Morchella conica, Russula brevipes, Lactarius sanguifluus, Macrolepiota procera, Cantharellus clavatus, Pleurotus djamor, Lentinus sajor-caju, Termitomyces tylerance, Morchella anguiceps, Termitomyces microcarpus, Agaricus blaezi, Sparassis crispa</td>
<td></td>
</tr>
<tr>
<td><strong>Veratric acid</strong></td>
<td>Sparassis crispa</td>
<td></td>
</tr>
<tr>
<td><strong>Gentisic acid</strong></td>
<td>Termitomyces heimii, Termitomyces mummiformis, Lactarius deliciosus, Pleurotus sajor-caju, Hydnum repandum, Lentinus squarrulosus, Sparassis crispa, Morchella conica, Russula brevipes, Lactarius sanguifluus, Macrolepiota procera, Cantharellus clavatus, Auricularia polytricha, Pleurotus djamor, Lactarius sanguifluus, Termitomyces tylerance, Morchella anguiceps, Termitomyces microcarpus, Helvella crispa, Termitomyces shimperi, Agaricus blaezi</td>
<td></td>
</tr>
<tr>
<td><strong>Cinnamic acid</strong></td>
<td>Agaricus bisporus (white), Agaricus bisporus (brown), Termitomyces heimii, Termitomyces mummiformis, Termitomyces shimperi, Pleurotus sajor-caju, Hydnum repandum, Lentinus squarrulosus, Agaricus bisporus (black), Agaricus bisporus (pink), Termitomyces heimii, Termitomyces mummiformis, Termitomyces shimperi, Pleurotus sajor-caju, Hydnum repandum, Lentinus squarrulosus</td>
<td></td>
</tr>
</tbody>
</table>

Puttaraju et al., 2006; Barros, Dueñas, Ferreira, Baptista & Santos-Buelga, 2008

Puttaraju et al., 2006; Kim et al., 2008

Puttaraju et al., 2006, Kim et al., 2008

Puttaraju et al., 2006; Heleno et al., 2012a; Heleno et al., 2012b
<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>p</em>-Coumaric acid</td>
<td><em>Cantharellus cibarius</em>, <em>Termitomyces heimii</em>, <em>Boletus edulis</em>,</td>
<td>Valentão et al., 2005; Puttaraju et al., 2006;</td>
</tr>
<tr>
<td></td>
<td><em>Sparassis crispa</em>, <em>Geastrum arinarius</em>, <em>Lactarius sanguifluus</em>,</td>
<td>Ribeiro et al., 2007; Kim et al., 2008; Heleno</td>
</tr>
<tr>
<td></td>
<td><em>Macrolepiota procera</em>, <em>Lentinus deliciosus</em>, <em>Pleurotus sajor-caju</em>,</td>
<td>et al., 2012; Heleno et al., 2012a; Heleno et</td>
</tr>
<tr>
<td></td>
<td><em>Lentinus squarrulosus</em>, <em>Sparassis crispa</em>, <em>Morchella conica</em>,</td>
<td>al., 2012b; Heleno et al., 2013</td>
</tr>
<tr>
<td></td>
<td><em>Cantharellus cibarius</em>, <em>Lactarius sanguifluus</em>, <em>Macrolepiota procera</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cantharellus clavatus</em>, <em>Pleurotus djamor</em>, <em>Lentinus sajor-caju</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Morchella conica</em>, <em>Fistulina hepatica</em>, <em>Agaricus arvensis</em>, *Agaricus</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>silvicola</em>, <em>Lepista nuda</em>, <em>Sparassis crispa</em>, <em>Ganoderma lucidum</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Coprinopsis atramentaria</em>, <em>Lactarius bertillonii</em>, <em>Lactarius vellereus</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rhodotus palmatus</em>, <em>Xerocomus chrysenteron</em>, <em>Morchella esculenta</em></td>
<td></td>
</tr>
<tr>
<td><em>o</em>-Coumaric acid</td>
<td><em>Inonotus obliquus</em></td>
<td>Kim et al., 2008</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td><em>Termitomyces heimii</em>, <em>Termitocytes microcarpus</em>, <em>Termitocytes shimperi</em>,</td>
<td>Puttaraju et al., 2006; Kim et al., 2008</td>
</tr>
<tr>
<td></td>
<td><em>Lactarius deliciosus</em>, <em>Pleurotus sajor-caju</em>, <em>Lentinus squarrulosus</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Sparassis crispa</em>, <em>Morchella conica</em>, <em>Cantharellus cibarius</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lactarius sanguifluus</em>, <em>Macrolepiota procera</em>, <em>Cantharellus clavatus</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pleurotus djamor</em>, <em>Flammulina velutipes</em>, <em>Inonotus obliquus</em></td>
<td></td>
</tr>
<tr>
<td>Caffeic acid</td>
<td><em>Termitomyces heimii</em>, <em>Termitocytes tylerance</em>, <em>Termitomyces microcarpus</em>,</td>
<td>Puttaraju et al., 2006; Ribeiro et al., 2007;</td>
</tr>
<tr>
<td></td>
<td><em>Termitomyces shimperi</em>, <em>Boletus edulis</em>, <em>Lentinus squarrulosus</em>,</td>
<td>Kim et al., 2008</td>
</tr>
<tr>
<td></td>
<td><em>Morchella conica</em>, <em>Russula brevipes</em>, <em>Cantharellus cibarius</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lactarius sanguifluus</em>, <em>Macrolepiota procera</em>, <em>Cantharellus clavatus</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pleurotus djamor</em>, <em>Lentinus sajor-caju</em>, <em>Morchella anguïcipes</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Fistulina hepatica</em>, <em>Flammulina velutipes</em>, <em>Sparassis crispa</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Phellinus linteus</em></td>
<td></td>
</tr>
<tr>
<td>5- Sulfosalicylic acid</td>
<td><em>Flammulina velutipes</em>, <em>Sparassis crispa</em>, <em>Phellinus linteus</em>,</td>
<td>Kim et al., 2008</td>
</tr>
<tr>
<td></td>
<td><em>Ganoderma lucidum</em></td>
<td></td>
</tr>
<tr>
<td>3,4 or 5- O-Caffeoylquinic acid</td>
<td><em>Cantharellus cibarius</em>, <em>Pleurotus ostreatus</em>, <em>Flammulina velutipes</em>,</td>
<td>Valentão et al., 2005; Kim et al., 2008</td>
</tr>
<tr>
<td></td>
<td><em>Phellinus linteus</em></td>
<td></td>
</tr>
</tbody>
</table>