Mushrooms do not contain flavonoids

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Chemical compounds studied in this article: Quercetin (PubChem CID: 5280343); Hesperidin (PubChem CID: 3594); Luteolin (PubChem CID: 5280445); Luteolin-7-O-glucoside (PubChem CID: 5280637); Gallic acid (PubChem CID: 370); Caffeic acid (PubChem CID: 689043); L-3,4-dihydroxyphenylalanine (PubChem CID: 6047) and ergosterol (PubChem CID: 444679).

Abbreviations: phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) and chalcone flavanone isomerase (CHI).

ABSTRACT

A literature and database review and several experiments were carried out because of the perturbing increase and exponential spreading of a major mistake thought scientific publications. Up to October 2015, more than 136 reports ensured that mushrooms contain flavonoids. However, 91% of the publications used an unspecific colorimetric method that was developed to quantify them in plants. However, no sequences encoding chalcone synthase or chalcone isomerase (key enzymes involved in the flavonoid biosynthetic pathway) were found in genomic databases even in completely sequenced mushrooms indicating that they do not have the enzymes needed for their production. Fruiting bodies obtained from substrates containing onion wastes and mycelia grown on media supplemented with specific flavonoids showed no flavonoid content after HPLC DAD/MS determinations indicating that they were also unable of absorbing them.

1. Introduction

Flavonoids are already for decades intimately associated with plant kingdom, particularly with land plants (Harborne, 1994; Robards & Antolovich, 1997; Winkel, 2006) and it is widely accepted by the scientific community that flavonoids are plant secondary metabolites. Although edible mushrooms belong to a completely different kingdom than plants, since 2005 an exponential increase in the number of publications has been noticed measuring these compounds that are almost restricted to the vegetal world in mushrooms. The fast spreading of this mistake is alarming since many other publications and even reviews are giving it for granted and justifying fungal bioactivities due to the action of these compounds (Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012; Guillaumon et al., 2010; Jayakumar, Thomas, Sheu, & Geraldine, 2011).

Flavonoids are compounds derived from the secondary metabolism in plants from precursors originated via two different pathways of primary metabolism, phenylpropanoid derivatives and malonyl-CoA. Malonyl-CoA is synthesised by carboxylation of acetyl-CoA, a central intermediate in the tricarboxylic acid cycle. Coumaroyl-CoA is synthesised from the amino acid phenylalanine by three enzymatic steps initiated by phenylalanine ammonia-lyase (PAL) within the so called phenylpropanoid pathway, involved in the biosynthesis of many other compounds besides flavonoids (lignans, coumarins, stilbenes etc.). Flavonoid synthesis starts by condensation of 4-coumaroyl-CoA with three molecules of malonylCoA yielding naringenin chalcone. This reaction is catalyzed by chalcone synthase (CHS). The generated chalcone is subsequently isomerised by chalcone flavanone isomerase (CHI) to yield a flavanone. From these central intermediates the pathway diverges into several side branches, each yielding a different class of flavonoids, isoflavones, anthocyanins etc. (Winkel, 2006).

Nowadays, flavonoid biosynthesis is considered as a classical example of a pathway that evolved gradually by genetic change through specialization of a duplicate gene and also through the evolution of the regulatory control on such gene. The climax of complexity may be exemplified in anthocyanin synthesis where six sequential biochemical reactions enabled by six different enzymes are involved (Winkel, 2006).

Land plants are believed to have evolved from organisms like green algae (Charales) and although extensive surveys were conducted, flavonoids were not found in algae (Markham, 1988). Furthermore, chalcone

synthase (the entry point to flavonoid biosynthesis) was not described in green algae (Charales) but it was in liverworts (Marchantiophyta) and mosses (Bryophyta) until more complex organisms such as flowering plants (Angiosperma). The complexity of the flavonoid pathways and the synthesized compounds fit with this evolutionary model (Rausher, 2006).

Mushrooms share with plants similarities in the biosynthetic pathways to generate phenolic compounds as precursors of melanins. The melanogenesis starts with the shikimate pathway derived from the Krebs cycle (as initial steps of the phenylpropanoid pathway) when the fungal hyphae are damaged or infected (Soler-Rivas, Jolivet, Arpin, Olivier, & Wichers, 1999). Then, phenolic compounds are substrates of polyphenol oxydases or peroxidases that catalyse oxidative reactions yielding diphenols, quinones and further intermediate complexes that spontaneously generate GHB-, DOPA-derivative melanins or other specific compounds.

The reported presence of flavonoids in mushrooms could be due to the facility of these organisms to absorb many nutrients and compounds from the substrate where they grow or from neighbouring plants by spreading their hyphae or forming mycorrhizae. Certain flavonoids are released by some plants to regulate these symbiotic plant-microbe interactions defining the species tolerated to grow on their roots (Grotewold, 2006). Although in taking of these compounds might negatively affect fungal growth because flavonoids are frequently produced by plant as protection against fungal infections indeed, they were widely reported as antifungal compounds (Grotewold, 2006).

Thus, in this work a detailed review of the literature was carried out to elucidate how this mistake might have appeared and evolved until its fast spreading acceptance. The methodological mistake was reproduced in order to explain the causes of the pitfall and other experiments and searches were carried out to confirm that mushrooms cannot synthesize flavonoids neither absorb them from neighbouring plants.

2. Experimental Section

2.1. Biological material

Mushroom strains used in this investigation were *Lentinus edodes*, *Pleurotus ostreatus*, *Pleurotus eryngii*, *Pleurotus pulmonarius*, *Agaricus bisporus*, *Agaricus blazei*, *Amanita ponderosa*, *Amanita caesarea*, *Lactarius*

deliciosus, *Craterellus cornucopioides*, *Cantharellus cibarius*, *Lyophyllum shimeji*, *Agrocybe aegerita*, *Auricularia judea*, *Calocybe gambosa*, *Ganoderma lucidum*, *Hydnum repandum*, *Lepiota procera*, *Grifola frondosa*, *Pholiota nameko*, *Hericium erinaceus*, *Boletus edulis*, and *Morchella conica*. Fruiting bodies from wild mushrooms were purchased on season from the local market in Madrid (Spain). The cultivable strains were grown at CTICH (Centro Tecnológico de Investigación del Champiñón de La Rioja, Autol, Spain). Fruiting bodies were frozen at -20°C and freeze-dried. Dehydrated samples were ground into fine powder as described in Gil-Ramírez et al. (2013) and stored at -20°C until further use.

Commercial fully grown spawn of *Pleurotus ostreatus* K8 was kindly donated by Micelios Fungisem S.A. (Autol, La Rioja, Spain) and was used to obtain fruiting bodies or mycelium subcultures.

2.2. Reagents

Malt extract agar (MEA), sodium hydroxide, quercetin, hesperidin, gallic, caffeic and formic acids, sodium nitrite, L-3,4-dihydroxyphenylalanine (L-DOPA) and Folin-Ciocalteu's reagent were purchased from Sigma-Aldrich (Madrid, Spain). Luteolin and luteolin-7-O-glucoside were obtained from Extrasynthese (Lyon Nord, France). $AlCl_3 \cdot H_2O$ was purchased at Panreac (Barcelona, Spain), ergosterol hydrate at Alfa Aesar GmbH & CoKG (Karlsruhe, Germany) and methanol (HPLC grade) at LAB-SCAN (Gliwice, Poland).

2.3. Systematic review of the literature

A quantitative and systematic search of the literature was carried out using the key words 'mushroom' and 'flavonoid' in the Web of ScienceTM (Thomson Reuters) by selecting 'all years' (system start searching from 1864 up to now). The search included listed records up to October 2015.

Records were reviewed one by one and selected only if the abstract or title was indicating that flavonoids (as total flavonoids or as specific identified flavonoid) were determined in mushrooms. Selected references were listed extracting the publication year, Impact factor (IF) of the journal that year (except for those articles published in 2015 where the indicated IF is from 2014), name of flavonoid identified (or total flavonoid determination), methodology utilized for their determination and detection device used, mushroom species analysed, type of extract (where indicated) and other considerations (see the Supplementary Table 1).

2.4. *In silico* search for enzymes involved in the flavonoid biosynthesis

A protein search was carried out using Pfam (<http://pfam.xfam.org/>) and Blast® (Basic local alignment search tool) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) looking for specific sequences of enzymes involved in the biosynthesis of phenolic compounds and flavonoids. The selected key enzymes were phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) and chalcone isomerase (CHI). The taxonomic distribution of the enzymes through the Eukaryote organisms was monitored using the *Sunburst species distribution* of Pfam. Afterwards, the CHS amino acid sequence (393 aa) from *Vitis vinifera* was obtained from Genbank (ref. seq. NP_001267879.1) (<http://www.ncbi.nlm.nih.gov/genbank/>) and utilized in Blastp as sequence to search for homologies (alignments) within model organisms, different plant and fungi families and certain specific species.

2.5. Lab scale production of fruiting bodies on flavonoid rich substrates

Specific substrate mixtures were prepared in triplicate as described by Ruiz-Rodriguez, Soler-Rivas, Polonia, & Wichers (2010) with a few modifications: wheat straw and onion waste (leftovers after peeling onions) were chopped (2 – 5 cm) and left overnight soaking up tap hot water. The excess of water was drained on a sieve during 20 min. Afterwards, the soaked lignocellulosic substrates were mixed and homogenized with 0% (control) and 14% (w/w) onion waste. Homogenized substrates (500 g) were placed in plastic bags and sterilized in autoclave (120°C) during 15 minutes.

The sterilized substrates were inoculated with 10% (w/w) of fully colonized spawn and left incubated in dark at 24°C. When substrates were fully colonized temperature was changed to 16°C and high humidity in the presence of light, in a day/night cycle to induce fructification. One hole (approx. 2 cm diam.) was made in one side of each bag. After few days (depending on substrate) pinheads were eye visible. Mushrooms obtained from the first flush were harvested, weighed and lyophilized. Afterwards, dried fruiting bodies were ground in a mortar with liquid nitrogen and stored at -20°C for further analysis.

2.6. Mycelium cultivation on Petri dishes supplemented with flavonoids

Mycelia obtained from mother cultures generated from one grain of the *P. ostreatus* spawn were inoculated on Petry plates containing MEA medium (50 g L⁻¹ of malt extract agar, Sigma-Aldrich, (Madrid, Spain)) and sterilized cellophane circles at the top of the semi-solid medium following the procedure

described by Ruiz-Rodríguez, Polonia, Soler-Rivas, & Wichers (2011). To test the effect of flavonoids or a phenolic compound, MEA medium was supplemented with increasing concentrations of luteolin, luteolin-7-O-glucoside (Extrasynthese, Lyon, France), quercetin, hesperidin or caffeic acid (Sigma-Aldrich) (from 0.25 up to 2 or 4 mg/mL depending on their saturation level) dissolved in ethanol (or MilliQ water for those water-soluble). Plates were inoculated in triplicate and incubated at 25°C.

2.7. Determination of fungal growth

Several cultivation parameters were evaluated during *P. ostreatus* cultivation on control substrates (containing only wheat straw) and on substrates including onion waste. The incubation time required before pinning (pinhead appearance) and for harvesting of the first flush was recorded. Yield, biological efficiency and productivity were monitored as described by Ruiz-Rodríguez et al. (2010).

Mycelial growth (minor and major diameters) was measured once a day until mycelia reached the plates edge (Ruiz-Rodríguez et al., 2011). Growth was later expressed as cm/day using the slope at the linear growth phase of the fungi. Mycelial biomass was also quantified by weighting the produced mycelia scratched from the cellophane when they reached the plate edges. Afterwards, fresh mycelia were frozen, freeze-dried and weighted again to calculate their dry weight. They were later ground in a mortar with liquid nitrogen and stored at -20°C for further analysis.

2.8. Determination of total phenol content

The total phenol concentration was determined by the Folin-Ciocalteu's phenol reagent method according to the procedure of Ramírez-Anguiano, Santoyo, Reglero, & Soler-Rivas (2007) using directly the powdered fruiting bodies or mycelia (10 mg) prepared as previously described. Gallic acid was used as standard for quantification.

2.9. Spectrophotometric determination utilized by literature as method to measure 'total flavonoid'

The colorimetric determination was carried out as described by Choi, Lee, Chun, Lee, & Lee (2006) since it was one of the first recorded publications that determined flavonoids in mushrooms using a modified protocol previously utilized to measure flavonoids in mulberry (Zhishen, Mengcheng, & Jianming, 1999). Briefly, powdered fruiting bodies (100 mg) were mixed with 10 ml methanol and stirred for 1h at room

temperature. Afterwards, solution was centrifuged (35000 rpm 15 min) and resulting supernatant filtered and dried until dryness. Two hundred fifty microliters of obtained mushroom extracts (diluted up to 15 mg/mL with methanol) were mixed with 1.25 mL of distilled water and 75 μ L of a 5% NaNO₂ solution. After 5 min, 150 μ L of a 10% AlCl₃ · H₂O was added. After 6 min, 500 μ L of 1M NaOH and 275 μ L of distilled water were added to the mixture. The solution was mixed and the intensity of red-pink color was measured at 415 and 510 nm. Quercetin was used as standard for quantification and ergosterol (ergosta-5,7,22-trien-3 β -ol, 0.7 mg/mL) and L-Dopa (L-3,4-dihydroxyphenylalanine, 0.1 mg/mL) were solubilized in ethanol and treated as mushroom extracts but not only its absorbance at those wavelengths were recorded but the complete spectrum from 300 to 600 nm to compare with results from Pękal & Pyrzynska (2014).

2.10. Flavonoid detection and quantification by HPLC-DAD

The presence of flavonoids in mushrooms was determined by HPLC in two different labs using two different devices and methods.

In the first one, powdered fruiting bodies or mycelia were mixed with methanol and treated as previously described for the spectrophotometrical methods. Dried extracts were dissolved in 100 μ L methanol and filtered (0.45 μ m PVDF filter (Millex HV13, Millipore, Bedford, MA, USA)) before HPLC analysis.

Mushroom extracts (10 μ L) were injected in a Varian liquid chromatograph, model 920-LC Galaxy, with a diode array (PAD) and fluorescence detectors (range scanned= 200-600 nm). Reverse phase chromatographic separation was performed with an AquaTM C18 analytical column (250 x 4.6 mm i.d. with an average particle size of 5 μ m) after a precolum AquaTM C18 (4 x 3 mm) both from Phenomenex (Madrid, Spain). As solvent (A) 2% formic acid (v/v) and (B) acetonitrile was used with the following gradient profile: from 0 to 20 min, 0 to 30% B, from 20 to 25 min, 30 to 60% B, from 25 to 26 min, 60% B, from 26 to 27, 60 to 100% B and 27 to 35 min washing with 100% B. The flow rate was 1 mL/min at 35°C. The absorbance changes were recorded in the vis-UV DAD and simultaneously at 2 wavelengths for the different types of flavonoids: 280 nm for flavanones, 350 nm for flavones but the complete spectral range was visualized to identify any possible flavonoid-related spectrum.

2.11. Flavonoid identification and quantification by HPLC-MS

The presence of flavonoids in mushrooms was also evaluated at the other lab by an HPLC–DAD–ESI/MS device using as starting material the same mushroom samples that above indicated. Firstly, powdered mushrooms (50 mg) were extracted with 1 mL of the extracting mixture MeOH:H₂O:HCOOH (25:24:1), homogenized using a vortex and sonicated in an ultrasonic bath for 60 min. Then, samples were kept overnight at 4°C and sonicated again for 60 min. The supernatant for analysis was separated using a centrifuge (9500 × g, 5 min) (model EBA 21, Hettich Zentrifugen) and filtered through a 0.45 µm PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) before the analyses were performed.

A chromatographic separation was performed using an HPLC system equipped with an Agilent 1200 series (Agilent Technologies, Waldbronn, Germany) and coupled to a mass detector in series. The HPLC system consisted of a binary capillary pump (G1376A), a degasser (G1379B), an autosampler (G1377A), a sample cooler (control temperature 4°C) (G1330B), and a photodiode array detector (G1315D) controlled by Chemstation software (v.B.0103-SR2). The mass detector was a Bruker, model UltraHCT (Bremen, Germany) ion trap spectrometer equipped with an electrospray ionisation (ESI) system and controlled by Bruker Daltonics Esquire software (v.6.1).

The separation was performed using C18 Kinetex 100A (150 × 4.6 mm i.d.) column, 5 µm particle size. The flow rate was 0.8 mL/min. Chromatographic separation was achieved using a binary gradient consisting of (A) 1% formic acid and (B) acetonitrile as HPLC grade solvents. The elution profile started with 10% B, reaching 30% B at 20 minutes, 60% B at 25 min and maintained up to 26 min. The injection volume was 8 µL. Chromatograms were recorded from 240 to 400 nm (set 340 nm for flavonoids).

The mass spectrometer settings were as follows: the pressure and flow rate of the dryer gas were set at 11 psi and 5 L/min respectively. The full-scan mass spectrum was from *m/z* 300 to 1400. Collision-induced fragmentation experiments were performed in the ion trap using He as collision gas, with voltage ramping cycles from 0.3 to 2 V. The heated capillary and voltage were maintained at 350°C and 4275 V, respectively. Other parameters for the detection were as follows: skimmer, 40 V; capillary exit, 108.5 V; octopole 1 DC, 12 V; octopole 2 DC, 1.7 V; octopole RF, 138.8 Vpp; lens 1, –5 V; lens 2, –60 V. The mass spectrometry data were acquired in the negative ionization mode for flavonols.

3. Results

3.1. Systematic literature review

After the literature search, a total of 136 scientific works were found determining specific or total flavonoids in mushrooms, although a few more (including reviews *i.e.* Fernandes et al. (2012); Guillamón et al. (2010); Jayakumar et al. (2011)) were noticed where flavonoid presence was assumed because it was described elsewhere. Attempts to detect flavonoids were carried out already in 2001 using HPLC (Mattila, et al., 2001) however, authors did not detect them. It was in 2005 when a report was firstly published indicating their presence (Y. M. Choi, Ku, Chang, & Lee, 2005)(see the Supplementary Table 1). The work was included in a low-indexed journal but at the following year, the same group managed to publish a similar work in a highly indexed journal (high for the area of *Food Science and Technology*)(Choi et al. 2006). Authors utilized a colorimetric method that was previously utilized to detect flavonoids in mulberry (Zhishen et al. 1999) and referred the determination of 'total flavonoids' as 'free and bound flavonoids'. The same procedure was then followed in 2007 by several groups to detect flavonoids in mushrooms pointing as 'method of reference' that utilized for mulberry or another used for propolis analysis (Park, Koo, Ikegaki, & Contado, 1997). Despite others, three of these works were published in journals with IF (Impact factor) >3 and 3 others in journals with IF>2 (Figure 1) promoting an exponential use of the colorimetric method to determine flavonoids in mushrooms. The increase was such that from 5 works published in 2008 it doubled the number in 2009 and raised up to 18 publications in 2010 and 2012 and even 22 in 2013 and 2014. Although most of the reports through all these years were published in journals with IF<1 (35%), many of them were also published in journals with IF>2 (21%) or IF>3 (11%) and even 4 of them were published in journals with IF>4 (3%) in 2008, 2009 and 2012.

When a close look was given to the methodologies utilized to determine flavonoids in mushrooms, 91% of the reports were based on colorimetric determinations utilizing $AlCl_3$ (or $Al(NO_3)_3$) as reactive agent and a spectrophotometer. Only 9.3% of the reports used more advanced systems such as HPLC (UV/DAD only two used MS as detection devices) (Leal et al. 2013; Liu, Jia, Kan, & Jin, 2013), UPLC-ESI-MS/MS (Özyürek, Bener, Güçlü, & Apak, 2014) and surprisingly, one manuscript indicated that these polyphenols and other triterpenic acids were determined by GC-MS (Kalogeropoulous, Yanni, Koutrotsios, & Aloupi, 2013).

Moreover, the amounts of flavonoids found in the mushrooms also differed in the range of $\mu\text{g/g dw}$ (Babu & Rao, 2013; Karaman, Stahl, Vulić, Vesić, & Čanadanović-Brunet, 2014) or mg/g (Kumari, Upadhyay, & Atri, 2013; Yongcai, Xin, & Yaung-lee, 2013) levels of 67.3 mg/g (Zhang et al. 2015) or 349.6 mg/g (Jeong et al. 2013) meaning that 6.7 or 34.9% of the mushroom dry weight were flavonoids. These levels were found in extracts obtained using different solvents including all polarities such as water, ethanol, methanol, chloroform, ethyl acetate, butanol, petroleum ether, acetone, mixtures of indicated solvent and other specific extracts (when it is well known that many flavonoids are virtually insoluble in some of the listed solvents).

In addition to the 'flavonoid method' some of the works also included a colorimetric method to determine 'anthocyanins' (Tevfik, Cihan, Oznur, & Ibrahim, 2011) or 'anthocyanidins' (Sharma & Gautam, 2015) in mushrooms such as *i.e. Agaricus arvensis* or *Cantharellus cibarius*, although their fruiting bodies did not show the red-violet-blue discoloration characteristic from berries and other food products containing these pigments. Mushroom colors are due to different compounds depending on the considered species but they have completely different molecular structures than antocyanins such as *i.e.* red terphenylquinones related to orange-red grevillins, yellow-orange pulvic or pulvinic acids and styrylpyrones, red-violet betacyanins or variegatic, xerocomic or atromentic acids or anthraquinones (Velíšek & Cejpek, 2011).

3.2. Systematic search in molecular databases for flavonoid-related gene homologies

In plants, the flavonoid biosynthesis required the presence of many enzymes but three of them play an essential role: the phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) and chalcone isomerase (CHI) (Winkel, 2006). The presence of these proteins through the Eukaryote organisms was investigated using the taxonomical distribution of Pfam 'Sunburst' species distribution. Results indicated that the 3 enzymes were mostly distributed through the Viridiplantae kingdom (Table 1), particularly the chalcone synthase and isomerase where respect. 93 and 87% of the known sequences were found in plant species being the rest still uncategorized.

PAL-like proteins were found in 12 fungal species belonging to the phyla Basidiomycota and Ascomycota where the macrofungi and most of the edible mushrooms are included (Table 1). In fact commonly

consumed mushrooms such as *Agaricus bisporus* (white button mushroom) and *Pleurotus eryngii* (king trumpet mushroom) expressed at least one sequence that match with the lyase aromatic family but no protein related to the PF02797 or PF02431 families (where respect. CHS and CHI are included) have been described so far.

Another attempt to try to find sequences encoding flavonoid-related enzymes was carried out using BLAST as search tool. The CHS sequence from *Vitis vinifera* obtained from Genebank was used as example to look for homologies within other Eukaryote organisms such as berries, citrus etc. as well as many fungal species. CHS sequences within plant species showed high homologies (Table 2) and most of them were identified as chalcone synthases including only one or several isoforms. However, homology with fungal sequences dropped down to 17 – 3% in completely sequenced mushrooms such as *A. bisporus* and *Pleurotus ostreatus* and no significant similarities were found in other edible mushrooms (although their genomes are still not completely sequenced). However, the sequence of the proteins that slightly matched grape CHS was not identified as CHS or any known enzyme, they were only hypothetical proteins.

Homologies with the *V. vinifera* chalcone synthase were also investigated through the known sequences of all the mushroom genera where flavonoids were reported using methods different than the colorimetric determination (*Lactarius*, *Morchella*, *Lepista*, *Chantarelle*, *Pholliota*, *Tremella*, *Boletus*, *Amanita*, *Russula*, *Gyromitra*, *Suillus*, *Cynomorium*, *Marasmius*, *Craterellus*, *Helvela*, *Clitocibe*, *Catathelasma*, *Stropharia*, *Laccaria*, *Auricularia*, *Calocybe*, *Hygrophorus* and truffles genus such as *Tuber* and *Terfezia* sp.) and no significant similarities were found.

3.3. Spectrophotometric determinations and HPLC analysis of mushroom fruiting bodies

The total phenolic content of several mushroom species was determined using a commonly utilized colorimetric method (Figure 2). Values ranged from 1 to more than 14 mg/g mushroom powder depending on the strain being *Boletus edulis* the specie with the highest phenol content followed by others such as *Amanita ponderosa*, *Agrocybe aegerita* or *Calocybe gambosa*. These values were in concordance with other authors (Barros, Dueñas, Ferreira, Baptista, & Santos-Buelga, 2009; Del Signore, Romeo, & Giaccio, 1997; Ramírez-Anguiano et al. 2007).

Similarly, the protocol used by several publications to determine “total flavonoid” was applied to the same mushroom species for comparison. Except for *Lactarius deliciosus*, most of the determined values were similar or lower than the phenolic compounds ranging from 0.1 to 6.7 mg/g. The profile did not correlate with the phenolic content since for instance *Amanita cesarea* showed similar phenolic composition than *P. ostreatus* or *Hericium erinaceus* but it doubled the values obtained using the method utilized to determine ‘total flavonoid’ in mushrooms.

On the other hand, when an ergosterol standard solution was prepared and treated as indicated to determine ‘total flavonoids’ it positively reacted with the protocol showing absorbance at both 415 and 510 nm (Figure 3). Ergosterol is the major sterol from fungal hyphae but its structure is completely different from a flavonoid. Other compounds typical for mushrooms such as L-Dopa showed similar spectrum than those indicated by Pękal & Pyrzynska (2014) for some flavonoids, confirming that *o*-diphenols might also induce false flavonoid-positives.

Moreover, when the selected mushroom species were prepared following typical methods to extract flavonoids in plants (as described in M&M) and injected in HPLC-DAD/MS, no flavonoid peaks were detected or identified in any of the 24 tested species.

3.4. Flavonoid absorption by fruiting bodies from the cultivation substrate

Pleurotus ostreatus was cultivated in wheat straw as control and straw supplemented with onion waste in order to test whether the latter mycelium could partially absorb any of the flavonoids present in the onion waste during its growth. Addition of the flavonoid-containing waste slightly increased 1 day the time for pinning (inducing 1 day delay in harvesting) but did not reduce significantly fruiting bodies yield (15.7% w/w) nor quality (90.4-85.6% water content). No significant differences were noticed in the levels of total phenols (2.6 - 3.4 mg/g) neither in the values determined with the method utilized to measure ‘total flavonoids’ in mushrooms cultivated with or without onion supplementation (respect. 1.8 - 2.2 mg/g).

The flavonoids present in the onion waste were extracted and analysed by HPLC/DAD-MS together with the substrate after mycelial colonization and the fruiting bodies obtained from both control and supplemented substrates. Some of the peaks detected in substrate (Figure 4a) were identified as flavonoids (quercetin 3,7 or 3,4’ diglucoside, quercetin 3-glucoside, isorhamnetin 3-diglucoside and

isorhamnetin 3-glucoside) and they practically disappeared after mushrooms were grown in it, leaving only traces of some of them detected, such as quercetin 3- glucoside (t_R : 16,02 min) (Figure 4b). Their disappearance from the substrate during the mycelial growth might be explained by transformation into a different chemical (*i.e.* degradation) or by fungal absorption. However, the latter possibility is unlikely since the HPLC chromatograms obtained after fruiting bodies analysis showed no peaks at any of the wavelengths used to monitor flavonoids nor spectrum or mass which might resemble them (Figure 4c).

3.5. Flavonoid absorption by mycelium from culture medium

Pleurotus ostreatus mycelium was grown on Petri plates supplemented with increasing concentrations of several flavonoids (quercetin, hesperidin, luteolin and luteolin-7-glucoside) and caffeic acid (as control) in order to enhance or force their absorption if that was possible. Quercetin or hesperidin addition up to 2mg/ml did not influence significantly mycelial growth, the slightly extended lag phase (4 days) and the growth reduction noticed (from 1.2 to approx. 0.5 cm/day in the exponential phase) was mostly due to the ethanol utilized to solubilize the flavonoids because it was also observed in control samples where only ethanol was added (0.4 cm/day). Ethanol (with or without flavonoid addition) induced larger biomass production and water retention capacity by the mycelium since they showed higher fresh and dry weights than the control.

Additions of luteolin or its glucosylated derivative to the cultivation medium resulted in inhibition of the mycelial growth correlating with the flavonoids concentration utilized. The inhibition was more drastic when the aglycone was used (Figure 5a) than when luteolin-7-glucoside was applied at similar concentrations. However, addition of the simple phenolic compound did not significantly influence mycelial growth or biomass production except for a slight reduction noticed when applied at the highest concentration tested (4mg/mL) (Figure 5b).

No significant differences were found when the total phenolic compounds were determined in the mycelia obtained from plates supplemented with any of the selected flavonoids or concentrations. Control samples with or without ethanol contained approx. 4.4 – 4.8 mg/g dw. Only caffeic acid addition induced a significant increase (up to 7.3 mg/g dw) but only when a low concentration was applied (0.25 mg/mL). However, when the samples were injected in HPLC-DAD and developed using a solvent gradient that

1 differently eluted the selected flavonoids and caffeic acid, none of the compounds were detected in the
2 mycelia grown at any of the selected flavonoid concentrations.
3
4

5 6 **4. Discussion**

7
8 The literature review carried out indicated that, at the present, approximately 136 scientific publications
9 are worldwide available ensuring that they can determine flavonoids in mushrooms. Moreover, during the
10 citations compilation many other articles were found (more than 50) where authors indicated in their
11 introduction section or under specific sub-headings (in reviews) that mushrooms contain flavonoids
12 because previous reports demonstrated their presence. However, in a more detailed observation of the
13 methodologies utilized to demonstrate their presence, almost 91% of the publications utilized an
14 unspecific colorimetric method developed to determine them in plants or plant products. The method
15 involved the measurement at 410 – 430 nm (some authors used other wavelengths such as 510 nm) of a
16 colorimetric reaction that takes place after addition of $AlCl_3$ which selectively reacts only with certain
17 flavonoids depending on the reaction medium utilized. The analytical method was proposed in the 60s
18 (Christ & Müller, 1960) and it was already indicated that was not accurate enough to determine 'total
19 flavonoids' neither in plants (Pękal & Pyrzyńska, 2014). The method is based on the nitration of any
20 aromatic ring bearing a catechol group with its 3 or 4 positions unsubstituted or sterically blocked. After
21 addition of $Al(III)$, a yellow complex is formed that turns red in the presence of $NaOH$ and can be quantified
22 at 510 nm. However, if $AlCl_3$ is added in neutral or acidic media (without $NaNO_2$) it should only be used to
23 determine flavonols (quercetin, rutin, galangin etc.) and luteolin. In the presence of $NaNO_2$ in alkaline
24 medium seems to react with rutin, luteolin and catechins but, also with other phenolic acids bearing
25 catecholic moieties such as chlorogenic acid, in fact in the past, this method was used for the
26 determination of o-diphenols (Barnum, 1977). Most of the protocols followed by authors that indicated
27 the presence of flavonoids in mushrooms used $NaNO_2$ and therefore, the more unspecific test. Moreover,
28 mushrooms contain not only chlorogenic acid but many other hydroxycinnamic acids (Puttaraju,
29 Venkateshaiah, Dharmesh, Urs, & Somasundaram, 2006), o-diphenols (*i.e.* glutaminyl-3,4-
30 dihydroxybenzene) and molecules including catechol residues such as pigments (*i.e.* variegatorubin,
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hispolon, 3,4',4-trihydroxypulvinone, inoscavin A etc) or melanin-precursors (*i.e.* 5,6-dihydroxyindole, leucodopachrome)(Soler-Rivas et al. 1999; Velíšek & Cejpek, 2011), even ergosterol (with a completely different chemical structure from a flavonoid or a phenolic compound) showed a positive response in the AlCl₃-test. This molecule is only partially soluble in methanol however; it might also contribute together with the other phenolic compounds to the absorbance determined as 'total flavonoids'. This might also explain the lack of linear correlation noticed between the amount of total phenolic compounds and the absorbances quantified as 'total flavonoids'.

Nevertheless, 13 publications identified specific flavonoids by HPLC/UPLC/GC using UV, DAD or MS as detection devices (although in one of them no device or method was described, HPLC was assumed because authors were able of quantifying quercetin (Zhishen et al. 1999)). These reports indicated the presence of compounds such as quercetin, luteolin, myricetin, naringenin, naringin, hesperetin, rutin, morin, kaempferol, chrysin and derivatives, genistein, apigenin or catechin in commonly consumed mushrooms *i.e.* *P. ostreatus*, *A. bisporus*, *B. edulis*, *C. cibarius*, *L. deliciosus*, and other less popular (see the Supplementary Table 1). The presence of these compounds in mushrooms could be due to two possibilities: that mushroom can synthetize them or absorb them from neighbouring plants or from those that establish symbiotic interactions via mycorrhizae (although not all the mushrooms species establish them).

In order to synthetize flavonoids, mushrooms should contain in their genome at least a copy of the three key enzymes within their biosynthetic pathway, phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) and chalcone isomerase (CHI) and nowadays only PAL has been reported in mushrooms according to genomic databases. No gene encoding CHS or CHI have been identified so far suggesting that they were not yet studied or not found because they were neither detected in those mushrooms that are, at the present, completely sequenced (*Agaricus bisporus* and *Pleurotus ostreatus*). These results indicate that mushrooms can generate phenolic compounds using their PAL but they cannot further transform them into flavonoids because they lacked the necessary enzymes.

Fungi evolved separately from plants long before the enzymatic mechanism specifically designed to produce most of the primitive flavonoids appeared. Moreover, although it is possible that, initially,

flavonoids were a metabolic by-product, nature found function for them in plants. For instance, they are signals for pollen tube formation, for bacteria attraction (nitrification), pest and plagues repellents etc. But, what do they do in fungi? Is there any single report informing about their role in fungal biology?

Then, if they do not synthesize them, the second possibility might be that they could absorb them from their substrate or from plants. However, when *P. ostreatus* was cultivated on onion waste containing flavonoids the obtained fruiting bodies lacked quercetin- or isorhamnetin-derivatives or any other flavonoid. Therefore, the flavonoids described at least in *P. ostreatus* (i.e. Huang, Cai, Xu, & Zhao (2012), Palacios et al. (2011) or Sapozhnikova, Byrdwell, Lobato, & Romig (2014)) cannot be explained by an absorption mechanism and as all fungi share a similar biology, these conclusions could be extended to the rest of species, or at least to those 24 strains analysed in this study since no trace of flavonoid was found using HPLC.

When *P. ostreatus* mycelium was forced to grow on semi-synthetic media with increasing concentrations of certain flavonoids no absorption was also noticed on the contrary, some of them inhibited the fungal growth confirming the fungicide properties that have been described for flavonoids in many other publications (Orhan, Özçelik, Özgen, & Ergun, 2010; Winkel, 2006). In several reports flavonoids were found in the range of micrograms per gram mushroom (dw) however in many others, they were present in mg/g reaching levels of 349.6 mg/g flavonoids (Jeong et al. 2013), where it seems unlikely that mushrooms could absorb that amount of toxic compounds (35% dw) without significant changes in the fruiting bodies quality and metabolism. If this was the case, mushrooms could have an extremely interesting commercial application as flavonoid source since in plants, they are present in large concentrations only under specific physiological conditions such as in the earliest developmental stages (Jourdan, McIntosh, & Mansell, 1985). When citrus fruits such as sour orange are still immature (diameter < 10 mm), values of i.e. neohesperidin are still below 30% dry matter and they are being used as raw material for industrial extraction processes (Ortuño et al. 1997).

5. Conclusions

Edible mushrooms cannot synthesise flavonoids because they do not have the main enzymes involved in their metabolic pathway and no significant absorption was noticed from fruiting bodies cultivated in

flavonoid-enriched substrates or from mycelia grown of flavonoid-supplemented lab media. Thus, spectrophotometric methods for 'total flavonoids' determination should not be applied to fungal samples. The flavonoids identified in several mushroom species using advance identification devices such as DAD and MS might be due to sample contaminations or other pitfalls within the utilized protocols. Scientists should be more careful when planning experiments to avoid the exponential spreading of large mistakes such as considering fungi as plants.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Appendix

Supplementary Table 1

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Table 1. Taxonomic distribution of sequences related to the three key enzymes involved in the biosynthesis of flavonoids extracted from Pfam (particularly those present in the fungal kingdom).

Enzyme	Function	PFamily	Kingdom *	Phyllum	Class	Order	Family	Genus	Specie	Seq.
PAL	Transform L-phenylalanine to ammonia and trans-cinnamic acid (first phenolic compound in the phenyl propanoid pathway)	Lyase aromatic (PF 00221)	No Kingdom	NS	NS	NS	NS	NS	20	37
			Viridiplantae	NS	NS	NS	NS	NS	312	1007
			Metazoa	NS	NS	NS	NS	NS	91	142
			Fungi	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	<i>Agaricus</i>	<i>bisporus</i>	1
							Tricholomataceae	<i>Tricholoma</i>	<i>matsutake</i>	3
							Amanitaceae	<i>Amanita</i>	<i>muscaria</i>	1
							Pleurotaceae	<i>Pleurotus</i>	<i>eryngii</i>	1
					Ustilaginomycetes	Ustilaginales	Ustilaginaceae	<i>Pseudozyma</i>	<i>brasiliensis</i>	1
				Ascomycota	No class	Sporidiobolales	No Family	<i>Rhodosporidium</i>	<i>toruloides</i>	1
								<i>Rhodotorula</i>	<i>mucilaginos</i>	1
								<i>glutinis</i>		3
								<i>Sporidiobolus</i>	<i>salmonicolor</i>	3
					Eurotiomycetes	Eurotiales	Aspergillaceae	<i>Penicillium</i>	<i>roqueforti</i>	1
					Sordariomycetes	Hypocreales	Nectriaceae	<i>Fusarium</i>	<i>graminearum</i>	1
					Lecanoromycetes	Lecanorales	Parmeliaceae	<i>Letharia</i>	<i>vulpina</i>	1
			Uncategorized	NS	NS	NS	NS	NS	28	102
CHS	Catalyzes the conversion of 4-coumaroyl-CoA and malonyl-CoA to naringenin chalcone (first flavonoid)	Chalcone and stilbene synthases, C-terminal domain (PF02797)	Viridiplantae	NS	NS	NS	NS	NS	751	2687
			Uncategorized	NS	NS	NS	NS	NS	61	199
CHI	Catalyzes the chemical reaction: a chalcone <-> a flavanone	Chalcone (PF02431)	Viridiplantae	NS	NS	NS	NS	NS	146	398
			Uncategorized	NS	NS	NS	NS	NS	15	55
			No Kingdom	NS	NS	NS	NS	NS	2	2

*Only inside Eukariota domain. PAL, phenylalanine ammonia-lyase; CHS, chalcone synthase; CHI, chalcone isomerase. NS, non-specified data.

Table 2. Results of the alignments of the Chalcone synthase (CHS) sequence from *Vitis vinifera* with other organisms using Blastp. Data included are those showing higher percentage of homology.

Organism	Term searched	Genus	Specie	Homology with	% alignment (query cover)
Plant	<i>Vitis vinifera</i>	<i>Vitis</i>	<i>vinifera</i>	Chalcone synthase	100
Plant	<i>Arabidopsis thaliana</i>	<i>Arabidopsis</i>	<i>thaliana</i>	Chalcone synthase	97
Plant	Mulberry	<i>Morus</i>	<i>alba</i> var. <i>multicaulis</i>	Chalcone synthase	98
		<i>Morus</i>	<i>notabilis</i>	Chalcone synthase	98
		<i>Morus</i>	<i>alba</i>	Chalcone synthase	65
Plant	Strawberry	<i>Fragaria</i>	<i>ananassa</i>	Chalcone synthase	98
Plant	Solanum	<i>Solanum</i>	<i>lycopersicum</i>	Chalcone synthase 1&2	98
		<i>Solanum</i>	<i>tuberosum</i>	Chalcone synthase	98
		<i>Solanum</i>	<i>pinnatisectum</i>	Chalcone synthase	98
Plant	Citrus	<i>Citrus</i>	<i>reticulata</i>	Chalcone synthase	98
		<i>Citrus</i>	<i>ichangensis</i>	Chalcone synthase	98
		<i>Citrus</i>	<i>maxima</i>	Chalcone synthase	98
		<i>Citrus</i>	<i>clementina</i>	Hypothetical protein CICLE v 10015535mg	98
Plant	<i>Arabidopsis thaliana</i>	<i>Arabidopsis</i>	<i>thaliana</i>	Chalcone synthase	97
Animal	Human	<i>Homo</i>	<i>sapiens</i>	No significant similarity found	0
Animal	Mouse	<i>Mus</i>	<i>musculus</i>	No significant similarity found	0
Animal	<i>Caenorhabditis elegans</i>	<i>Caenorhabditis</i>	<i>elegans</i>	No significant similarity found	0
Fungi	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces</i>	<i>cerevisiae</i>	No significant similarity found	0
Fungi	<i>Agaricus</i>	<i>Agaricus</i>	<i>bisporus</i> var. <i>burnettii</i> JB137-S8	Hypothetical protein AGABI2DRAFT 11251	7
		<i>Agaricus</i>	<i>bisporus</i> var. <i>bisporus</i> H97	Hypothetical protein AGABI2DRAFT 193398	7
		<i>Agaricus</i>	<i>bisporus</i> var. <i>burnettii</i> JB137-S8	Hypothetical protein AGABI2DRAFT 74201	3
		<i>Agaricus</i>	<i>bisporus</i> var. <i>bisporus</i> H97	Hypothetical protein AGABI2DRAFT 207020	3
Fungi	<i>Pleurotus ostreatus</i>	<i>Pleurotus</i>	<i>ostreatus</i> PC15	Hypothetical protein PLEOSDRAFT 1059391	17
Fungi	<i>Pleurotus eryngii</i> *	<i>Pleurotus</i>	<i>eryngii</i> var. <i>tuoliensis</i>	Homeodomain 1 mating type protein (genome not completely sequenced)	3
Fungi	<i>Lentinula edodes</i> *	<i>Lentinula</i>	<i>edodes</i>	No significant similarity found	0
Fungi	<i>Ganoderma lucidum</i> *	<i>Ganoderma</i>	<i>lucidum</i>	No significant similarity found	0
Fungi	<i>Lactarius deliciosus</i> *	<i>Lactarius</i>	<i>deliciosus</i>	No significant similarity found	0
Others	Microbes	<i>Pseudomonas</i>	<i>pseudoalcaligenes</i>	Hypothetical protein N619_00405	55
		<i>Gordonia</i>	<i>rhizosphera</i>	Stilbene synthase	34

*Genomes from mushrooms not completely sequenced (in 2015).

Figure captions

Figure 1. Number of scientific works published up to October 2015 determining flavonoids in mushrooms (according to Web of Science). References were sorted by the impact factor of the journal where they were published the year that they were published (except for 2015).

Figure 2. Total phenols and the colorimetric results obtained after using the method to determine 'total flavonoids' in several mushrooms species (at 510 nm).

Figure 3. VIS spectra of quercetin, L-DOPA and ergosterol standard compounds when they were treated with $\text{Al}(\text{Cl}_3)_3$ and NaNO_2 solutions as indicated in the test used to measure 'total flavonoids' in mushrooms.

Figure 4. HPLC chromatogram at 340 nm of a) onion waste b) substrate supplemented with onion waste after cultivation of *P. ostreatus* and c) *P. ostreatus* fruiting bodies grown on substrate supplemented with onion waste (Rt= 8.76 min. quercetin 3,7- or 3,4'-diglucoside; Rt= 10.02 min. quercetin 3,7- or 3,4'-diglucoside; Rt= 10.81 min. isorhamnetin 3-diglucoside (or 3-sophoroside); Rt=16.02 min. quercetin 3-glucoside and Rt= 17.39 min. isorhamnetin 3-glucoside).

Figure 5. Mycelial growth of *P. ostreatus* grown on MEA media supplemented with different concentrations of a) luteolin dissolved in ethanol and b) caffeic acid.

Table 1. Taxonomic distribution of sequences related to the three key enzymes involved in the biosynthesis of flavonoids extracted from Pfam (particularly those present in the fungal kingdom).

Enzyme	Function	PFamily	Kingdom *	Phylum	Class	Order	Family	Genus	Specie	Seq.
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			Metazoa	NS	NS	NS	NS	NS	91	142
			Fungi	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	<i>Agaricus</i>	<i>bisporus</i>	1
							Tricholomataceae	<i>Tricholoma</i>	<i>matsutake</i>	3
							Amanitaceae	<i>Amanita</i>	<i>muscaria</i>	1
							Pleurotaceae	<i>Pleurotus</i>	<i>eryngii</i>	1
					Ustilaginomycetes	Ustilaginales	Ustilaginaceae	<i>Pseudozyma</i>	<i>brasiliensis</i>	1
				Ascomycota	No class	Sporidiobolales	No Family	<i>Rhodosporidium</i>	<i>toruloides</i>	1
								<i>Rhodotorula</i>	<i>mucilaginoso</i>	1
									<i>glutinis</i>	3
								<i>Sporidiobolus</i>	<i>salmonicolor</i>	3
					Eurotiomycetes	Eurotiales	Aspergillaceae	<i>Penicillium</i>	<i>roqueforti</i>	1
					Sordariomycetes	Hypocreales	Nectriaceae	<i>Fusarium</i>	<i>graminearum</i>	1
					Lecanoromycetes	Lecanorales	Parmeliaceae	<i>Letharia</i>	<i>vulpina</i>	1
			Uncategorized	NS	NS	NS	NS	NS	28	102
CHS	Catalyzes the conversion of 4-coumaroyl-CoA and malonyl-CoA to naringenin chalcone (first flavonoid)	Chalcone and stilbene synthases, C-terminal domain (PF02797)	Viridiplantae	NS	NS	NS	NS	NS	751	2687
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CHI	Catalyzes the chemical reaction: a chalcone <-> a flavanone	Chalcone (PF02431)	Viridiplantae	NS	NS	NS	NS	NS	146	398
			Uncategorized	NS	NS	NS	NS	NS	15	55
			No Kingdom	NS	NS	NS	NS	NS	2	2

*Only inside Eukariota domain. PAL, phenylalanine ammonia-lyase; CHS, chalcone synthase; CHI, chalcone isomerase. NS, non-specified data.

Table 2

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Table 2. Results of the alignments of the Chalcone synthase (CHS) sequence from *Vitis vinifera* with other organisms using Blastp. Data included are those showing higher percentage of homology.

Organism	Term searched	Genus	Specie	Homology with	% alignment (query cover)
Plant	<i>Vitis vinifera</i>	<i>Vitis</i>	<i>vinifera</i>	Chalcone synthase	100
Plant	<i>Arabidopsis thaliana</i>	<i>Arabidopsis</i>	<i>thaliana</i>	Chalcone synthase	97
Plant	Mulberry	<i>Morus</i>	<i>alba</i> var. <i>multicaulis</i>	Chalcone synthase	98
		<i>Morus</i>	<i>notabilis</i>	Chalcone synthase	98
		<i>Morus</i>	<i>alba</i>	Chalcone synthase	65
Plant	Strawberry	<i>Fragaria</i>	<i>anassa</i>	Chalcone synthase	98
Plant	<i>Solanum</i>	<i>Solanum</i>	<i>lycopersicum</i>	Chalcone synthase 1&2	98
		<i>Solanum</i>	<i>tuberosum</i>	Chalcone synthase	98
		<i>Solanum</i>	<i>pinnatisectum</i>	Chalcone synthase	98
Plant	Citrus	<i>Citrus</i>	<i>reticulata</i>	Chalcone synthase	98
		<i>Citrus</i>	<i>ichangensis</i>	Chalcone synthase	98
		<i>Citrus</i>	<i>maxima</i>	Chalcone synthase	98
		<i>Citrus</i>	<i>clementina</i>	Hypothetical protein CICLE v 10015535mg	98
Plant	<i>Arabidopsis thaliana</i>	<i>Arabidopsis</i>	<i>thaliana</i>	Chalcone synthase	97
Animal	Human	<i>Homo</i>	<i>sapiens</i>	No significant similarity found	0
Animal	Mouse	<i>Mus</i>	<i>musculus</i>	No significant similarity found	0
Animal	<i>Caenorhabditis elegans</i>	<i>Caenorhabditis</i>	<i>elegans</i>	No significant similarity found	0
Fungi	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces</i>	<i>cerevisiae</i>	No significant similarity found	0
Fungi	<i>Agaricus</i>	<i>Agaricus</i>	<i>bisporus</i> var. <i>burnettii</i> JB137-S8	Hypothetical protein AGABQDRAFT 11251	7
		<i>Agaricus</i>	<i>bisporus</i> var. <i>bisporus</i> H97	Hypothetical protein AGABQDRAFT 193398	7
		<i>Agaricus</i>	<i>bisporus</i> var. <i>burnettii</i> JB137-S8	Hypothetical protein AGABQDRAFT 74201	3
		<i>Agaricus</i>	<i>bisporus</i> var. <i>bisporus</i> H97	Hypothetical protein AGABQDRAFT 207020	3
Fungi	<i>Pleurotus ostreatus</i>	<i>Pleurotus</i>	<i>ostreatus</i> PC15	Hypothetical protein PLEODRAFT 1059391	17
Fungi	<i>Pleurotus eryngii</i> *	<i>Pleurotus</i>	<i>eryngii</i> var. <i>tuoliensis</i>	Homeodomain 1 mating type protein (genome not completely sequenced)	3
Fungi	<i>Lentinula edodes</i> *	<i>Lentinula</i>	<i>edodes</i>	No significant similarity found	0
Fungi	<i>Ganoderma lucidum</i> *	<i>Ganoderma</i>	<i>lucidum</i>	No significant similarity found	0
Fungi	<i>Lactarius deliciosus</i> *	<i>Lactarius</i>	<i>deliciosus</i>	No significant similarity found	0
Others	Microbes	<i>Pseudomonas</i>	<i>pseudocaligenes</i>	Hypothetical protein N619_00405	55
		<i>Gordonia</i>	<i>rhizosphaera</i>	Stilbene synthase	34

*Genomes from mushrooms not completely sequenced (in 2015).

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Figure 1.

Figure 1.

Figure 2
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Figure 2.

Figure 3.

Figure 4
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Figure 4.

Figure 5.

a)

b)

Appendix- Supplementary Table 1

Number	Year	Journal	Impact factor	First author	Title	Volumen, Issue, pages	Compounds found in mushrooms	Method utilized to measure flavonoids	Detection device or wavelength used	Solvents used for extraction	Mushroom specie studied
1	2005	Food Science and Biotechnology	0.645	Choi, YM. et al.	Antioxidant activities and total phenolics of ethanol extracts from several edible mushrooms produced in Korea.	14, 5, 700-703	Total flavonoids	Colorimetric	NF	Ethanol	<i>Ganoderma lucidum</i> and <i>Pleurotus eryngii</i>
2	2006	Food Chemistry	2.433	Choi, Y. et al.	Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (<i>Lentinus edodes</i>) mushroom.	99, 2, 381–387	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	80% Ethanol	<i>Lentinus edodes</i>
3	2006	Journal of Culinary Science and Technology	NF	Ganguli, A. et al.	Antioxidant activities and total phenolics of pickles produced from the edible mushroom, <i>Agaricus bisporus</i> .	5, 2-3, 131-142	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Ethanol	<i>Agaricus bisporus</i>
4	2006	Annals of Microbiology	0.427	Mercan, N. et al.	Antioxidant and antimicrobial properties of ethanolic extract from <i>Lepista nuda</i> (Bull.) Cooke.	56, 4, 339-344	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Ethanol	<i>Lepista nuda</i>
5	2006	African Journal of Biotechnology	NF	Gezer, K. et al.	Free-radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey.	5, 20, 1924-1928	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Ethanol	Wild edible mushrooms
6	2006	African Journal of Biotechnology	NF	Turkoglu, A. et al.	Antioxidant and antimicrobial activities of <i>Morchella conica</i> Pers.	5, 11, 1146-1150	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Ethanol	<i>Morchella conica</i>
7	2007	Food Chemistry	3.052	Turkoglu, A. et al.	Antioxidant and antimicrobial activities of <i>Laetiporus sulphureus</i> (Bull.) Murrill	101, 1, 267–273	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Ethanol	<i>Laetiporus sulphureus</i>
8	2007	Food and Chemical Toxicology	2.186	Barros, L. et al.	Effect of <i>Lactarius piperatus</i> fruiting body maturity stage on antioxidant activity	45, 9, 1731–1737	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>Lactarius piperatus</i>

Appendix- Supplementary Table 1

Number	Year	Journal	Impact factor	First author	Title	Volumen, Issue, pages	Compounds found in mushrooms	Method utilized to measure flavonoids	Detection device or wavelength used	Solvents used for extraction	Mushroom specie studied
					measured by several biochemical assays.						
9	2007	Food Chemistry	3.052	Barros, L. et al.	Bioactive properties of the medicinal mushroom <i>Leucopaxillus giganteus</i> mycelium obtained in the presence of different nitrogen sources.	105, 1, 179–186	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>Leucopaxillus giganteus</i>
10	2007	Food Chemistry	3.052	Lim, HW. et al.	Free radical-scavenging and inhibition of nitric oxide production by four grades of pine mushroom (<i>Tricholoma matsutake</i> Sing.)	103, 4, 1337–1342	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Ethyl acetate or butanol	<i>Tricholoma matsutake</i>
11	2007	Journal of Agricultural and Food Chemistry	2.532	Barros, L. et al.	Effect of fruiting body maturity stage, on chemical composition and antimicrobial activity of <i>Lactarius</i> sp mushrooms.	55, 21, 8766-8771	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>Lactarius deliciosus</i> and <i>L. piperatus</i>
12	2007	Plant Archives	NF	Gowdaman, V. et al.	Antibacterial metabolites from wood rot fungus <i>Phellinus hohnelli</i> .	7, 2, 763-765	Total flavonoids	Colorimetric	NF	Ethanol	<i>Phellinus hohnelli</i>
13	2007	Food and Chemical Toxicology	2.186	Barros, L. et al.	Effect of <i>Lactarius piperatus</i> fruiting body maturity stage on antioxidant activity measured by several biochemical assays.	45, 9, 1731-1737	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>L. piperatus</i>
14	2007	European Food Research and Technology	1.159	Barros, L. et al.	Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts	225, 2, 151 EP 156	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>L. deliciosus</i> , <i>Sarcodon imbricatus</i> and <i>Tricholoma portentosum</i>

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Number	Year	Journal	Impact factor	First author	Title	Volumen, Issue, pages	Compounds found in mushrooms	Method utilized to measure flavonoids	Detection device or wavelength used	Solvents used for extraction	Mushroom specie studied
15	2008	Bioresource Technology	4.453	Sarikurkcui, C. et al.	Evaluation of the antioxidant activity of four edible mushrooms from the Central Anatolia, Eskisehir – Turkey: <i>Lactarius deterrimus</i> , <i>Suillus collitinus</i> , <i>Boletus edulis</i> , <i>Xerocomus chrysenteron</i> .	99, 14, 6651–6655	Total flavonoids	2% AlCl ₃	UV/VIS 415 nm	Methanol	<i>Lactarius deterrimus</i> , <i>Suillus collitinus</i> , <i>Boletus edulis</i> and <i>Xerocomus chrysenteron</i>
16	2008	Food Chemistry	2.696	Barros, L. et al.	Antioxidant activity of <i>Agaricus</i> sp. mushrooms by chemical, biochemical and electrochemical assays.	111, 1, 61–66	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>Agaricus bisporus</i> , <i>Agaricus arvensis</i> , <i>Agaricus romagnesi</i> , <i>Agaricus sylvaticus</i> and <i>Agaricus silvicola</i>
17	2008	Food and Chemical Toxicology	2.321	Barros, L. et al.	Wild and commercial mushrooms as source of nutrients and nutraceuticals.	46, 8, 2742–2747	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>B. edulis</i> , <i>Calocybe gambosa</i> , <i>Cantharellus cibarius</i> , <i>Craterellus cornucopioides</i> , <i>Marasmius oreades</i> , <i>A. bisporus</i> , <i>A. sylvaticus</i> and <i>A. silvicola</i>
18	2008	Journal of Agricultural and Food Chemistry	2.562	Kim, MY. et al.	Phenolic compound concentration and antioxidant activities of edible and	56, 16, 7265–7270	Total flavonoids	HPLC-DAD	UV 280 nm	80% Methanol	<i>P. eryngii</i> , <i>G. lucidum</i> and <i>A. bisporus</i>

Appendix- Supplementary Table 1

Number	Year	Journal	Impact factor	First author	Title	Volumen, Issue, pages	Compounds found in mushrooms	Method utilized to measure flavonoids	Detection device or wavelength used	Solvents used for extraction	Mushroom specie studied
					medicinal mushrooms from Korea.						
19	2008	Food Science and Techonology International	0.702	Barros, L. et al.	Phenolics and antioxidant activity of mushroom <i>Leucopaxillus giganteus</i> mycelium at different carbon sources.	14, 1, 47- 55	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Water	<i>L. giganteus</i>
20	2009	Innovative Food Science and Emerging Technologies	2.174	Jayakumar, T. et al.	<i>In-vitro</i> antioxidant activities of an Ethanolic extract of the oyster mushroom <i>Pleurotus ostreatus</i> .	10, 2, 228–234	Rutin and chrysin	HPLC	UV 300 nm	95% Ethanol	<i>Pleurotus ostreatus</i>
21	2009	Food and Chemical Toxicology	2.114	Gursoy, N. et al.	Antioxidant activities, metal contents, total phenolics and flavonoids of seven <i>Morchella</i> species.	47, 9, 2381–2388	Total flavonoids	AlCl ₃	UV/VIS 415 nm	Methanol	<i>Morchella rotunda</i> , <i>Morchella crassipes</i> , <i>Morchella esculenta</i> var. <i>umbrina</i> , <i>Morchella deliciosa</i> , <i>Morchella elata</i> , <i>M.conica</i> and <i>Morchella angusticeps</i> .
22	2009	Micologia Aplicada International	NF	Bruijn, J. et al.	Antioxidant properties of extracts obtained from <i>Grifola gargal</i> mushrooms.	21, 1, 11-18	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Acetone, water, ethanol, ethyl acetate, n-hexane or ethanol:water mixtures	<i>Grifola gargal</i>

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Number	Year	Journal	Impact factor	First author	Title	Volumen, Issue, pages	Compounds found in mushrooms	Method utilized to measure flavonoids	Detection device or wavelength used	Solvents used for extraction	Mushroom specie studied
23	2009	Asian Journal of Food and AgroIndustry	NF	Hip, SY. et al.	Phenolic profiles of selected edible wild mushrooms as affected by extraction solvent, time and temperature.	2, 3, 392-401	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	510 nm	Mixtures of methanol, ethanol, acetone, water or absolute hexane	<i>Pleurotus</i> sp., <i>Hygrocybe conica</i> , <i>Schizophyllum commune</i> and <i>Lentinus ciliatus</i>
24	2009	African Journal of Biotechnology	0.565	Jagadish, L.K., et al.	Comparitive study on the antioxidant, anticancer and antimicrobial property of <i>Agaricus bisporus</i> (J. E. Lange) Imbach before and after boiling.	8, 4, 654-661	Total flavonoids	AlCl ₃	UV 367 nm	Ethanol	<i>A. bisporus</i>
25	2009	Bioresource Technology	4.253	Zheng, W. et al.	Accumulation of antioxidant phenolic constituents in submerged cultures of <i>Inonotus obliquus</i> .	100, 3, 1327-1335	Total flavonoids	HPLC-DAD	UV 280-310 nm	70% Aqueous acetone	<i>Inonotus obliquus</i>
26	2009	International Journal of Food Sciences and Nutrition	1.313	Liang, L. et al.	Antioxidant activities of extracts and subfractions from <i>Inonotus obliquus</i> .	60, 2, 175-184	Total flavonoids	Colorimetric	NF	Ethyl acetate, n-butanol or water	<i>I. obliquus</i>
27	2009	International Journal of Medicinal Mushrooms	0.661	Percario, S. et al.	Antioxidant activity of edible blushing wood mushroom, <i>Agaricus sylvaticus</i> Schaeff. (Agaricomycetideae) <i>in vitro</i> .	11, 2,133-139	Total flavonoids	AlCl ₃	UV/VIS 415 nm	Water	<i>A. sylvaticus</i>
28	2009	Nutrition and Food Science	NF	Singla, R. et al.	Phenolics and antioxidant activity of a ready-to-eat snack food prepared from the edible mushroom (<i>Agaricus bisporus</i>).	39, 3, 227-234	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	NF	<i>A. bisporus</i>

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29	2009	Environmental Research	3.237	Froufe, H.J.C. et al.	A QCAR model for predicting antioxidant activity of wild mushrooms.	20, 5-6, 579-590	Total flavonoids	Colorimetric	NF	NF	Wild mushrooms
30	2010	Food Chemistry	3.458	Jiang, T. et al.	Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (<i>Lentinus edodes</i>).	122, 3, 761–767	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Ethanol or water	<i>L. edodes</i>
31	2010	Postharvest Biology and Technology	2.256	Jiang, T. et al.	Influence of UV-C treatment on antioxidant capacity, antioxidant enzyme activity and texture of postharvest shiitake (<i>Lentinus edodes</i>) mushrooms during storage.	56, 3, 209–215	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Ethanol	<i>L.edodes</i>
32	2010	Journal of Agricultural and Food Chemistry	2.816	Chen, XH. et al.	Chemical composition and antioxidant activities of <i>Russula griseocarnosa</i> sp. nov.	58, 11, 6966–6971	Quercetine	HPLC-DAD	UV 273 nm	Acetonitrile and hydrochloric acid	<i>Russula griseocarnosa</i>
33	2010	Journal of Central European Agriculture	NF	Mujić, I. et al.	Antioxidant properties of selected edible mushroom species.	11, 4, 387-392	Total flavonoids	AlCl ₃	NF	50% Ethanol	<i>L. edodes</i> , <i>Hericium erinaceus</i> and <i>Agrocybe aegerita</i>

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Number	Year	Journal	Impact factor	First author	Title	Volumen, Issue, pages	Compounds found in mushrooms	Method utilized to measure flavonoids	Detection device or wavelength used	Solvents used for extraction	Mushroom specie studied
34	2010	Food Science and Biotechnology	0.505	Gursoy, N. et al.	Evaluation of antioxidant activities of 3 edible mushrooms: <i>Ramaria flava</i> (Schaef.: Fr.) Quél., <i>Rhizopogon roseolus</i> (Corda) T.M. Fries., and <i>Russula delica</i> Fr.	19, 3, 691-696	Total flavonoids	AlCl ₃	UV/VIS 415 nm	Methanol	<i>Ramaria flava</i> , <i>Rhizopogon roseolus</i> and <i>Russula delica</i>
35	2010	Pharmacognosy Research	NF	Ramesh, CH. et al.	Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of western ghats of Karnataka, India.	2, 2, 107–112	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Methanol	<i>Lycoperdon perlatum</i> , <i>Cantharellus cibarius</i> , <i>Clavaria vermiculiris</i> , <i>Ramaria Formosa</i> , <i>Marasmius oreades</i> and <i>Pleurotus pulmonarius</i>
36	2010	Indian Journal of Experimental Biology	0.702	Pal, J. et al.	<i>In vitro</i> free radical scavenging activity of wild edible mushroom, <i>Pleurotus squarrosulus</i> (Mont.) Singer	48, 12, 1210-1218	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Cold/hot water and methanol	<i>Pleurotus squarrosulus</i>
37	2010	Asian Journal of Chemistry	0.247	Chen, XH. et al.	Chemical composition and antioxidant activity of two edible mycorrhizal fungi from South China.	22, 9, 6867-6878	Total flavonoids	Colorimetric	NF	NF	<i>Russula virescens</i> and <i>Cantharellus cibarius</i>
38	2010	Pharmacognosy Magazine	0.432	Ozen, T. et al.	Antioxidant activities of <i>Sarcodon imbricatum</i> wildly grown in the black sea region of Turkey.	6 ,22, 89-97	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Methanol	<i>S. imbricatus</i>

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Number	Year	Journal	Impact factor	First author	Title	Volumen, Issue, pages	Compounds found in mushrooms	Method utilized to measure flavonoids	Detection device or wavelength used	Solvents used for extraction	Mushroom specie studied
39	2010	Molecules	1.988	Li, B. et al.	Antioxidant properties of cap and stipe from <i>Coprinus comatus</i> .	15, 3, 1473-1486	Total flavonoids	$\text{NaNO}_2 + 10\% \text{Al}(\text{NO}_3)_3 \cdot \text{H}_2\text{O}$	VIS 510 nm	Ethanol or hot water	<i>Coprinus comatus</i>
40	2010	Journal of Food Composition and Analysis	1.948	Al-Laiith, AAA.	Antioxidant components and antioxidant/antiradical activities of desert truffle (<i>Tirmania nivea</i>) from various Middle Eastern origins.	23, 1, 15-22	Total flavonoids	Comparison of free phenolic content after formaldehyde precipitation.	Folin–Ciocalteu method (765 nm)	80% Ethanol	<i>Tirmania nivea</i>
41	2010	International Journal of Food Properties	0.947	Singla, R. et al.	Antioxidant activities and polyphenolic properties of raw and osmotically dehydrated dried mushroom (<i>Agaricus bisporus</i>) snack food.	3, 6, 1290-1299	Total flavonoids	$\text{NaNO}_2 + 10\% \text{AlCl}_3 \cdot \text{H}_2\text{O}$	VIS 510 nm	99% Ethanol	<i>A. bisporus</i>
42	2010	International Journal of Medicinal Mushrooms	0.639	Ebrahimzadeh, MA. et al.	Antioxidant and free radical scavenging activities of culinary-medicinal mushrooms, Golden Chanterelle <i>Cantharellus cibarius</i> and Angel's Wings <i>Pleurotus porrigens</i> .	12, 3, 265-272	Total flavonoids	Colorimetric	NF	Ethyl acetate or methanol	<i>C. cibarius</i> and <i>Pleurotus porrigens</i>
43	2010	International Journal of Medicinal Mushrooms	0.639	Lai, LK. et al.	Anti-Human Papillomavirus (HPV) 16 E6 activity of Ling Zhi or Reishi medicinal mushroom, <i>Ganoderma lucidum</i> (W. Curt.: Fr.) P. Karst. (Aphyllphoromycetideae) extracts.	12, 3, 279-286	Total flavonoids	Colorimetric	NF	Dichloromethane	<i>G. lucidum</i>

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44	2010	Journal of Food Science	1.733	Lung, MY. et al.	Antioxidant properties of edible Basidiomycete <i>Phellinus igniarius</i> in submerged cultures.	75, 1, 18-24	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	UV/VIS 415 nm	Hot water and methanol	<i>Phellinus igniarius</i> (mycellium)
45	2010	Journal of Nutrition and Metabolism	NF	Robaszkiewicz, A. et al.	The role of polyphenols, beta-carotene, and lycopene in the antioxidative action of the extracts of dried, edible mushrooms.	2010, -, 9 (ID 173274)	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 515 nm	Hot water and methanol	Polish mushrooms
46	2011	Food Chemistry	3.655	Guo, T. et al.	Antioxidant activities of extract and fractions from <i>Tuber indicum</i> Cooke & Massee.	127, 4, 1634–1640	Total flavonoids	NaNO ₂ + 10% Al(NO ₃) ₃ · H ₂ O	VIS 510nm	Ethanol	<i>Tuber indicum</i>
47	2011	Food Chemistry	3.655	Oke, F. et al.	Protective effect of two edible mushrooms against oxidative cell damage and their phenolic composition.	128, 3, 613–619	Luteolin	HPLC-DAD	UV 278 nm	Methanol or water	<i>P. eryngii</i> and <i>Auricularia auricula-judae</i>
48	2011	Food and Chemical Toxicology	2.999	Öztürk, M. et al.	<i>In vitro</i> antioxidant, anticholinesterase and antimicrobial activity studies on three <i>Agaricus</i> species with fatty acid compositions and iron contents: A comparative study on the three most edible mushrooms.	49, 6, 1353–1360	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Hexane, ethyl acetate or aqueous methanol	<i>Agaricus essettei</i> , <i>Agaricus bitorquis</i> and <i>A. bisporus</i> .

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49	2011	Food Chemistry	3.655	Palacios, I. et al.	Antioxidant properties of phenolic compounds occurring in edible mushrooms.	128, 3, 674–678	Total flavonoids	AlCl ₃	NF	Methanol	<i>A. bisporus</i> , <i>B. edulis</i> , <i>C. gambosa</i> , <i>C. cibarius</i> , <i>C. cornucopoid</i> , <i>Hygrophorus marzuolus</i> , <i>L. deliciosus</i> and <i>P. ostreatus</i> .
							Catechin and myricetin	HPLC-DAD	280 y 320nm		
50	2011	International Food Research Journal	0.282	Imran, MM. et al.	Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms <i>Pleurotus florida</i> and <i>Pleurotus eous</i> .	18, 2, 579-582	Total flavonoids	AlCl ₃	UV/VIS 415 nm	Methanol	<i>Pleurotus florida</i> and <i>Pleurotus eous</i>
51	2011	Journal of Chemical and Pharmaceutical Research	NF	Sathyaprabha, G. et al.	Analysis of antioxidant activity, total phenol, total flavonoid and screening of phytocomponents in <i>Pleurotus platypus</i> and <i>Pleurotus eous</i> .	3, 6, 1-6	Total flavonoids	Detection: ammonia solution + sulphuric acid	Presence of yellow color	Water	<i>P. eous</i> and <i>Pleurotus platypus</i>
								Cuantification : NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm		
52	2011	International Journal of Molecular Sciences	2.6	Lung, MY. et al.	Antioxidant properties of the edible Basidiomycete <i>Armillaria mellea</i> in submerged cultures.	12, 10, 6367-6384	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	UV/VIS 415 nm	Hot water or methanol	<i>Armillaria mellea</i> (micellium)
53	2011	Molecules	2.386	Yeh, JY. et al.	Antioxidant properties and antioxidant compounds of various extracts from the edible Basidiomycete <i>Grifola Frondosa</i> (Maitake).	16, 4, 3197-3211	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	UV/VIS 415 nm	Cold/hot water or methanol	<i>Grifola frondosa</i>

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54	2011	African Journal of Biotechnology	NI	Alam, N. et al.	Evaluation of the antioxidant and antityrosinase activities of three extracts from <i>Pleurotus nebrodensis</i> fruiting bodies.	10, 11, 2978-2986	naringenin, hesperetin	HPLC-DAD	UV 280 nm	60% acetone, 80% methanol or hot water	<i>Pleurotus nebrodensis</i>
55	2011	Combinatorial Chemistry & High Throughput Screening	1.785	Ozen, T. et al.	Screening of antioxidant, antimicrobial activities and chemical contents of edible mushrooms wildly grown in the Black Sea region of Turkey.	14, 2, 72-84	Total flavonoids and anthocyanins	Colorimetric	NF	NF	Twelve commonly consumed wild edible mushrooms
56	2011	International Journal of Agriculture and Biology	0.94	Jahangir, MM. et al.	Effect of spermine on bioactive components and antioxidant properties of sliced buttonmushroom (<i>Agaricus bisporus</i>) during storage.	13, 5, 771-775	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>A. bisporus</i>
57	2011	Journal of Food Nutrition and Research	0.679	Lagnika, C. et al.	Effect of high argon pressure and modified atmosphere packaging on the white mushroom (<i>Agaricus bisporus</i>) physico-chemical and microbiological properties.	50, 3, 167-176	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Water	<i>A. bisporus</i>
58	2011	Journal of Food Quality	0.54	Da Silva, AC. et al.	Antioxidant properties of <i>Lentinus edodes</i> and <i>Agaricus blazei</i> extracts.	34, 6, 386-394	Total flavonoids	Colorimetric	NF	NF	<i>L. edodes</i> and <i>Agaricus blazei</i>

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59	2012	Food Chemistry	3.334	Dong, J. et al.	Nitric oxide fumigation stimulates flavonoid and phenolic accumulation and enhances antioxidant activity of mushroom.	135, 3, 1220–1225	Total flavonoids	$\text{NaNO}_2 + 10\% \text{Al}(\text{NO}_3)_3 \cdot \text{H}_2\text{O}$	VIS 510nm	Methanol	<i>R. griseocarnos a</i>
60	2012	Food Chemistry	3.334	Pereira, E. et al.	Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats.	130, 2, 394–403	Total flavonoids	$\text{NaNO}_2 + 10\% \text{AlCl}_3 \cdot \text{H}_2\text{O}$	VIS 510 nm	Methanol	Twelve Portuguese wild edible mushrooms
61	2012	Asian Pacific Journal of Tropical Biomedicine	0.502	Shirmila, JG. et al.	Identification and determination of antioxidant constituents of bioluminescent mushroom.	2, 1, supplement, 386–391	Total flavonoids	AlCl_3	UV/VIS 420 nm	Methanol	<i>Omphalotus nidiformis</i>
62	2012	Food and Chemical Toxicology	3.01	Liu, YT. et al.	Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity.	50, 5, 1238–1244	Total flavonoids, quercetin and catechin	HPLC-DAD	UV 273 nm	Ethanol	<i>Clitocybe maxima</i> , <i>Catathelasma ventricosum</i> , <i>Stropharia rugoso-annulata</i> , <i>C. cornucopioides</i> and <i>Laccaria amethystea</i>
63	2012	Iranian Journal of Pharmaceutical Research	0.54	Kosanić, M. et al.	Mushrooms as possible antioxidant and antimicrobial agents.	11, 4, 1095–1102	Total flavonoids	AlCl_3	UV/VIS 415 nm	Acetone and methanol	<i>Boletus aestivalis</i> , <i>B. edulis</i> and <i>Leccinum carpini</i>

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64	2012	Food Science and Biotechnology	0.695	Li, H et al.	Targeted phenolic analysis in <i>Hericium erinaceum</i> and its antioxidant activities.	21, 3, 881-888	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Methanol	<i>H. erinaceum</i>
65	2012	Food Analytical Methods	1.969	Tel, G.et al.	Antioxidant and cholinesterase inhibition activities of three <i>Tricholoma</i> species with total phenolic and flavonoid contents: The edible mushrooms from Anatolia.	5, 3, 495-504	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Methanol	<i>Tricholoma fracticum</i> , <i>Tricholoma imbricatum</i> and <i>Tricholoma terreum</i>
66	2012	Revista Iberoamericana de Micología	1.312	Ayala-Zavala, FJ. et al.	Antioxidant and antifungal potential of methanol extracts of <i>Phellinus</i> spp. from Sonora, Mexico.	29, 3, 132-138	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	UV/VIS 415 nm	Methanol	<i>Phellinus gilvus</i> , <i>Phellinus rimosus</i> and <i>Phellinus badius</i>
67	2012	Food Science and Biotechnology	0.695	Sudha, G. et al.	Antioxidant activity of various extracts from an edible mushroom <i>Pleurotus eous</i> .	21, 3, 661-668	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Ethyl acetate, methanol or hot water	<i>P. eous</i>
68	2012	Current topics in Nutraceutical Research	0.262	Akyuz, M. et al.	Flavonoids contents and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of some edible mushrooms from Turkey: <i>A. bisporus</i> and <i>Pleurotus</i> spp.	10, 2, 133-136	Rutin, myricetin, morin, quercetin, kaempferol, catechin, naringenin and naringin	HPLC-DAD	UV 254-280 nm	80 % Methanol	<i>A. bisporus</i> , <i>P. eryngii</i> var. <i>eryngii</i> , <i>P. eryngii</i> var. <i>ferulae</i> , <i>Pleurotus sajor-caju</i> , <i>P. ostreatus</i> and <i>Pleurotus florida</i>

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69	2012	Food and Bioprocess Technology	4.115	Singla, R. et al.	Physicochemical and nutritional characteristics of organic acid-treated button mushrooms (<i>Agaricus bisporous</i>).	5, 2, 808-815	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	NF	<i>A. bisporus</i>
70	2012	Journal of Food Science and Biotechnology	0.695	Huang, XD. et al.	Study on antioxidation effect <i>in vitro</i> and <i>in vivo</i> of total flavonoids from <i>Inonotus obliquus</i> .	31, 10, 1062-1068	Total flavonoids	NF	NF	NF	<i>I. obliquus</i>
71	2012	International Journal of Medicinal Mushrooms	0.838	Yin, X. et al.	Immunomodulatory activities of different solvent extracts from <i>Tricholoma matsutake</i> (S. Ito et S. Imai) Singer (higher Basidiomycetes) on normal mice.	14, 6, 549-556	Total flavonoids	Colorimetric	NF	Water or n-butyl alcohol.	<i>T. matsutake</i>
72	2012	International Journal of Medicinal Mushrooms	0.838	Ayala-Zavala, F.J. et al.	Polar fractionation affects the antioxidant properties of methanolic extracts from species of genus <i>Phellinus</i> Quel. (higher Basidiomycetes).	14, 6, 563-573	Total flavonoids	Colorimetric	NF	Water or ethyl acetate	<i>Phellinus fastuosus</i> , <i>Phellinus grenadensis</i> , <i>Phellinus merrillii</i> , and <i>Phellinus badius</i>
73	2012	Journal of the Korean Society of Food Science and Nutrition	NF	Baek, GH. et al.	Pharmacological activity of chaga mushroom on extraction conditions and immunostimulating polysaccharide.	41, 10, 1378-1387	Total flavonoids	Colorimetric	NF	Hot water or hot ethanol	<i>I. obliquus</i>
74	2012	Journal of the Korean Society of Food Science and Nutrition	NF	Hong, MH. et al.	Antioxidant properties and ubiquinone contents in different parts of several commercial mushrooms.	41, 9, 1235-1241	Total flavonoids	Colorimetric	NF	Methanol	14 commercial mushrooms

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75	2012	International Journal of Medicinal Mushrooms	0.838	Wang, Y. et al.	Contents of some metabolites in the peel and flesh of the medicinal mushroom <i>Wolfiporia cocos</i> (F.A. Wolf) Ryvarden et Gilb. (Higher Basidiomycetes).	14, 1, 79-83	Total flavonoids	Colorimetric	NF	NF	<i>Wolfiporia cocos</i>
76	2013	Food Research International	3.05	Leal, AR. et al.	Portuguese wild mushrooms at the "pharma-nutrition" interface: nutritional characterization and antioxidant properties.	50, 1, 1-9	Chrysin derivatives	HPLC-DAD/MS	UV 280-370; comparison of mass spectras and retention times	80% Methanol	<i>Amanita crocea</i> , <i>Amanita mairei</i> , <i>Boletus porosporus</i> , <i>Boletus regius</i> , <i>Gyromitra esculenta</i> , <i>Helvella lacunosa</i> , <i>Russula aurea</i> and <i>R. virescens</i>
77	2013	Food and Chemical Toxicology	2.61	Liu, J. et al.	<i>In vitro</i> and <i>in vivo</i> antioxidant activity of ethanolic extract of white button mushroom (<i>Agaricus bisporus</i>).	51, -,310-316	Myricetin and catechin	HPLC-ESI-MS/MS	Comparison of mass spectras and retention times	80% Ethanol	<i>A. bisporus</i>
78	2013	Journal of Natural Products and Plant Resources	NF	Abugria, DA. et al.	Extraction of total phenolic and flavonoids from edible wild and cultivated medicinal mushrooms as affected by different solvents.	3, 3, 37-42	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Mehtnaol or water	<i>A. bisporus</i> , <i>Trametes versicolor</i> , <i>Ganoderma applanatum</i> and <i>Fomes fomentarius</i> .

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79	2013	International Food Research Journal	NF	Gan, C. et al.	Antioxidant analysis of different types of edible mushrooms (<i>Agaricus bisporous</i> and <i>Agaricus brasiliensis</i>).	20, 3, 1095-1102	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	60% Ethanol or water	<i>A. bisporus</i> and <i>Agaricus brasiliensis</i>
80	2013	Tropical Journal of Pharmaceutical Research	0.495	Wong, FC. et al.	Evaluation of bioactivities and phenolic content of selected edible mushrooms in Malaysia.	12, 6, 1011-1016	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	90 % Ethanol	<i>Hypsizyugus tessulatus</i> , <i>P. eryngii</i> , <i>P. florida</i> , <i>Auricularia polytricha</i> and <i>Flammulina velutipes</i>
81	2013	International Journal of Pharmacy and Pharmaceutical Sciences	NF	Shirmila, J, et al.	<i>In vitro</i> antioxidant activities, total phenolics and flavonoid of wild edible mushroom <i>Macrolepiota Mastoidea</i> (Fr.) Singer.	5, 2, 161-166	Total flavonoids	AlCl ₃	UV/VIS 415 nm	Methanol	<i>Macrolepiota mastoidea</i>
82	2013	Journal of Soil Science and Plant Nutrition	0.582	Akyüz, M.	Nutritive value, flavonoid content and radical scavenging activity of the truffle (<i>Terfezia boudieri</i> Chatin).	13, 1, 143-151	Myricetin, kaempferol, naringin and naringenin	HPLC-DAD	UV 254-280 nm	80% Methanol	<i>Terfezia boudieri</i>
83	2013	Mycosphere	NF	Arbaayah, HH. et al.	Antioxidant properties in the oyster mushrooms (<i>Pleurotus</i> spp.) and split gill mushroom (<i>Schizophyllum commune</i>) ethanolic extracts.	4, 4, 661–673	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Ethanol	<i>Pleurotus djamor</i> var. <i>djamor</i> and <i>Schizophyllum commune</i>
84	2013	Journal of Food Research	1.791	Wandati, TW. et al.	Phytochemicals in edible wild mushrooms from selected areas in Kenya.	2, 3, 137-144	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	UV/VIS 415 nm	Ethanol	<i>P. florida</i> and <i>A. bisporus</i>

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85	2013	Food and Agricultural Immunology	0.984	Llaurado, G. et al.	Phytochemical screening and effects on cell-mediated immune response of <i>Pleurotus</i> fruiting bodies powder.	24, 3, 295-304	Total flavonoids	Colorimetric	NF	NF	<i>Pleurotus</i>
86	2013	Molecules	2.095	Hasnat, A. et al.	Acetylcholinesterase inhibition and <i>in vitro</i> and <i>in vivo</i> antioxidant activities of <i>Ganoderma lucidum</i> grown on germinated brown rice.	18, 6, 6663-6678	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 517 nm	Water	<i>G. lucidum</i>
							Quercetin, kaempferol, cateuin, myricetin, rutin, epicatechin	HPLC-DAD/MS	UV 280 nm; comparison of mass spectras and retention times		
87	2013	Food and Chemical toxicology	2.61	Kalogeropoul os, N. et al.	Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece.	55, -, 378-385	Chrysin, genistein, kaempferol and quercetin	GC-MS	Comparison of mass spectras and retention times	Methanol	<i>L. deliciosus</i> , <i>Lactarius sanguifluus</i> , <i>Lactarius semisanguifluus</i> , <i>R. Suilica</i> and <i>Suillus bellinii</i>
88	2013	Journal of Food Science and Technology	2.024	Babu, DR. et al.	Antioxidant properties and electrochemical behavior of cultivated commercial Indian edible mushrooms.	50, 2, 301-308	Total flavonoids	AlCl ₃	UV/VIS 415 nm	95% Etahnol	<i>A. bisporus</i> , <i>Hypsizygyus ulmarius</i> and <i>Calocybe indica</i>
89	2013	Food Science and Biotechnology	0.656	Singdevsachan, SK. et al.	Nutritional and bioactive potential of two wild edible mushrooms (<i>Lentinus sajor-caju</i> and <i>Lentinus torulosus</i>) from Similipal Biosphere Reserve, India.	22, 1, 137-145	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Ethanol, methanol or water	<i>Lentinus sajor-caju</i> and <i>Lentinus torulosus</i>

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90	2013	International Journal of Medicinal Mushrooms	1.123	Durgo, K. et al.	Cytotoxicity of blended versus single medicinal mushroom extracts on human cancer cell lines: contribution of polyphenol and polysaccharide content.	15, 5, 435-448	Total flavonoids	Colorimetric	NF	NF	<i>L. edodes</i> , <i>G. lucidum</i> , <i>A. bisporus</i> , <i>G. frondosa</i> , <i>T. massutake</i> and <i>P. ostreatus</i>
91	2013	BioMed Research International	2.706	Vamanu, E. et al.	Antioxidant capacity and the correlation with major phenolic compounds, anthocyanin, and tocopherol content in various extracts from the wild edible <i>Boletus edulis</i> mushroom.	2013, -, 11 (ID 313905)	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	UV/VIS 410 nm	Ethanol, methanol or cold/hot water	<i>B. edulis</i>
92	2013	International Journal of Medicinal Mushrooms	1.123	Lai, MN. et al.	Antioxidant and antiedema properties of solid-state cultured honey mushroom, <i>Armillaria mellea</i> (Higher Basidiomycetes), extracts and their polysaccharide and polyphenol contents.	15, 1, 1-8	Total flavonoids	Colorimetric	NF	Ethanol or water	<i>A. mellea</i>
93	2013	International Journal of Medicinal Mushrooms	1.123	Kumari, B. et al.	Evaluation of nutraceutical components and antioxidant potential of North Indian wild culinary-medicinal termitophilous mushrooms.	15,2, 191-197	Total flavonoids	Colorimetric	NF	NF	<i>Termitomyces microcarpus</i> , <i>Termitomyces radicans</i> , <i>Termitomyces badius</i> , <i>Termitomyces medius</i> , <i>Termitomyces heimii</i> and <i>Termitomyces striatus</i>

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94	2013	International Journal of Medicinal Mushrooms	1.123	Jeong, SC. et al.	Antioxidant and immunomodulating activities of exo- and endopolysaccharide fractions from submerged mycelia cultures of culinary-medicinal mushrooms.	15, 3, 251-266	Total flavonoids	Colorimetric	NF	NF	<i>Cryptosporus volvatus</i> and <i>Cordyceps militaris</i>
95	2013	Journal of the Korean Society of Food Science and Nutrition	NI	Qi, Y. et al.	Antioxidant and anticancer effects of edible and medicinal mushrooms.	42, 5, 655-662	Total flavonoids	Colorimetric	NF	Methanol	<i>L. edodes</i> , <i>A. blazei</i> , <i>C. militaris</i> , <i>G. lucidum</i> , <i>I. obliquus</i> and <i>Phellinus linteus</i>
96	2013	Journal of Food Biochemistry	0.853	Debnath, T. et al.	Antioxidant activity of <i>Inonotus obliquus</i> grown on germinated brown rice extracts.	37, 4, 456-464	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 517 nm	Ethanol, methanol or water	<i>I. obliquus</i>
97	2014	Food Chemistry	3.391	Woldegiorgis, AZ. et al.	Antioxidant property of edible mushrooms collected from Ethiopia.	157, 15, 30–36	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>P. ostreatus</i> , <i>L. edodes</i> , <i>L. sulphureus</i> , <i>Agaricus campestris</i> , <i>Termocyes clypeatus</i> , <i>T. microcarpus</i> and <i>Termocyes letestui</i>
98	2014	Food Chemistry	3.391	Özyürek, M. et al.	Antioxidant/antiradical properties of microwave-assisted extracts of three wild edible mushrooms.	157, 15, 323–331	Rutin, quercetin, hesperidin, naringenin, kaempferol and apigenin	UPLC–ESI-MS/M	Comparison of mass spectras and retention times	80% Methanol	<i>T. boudieri</i> Chatin, <i>B. edulis</i> and <i>Lactarius volemus</i>

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99	2014	Journal of Food Science and Technology	2.203	Babu, DR. et al.	Antioxidant and electrochemical properties of cultivated <i>Pleurotus spp.</i> and their sporeless/low sporing mutants.	51, 11, 3317-3324	Total flavonoids	AlCl ₃	UV/VIS 415 nm	95% Ethanol	<i>P. florida</i> , <i>P. sajor-caju</i> , <i>Pleurotus cystidiosus</i> and <i>P. djamor</i>
100	2014	International Journal of Medicinal Mushrooms	0.927	Rathor, R. et al.	Scientific validation of the Chinese caterpillar medicinal mushroom, <i>Ophiocordyceps sinensis</i> (Ascomycetes) from India: immunomodulatory and antioxidant activity.	16, 6, 541-553	Total flavonoids	Colorimetric	NF	Water	<i>Ophiocordyceps sinensis</i>
101	2014	International Journal of Medicinal Mushrooms	0.927	Dong, CH. et al.	A comparative study of the antimicrobial, antioxidant, and cytotoxic activities of methanol extracts from fruit bodies and fermented mycelia of caterpillar medicinal mushroom <i>Cordyceps militaris</i> (Ascomycetes).	16, 5, 485-495.	Total flavonoids	Colorimetric	NF	Methanol	<i>C. militaris</i>
102	2014	International Journal of Medicinal Mushrooms	0.927	Gupta, A. et al.	Wound healing activity of an aqueous extract of the Lingzhi or Reishi medicinal mushroom <i>Ganoderma lucidum</i> (higher Basidiomycetes).	6, 4, 345-354.	Total flavonoids	Colorimetric	NF	Water	<i>G. lucidum</i>
103	2014	LWT-Food Sciences and Technology	2.416	Jaworska, G. et al.	Vitamins, phenolics and antioxidant activity of culinary prepared <i>Suillus luteus</i> (L.) Roussel mushroom.	59, 2 (1), 701-706	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>Suillus luteus</i>

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104	2014	Journal of Food Science and Technology	2.203	Gogavekar, SS. et al	Important nutritional constituents, flavour components, antioxidant and antibacterial properties of <i>Pleurotus sajor-caju</i> .	51, 8, 1483-1491	Total flavonoids	AlCl ₃	UV/VIS 415 nm	Methanol	<i>P. sajor-caju</i>
105	2014	Journal of Consumer Protection and Food Safety	0.719	Singdevsachan, SK. et al.	Evaluation of nutritional and nutraceutical potentials of three wild edible mushrooms from Similipal Biosphere Reserve, Odisha, India.	9, 2, 111-120	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Ethanol, methanol or water	<i>Russula vesca</i> , <i>R. delica</i> and <i>Termitomyces eurrhizus</i>
106	2014	International Journal of Food Sciences and Nutrition	1.206	Karaman, M. et al.	Wild-growing lignicolous mushroom species as sources of novel agents with antioxidative and antibacterial potentials.	65, 3, 311-319	Total flavonoids	Colorimetric	NF	NF	<i>Meripilus giganteus</i> , <i>A. aegerita</i> , <i>F. fomentarius</i> and <i>Xylaria polymorpha</i>
107	2014	Journal of Agricultural and Food Chemistry	2.912	Sapozhnikova, Y. et al.	Effects of UV-B radiation levels on concentrations of phytosterols, ergothioneine, and polyphenolic compounds in mushroom powders used as dietary supplements.	62, 14, 3034-3042	Total flavonoids	LC-ESI-MS/M	Comparison of mass spectras and retention times	80% Methanol	<i>A. bisporus</i> , <i>P. ostreatus</i> and <i>L. edodes</i>
108	2014	Journal of Food Science	1.696	Li, H. et al.	Antioxidant and anti-inflammatory activities of methanol extracts of <i>Tremella fuciformis</i> and its major phenolic acids.	79, 4, C460-C468	Total flavonoids	AlCl ₃	VIS 640 nm	80% Methanol	<i>Tremella fuciformis</i>
109	2014	Journal of Food Biochemistry	0.741	Hasnat, MA. et al.	DNA protection, total phenolics and antioxidant potential of the mushroom <i>Russula virescens</i> .	38, 1, 6-17	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 517 nm	75% Ethanol or water	<i>R. virescens</i>

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110	2014	Food Analytical Methods	1.956	Cayan, F. et al.	Application of GC, GC-MSD, ICP-MS and spectrophotometric methods for the determination of chemical composition and <i>in vitro</i> bioactivities of <i>Chroogomphus rutilus</i> : the edible mushroom species.	7, 2, 449-458	Total flavonoids	10% AINO ₃	UV/VIS 415 nm	Ethaol, hexane, ethyl acetate or water	<i>Chroogomphus rutilus</i>
111	2014	Drying Technologies	1.518	Jaworska, G. et al.	Effect of different drying methods and 24-month storage on water activity, rehydration capacity, and antioxidants in <i>Boletus edulis</i> mushrooms.	32, 3, 291-300	Total flavonoids	Colorimetric	NF	NF	<i>B. edulis</i>
112	2014	Journal of Pure and Applied Microbiology	0.073	Thakur, M. et al.	Qualitative phytochemical screening, total phenolic content, <i>in vitro</i> antioxidant activity and antimicrobial activities in methanolic extracts of <i>Lactarius sanguifluus</i> (Paulet) Fr.	8, 6, 4735-4741	Total flavonoids	Colorimetric	NF	Methanol	<i>L. sanguifluus</i>
113	2014	Journal of Applied Botany and Food Quality	0.758	Kruzselyi, D. et al.	Complex chemical evaluation of the summer truffle (<i>Tuber aestivum</i> Vittadini) fruit bodies.	87, -, 291-295	Total flavonoids	AlCl ₃	UV/VIS 415 nm	Methanol	<i>Tuber aestivum</i> Vittadini
114	2014	Archives of Biological Sciences	0.718	Milovanovic, I. et al.	Antioxidant, antigungal and anticancer activities of Se-enriched <i>Pleurotus spp.</i> mycelium extracts.	66, 4, 1379-1388	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	96% Ethanol	<i>P. eryngii</i> , <i>P. ostreatus</i> and <i>P. pulmonarius</i>

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Number	Year	Journal	Impact factor	First author	Title	Volumen, Issue, pages	Compounds found in mushrooms	Method utilized to measure flavonoids	Detection device or wavelength used	Solvents used for extraction	Mushroom specie studied
115	2014	Scientific World Journal	NF	Phan, CW. et al.	Intrastrain comparison of the chemical composition and antioxidant activity of an edible mushroom, <i>Pleurotus giganteus</i> , and its potent neurotogenic properties.	2014, -, 111 (ID 37865 1)	Total flavonoids	AlCl ₃	VIS 435 nm	Ethanol	<i>Pleurotus giganteus</i>
116	2014	Food Science and Biotechnology	0.653	Vamanu, E. et al.	Biological activity of fluidized bed ethanol extracts from several edible mushrooms.	23, 5, 1483-1490	Total flavonoids	AlCl ₃	UV/VIS 415 nm	70% Ethanol	<i>P. ostreatus</i> , <i>A. bisporus</i> , <i>M. oreades</i> , <i>C. cornucopioides</i> , and <i>Tuber melanosporum</i>
							Rutin and catechin	HPLC-DAD	Comparison of retention times		<i>T. melanosporum</i> and <i>P. ostreatus</i>
117	2014	Food Science and Technology	0.393	Miao, QJ. et al.	Research on total flavonoids bioactivity of edible fungus.	7 , 39, 206-209	Total flavonoids	Colorimetric	NF	NF	<i>Pleurotus citrinopileatus</i> , <i>Pleurotus geesteranus</i> , <i>A. auricula</i> and <i>P. ostreatus</i>
118	2014	LWT-Food Sciences and Technology	2.416	Lin, JT. et al.	Chemical composition, antioxidant and anti-inflammatory properties for Ethanolic extracts from <i>Pleurotus eryngii</i> fruiting bodies harvested at different time.	55, 1, 374-382	Total flavonoids	HPLC-DAD	UV 220-450 nm and comparison of retention times	Ethanol	<i>P. eryngii</i>

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119	2015*	Industrial Crops and Products	2.837	Tel-Cayan, G. et al.	Phytochemical investigation, antioxidant and anticholinesterase activities of <i>Ganoderma adspersum</i> .	76, -, 749–754	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Methanol	<i>Ganoderma adspersum</i>
120	2015*	Food Chemistry	3.391	Jiang, T. et al.	Fumigation with essential oils improves sensory quality and enhanced antioxidant ability of shiitake mushroom (<i>Lentinus edodes</i>).	172, -, 692–698	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	80% Ethanol	<i>L. edodes</i>
121	2015*	International Journal of Medicinal Mushrooms	0.927	Ebrahimzadeh, MA. et al.	Antioxidant activity of different fractions of methanolic extract of the golden chanterelle mushroom <i>Cantharellus cibarius</i> (Higher Basidiomycetes) from Iran.	17, 6, 557-565	Total flavonoids	Colorimetric	NF	Metanol, hexane, chloroform, ethyl acetate, n-butanol or water	<i>C. cibarius</i>
122	2015*	BioMed Research International	1.579	Sharma, SK. et al.	Chemical, bioactive, and antioxidant potential of twenty wild culinary mushroom species.	2015, -, 12 (ID 346508)	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Water	20 culinary mushroom species
							Anthocyanidins	Colorimetric	VIS 546 nm	Ethyl alcohol and CIH	
123	2015*	Food and Function	2.791	Kozarski, M. et al.	Nutraceutical properties of the methanolic extract of edible mushroom <i>Cantharellus cibarius</i> (Fries): primary mechanisms.	6, 6, 1875-86	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>C. cibarius</i>
124	2015*	International Journal of Medicinal Mushrooms	0.927	Khaund, P. et al.	Functional nutraceutical profiling of wild edible and medicinal mushrooms consumed by ethnic tribes in India.	17, 2, 187-197	Total flavonoids	Colorimetric	NF	Ethanol	10 wild and edible mushrooms

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125	2015*	International Journal of Medicinal Mushrooms	0.927	Wu, T. et al.	Antidiabetic and antioxidant activities of eight medicinal mushroom species from China.	17, 2, 129-40	Total flavonoids	Colorimetric	NF	NF	<i>G. lucidum</i> and <i>T. fuciformis</i>
126	2015*	International Journal of Medicinal Mushrooms	0.927	Mishra ,KK. et al.	Antioxidant activities and bioactive compound determination from caps and stipes of specialty medicinal mushrooms <i>Calocybe indica</i> and <i>Pleurotus sajor-caju</i> (higher Basidiomycetes) from India.	16, 6, 555-67.	Tannins	Colorimetric	NF	Methanol or water	<i>C. indica</i> and <i>P. sajor-caju</i>
127	2015*	Brazilian Journal of Microbiology	0.592	Tajalli, F. et al.	Antioxidant capacity of several Iranian, wild and cultivated strains of the button mushroom.	46, 3, 769-776	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>A. bisporus</i>
							Anthocyanidins	NaCH ₃ CO ₂	VIS 510-700 nm		
128	2015*	Internatinal Journal of Food Science and Technology	1.384	Mokochinski, JB. et al.	Production of <i>Agaricus brasiliensis</i> mycelium from food industry residues as a source of antioxidants and essential fatty acids.	50, 9, 2052-2058	Total flavonoids	AlCl ₃	VIS 425 nm	Methanol	<i>A. brasiliensis</i>
129	2015*	LWT-Food Sciences and Technology	2.416	Gasecka, M. et al.	The effect of selenium on phenolics and flavonoids in selected edible white rot fungi.	63, 1, 726-731	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Methanol	<i>Pholiota nameko</i> , <i>P. eryngii</i> and <i>P. ostreatus</i>
130	2015*	Pharmaceutil Biology	1.241	Tel, G. et al.	Antioxidant and anticholinesterase activities of five wild mushroom species with total bioactive contents.	53, 6, 824-830	Total flavonoids	Colorimetric	NF	Methanol or ethyl acetate	<i>G. lucidum</i> and <i>Funalia trogii</i>

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131	2015*	Food Chemistry	3.391	Sarikurkcü, C. et al.	Metal concentration and antioxidant activity of edible mushrooms from Turkey.	175, -, 549-555	Total flavonoids	AlCl ₃	UV/VIS 415 nm	Methanol or water	<i>Polyporus sulphureus</i> , <i>Macrolepiota procera</i> , <i>L. perlatum</i> and <i>Gomphus clavatus</i>
132	2015*	Pharmacognosy Magazine	1.256	Sadi, G. et al.	Cytotoxicity of some edible mushrooms extracts over liver hepatocellular carcinoma cells in conjunction with their antioxidant and antibacterial properties.	11, 4, supplement, 6-18	Total flavonoids	Colorimetric	NF	Methanol, acetone, n-hexane, chloroform or water	<i>C. comatus</i> , <i>T. fracticum</i> , <i>Rhizopogon luteolus</i> and <i>Lentinus tigrinus</i>
133	2015*	International Journal of Food Science and Technology	1.384	Zhang, N. et al.	Chemical composition and antioxidant properties of five edible Hymenomycetes mushrooms.	50, 2, 465-471	Total flavonoids	NaNO ₂ + 10% AlCl ₃ · H ₂ O	VIS 510 nm	Ethanol	<i>L. edodes</i> , <i>T. fuciformis</i> , <i>A. polytricha</i> , <i>G. lucidum</i> and <i>I. obliquus</i>
134	2015*	International Journal of Medicinal Mushrooms	0.927	Ganeshpurkar, A. et al.	Antioxidant potential of white oyster culinary-medicinal mushroom, <i>Pleurotus florida</i> (Higher Basidiomycetes).	17, 5, 491-498	Total flavonoids	Colorimetric	NF	50% Ethanol	<i>P. florida</i>
135	2015*	International Journal of Medicinal Mushrooms	0.927	Milovanovic, I. et al.	Effects of Selenium presence in mycelia of <i>Ganoderma</i> species (Higher Basidiomycetes) on their medicinal properties.	17, 1, 11-20	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	96% Ethanol	<i>G. applanatum</i> and <i>G. lucidum</i>
136	2015*	Annals of Clinical Microbiology and Antimicrobials	2.189	Chowdhury, MMH. et al.	Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh.	14, 1, 8	Total flavonoids	10% Al(NO ₃) ₃	UV/VIS 415 nm	Methanol	<i>P. ostreatus</i> , <i>L. edodes</i> and <i>H. tessulatus</i>

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