

## Phenolic acids – Occurrence and Significance in the World of Higher Fungi

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### **Abstract**

Phenolic acids constitute a chemically diverse group of bioactive compounds. The activity of phenolic acids is related to the presence of hydroxycarboxylic groups and their position on the aromatic ring. These are compounds commonly found in nature – they are present in many species of plants and fungi. In the world of higher fungi, they constitute the most numerous group among phenolic compounds. The most common phenolic acids in the world of fungi are caffeic acid, gallic acid, gentisic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, and a compound with a structure similar to phenolic acids – *t*-cinnamic acid. Phenolic acids found in fruiting bodies of mushrooms show, among others, antioxidant, antimicrobial, and anti-cancer properties.

**Keywords: phenolic acids, higher fungi, biological activity**

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### Definition, biosynthesis and chemical structure

Phenolic acids (phenolcarboxylic acids) are substances containing an aromatic ring substituted by at least one hydroxyl and a carboxyl group. Depending on the number of carbon atoms in the side chain, they can be divided into hydroxybenzoic acids and hydroxycinnamic acids [1]. The structures of the selected phenolic acids are shown in Table 1. The precursors of most phenolic acids are aromatic amino acids – tyrosine and phenylalanine, which are formed by the transformation of erythrose phosphate and phosphoenolpyruvic acid through the shikimic acid pathway. From phenylalanine and tyrosine, as a result of their deamination, are formed cinnamic acid or p-coumaric acid and its hydroxyl derivatives. Other phenolic acids can be products of the transformation of malonic acid or chorismic acid and also polyketide way [2].

### Occurrence in the world of fungi

Phenolic acids are found in nature in both free forms and the form of glycosides and esters (depsides and depsidones). In the fruiting bodies of higher fungi belonging to Basidiomycota, free phenolic acids are usually present in small amounts. Their content depends on the species and degree of ripeness of the fruiting bodies. It has been shown, that at high temperatures, as well as after acid hydrolysis, ester bonds are broken, and free phenolic acids are released [1].

Hydroxybenzoic acid derivatives most often occur in a bonded form as components of complex structures such as lignin and hydrolysable tannins. They also form complexes with simple sugars or ester complexes with organic acids. Hydroxycinnamic acid derivatives also occur in a bound form. Most often they form complexes with compounds such as chitin or proteins, while with organic, tartaric, or quinic acids they form ester connections (e.g. 5-O-caffeoylquinic acid – chloro-

genic acid). Besides, complexes of phenolic acids with sterols and fatty acids have been identified [1-3]

Based on numerous analyses, proved that phenolic acids are the dominant quantitative group of phenolic derivatives found in higher fungi [4,5]. In the studies carried out by Barros et al., 16 species of edible mushrooms were analyzed. Apart from phenolic acids, no flavonoids and other phenolic derivatives were found [3]. Similar results were obtained in the course of studies conducted by researchers at the University of Helsinki – apart from phenolic acids, the presence of flavonoids or lignans was not observed in the tested mushrooms [6].

The most common phenolic acids in fruiting bodies of higher fungi are p-hydroxybenzoic acid, gallic acid, protocatechuic acid, p-coumaric acid, vanillic acid, ferulic acid, gentisic acid, caffeic acid, and a compound with a structure similar to phenolic acids – t-cinnamic acid. Less frequent in fruiting bodies are syringic acid, chlorogenic acid, ellagic acid and veratric acid. Protocatechuic acid and gallic acid are the most abundant in the arboreal species of Basidiomycota [5,7-9]. The occurrence of phenolic acids and cinnamic acid in selected higher fungi is presented in Table 2.

In the fruiting bodies analyzed by Barros et al. phenolic acids content ranged from 2.3 mg/100 g of dry weight (d.w) in *Lactarius deliciosus* to 35.7 mg/100 g d.w in *Ramaria botrytis*. p-Hydroxybenzoic acid dominated in 50% of the analyzed species and its highest content was observed in *Agaricus silvicola* (23.9 mg/100 g d.w.). Protocatechuic and vanillic acids were identified in two species studied, while p-coumaric acid was identified in three species. In *Cantharellus cibarius*, *Lycoperdon perlatum*, *Macrolepiota procera* confirmed the presence of t-cinnamic acid (1.5; 1.4; 2.2 mg/100 g d.w.) respectively [3,10]. The presence of p-hydroxybenzoic acid has also been confirmed in other species of i.a. *Tricholoma equestre* and *Amanita rubescens* [11]. Noechlorogenic acid (3-O-caffeoylquinic acid) is the dominant phenolic acid in *Cantharellus cibarius* fruiting bodies, while 4-O-caffeoylquinic, chlorogenic, caffeic

and p-coumaric acids are present in much smaller quantities. The total phenolic acid content of *Cantharellus cibarius* is approximately 2.0 mg/100 g d.w [8].

One of the phenolic acid-richest species is *Fistulina hepatica*, in which the content of these compounds was about 55.1 mg/100 g d.w., of which about 50% is elagic acid, 24% caffeic acid, 26% p-coumaric acid [7].

In the studies conducted by Puttaraju et al., the chemical composition and antioxidant activity of 23 species of edible fungi were analyzed. The dominant phenolic acids in the extracts obtained from fruiting bodies were gallic, protocatechuic and gentisin acids, the presence of which has been confirmed in most species. t-Cinnamic, syringic, caffeic, ferulic, and p-coumaric acid were identified in low or trace amounts. Besides, studies have proven a higher content of phenolic acids in aqueous extracts compared to methanolic extracts [12].

In *Agaricus bisporus*, *Lentinus edodes*, *Pleurotus ostreatus*, four phenolic acids were identified in relatively low amounts (from 0.1 mg/100 g d.w. to 0.8 mg/100 g d.w.). Quantitatively dominant acid was p-hydroxybenzoic acid, in a low amount, was determined t-cinnamic and protocatechuic acids and caffeic acid [6].

Kim et al. carried out quantitative determination of phenolic acids in five species of edible fungi *Pleurotus ostreatus*, *Agaricus bisporus*, *Flammulina velutipes*, *Pleurotus eryngii*, *Lentinus edodes*, as well as five species of fungi of medicinal use: *Agaricus blazei*, *Sparassis crispa*, *Phellinus linteus*, *Ganoderma lucidum*, *Inonotus obliquus*. The phenolic acids content was shown to be higher in the species of medicinal mushrooms. Protocatechuic acid was present in all studied species. In addition, the chemical analysis showed the presence gentisin acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, t-cinnamic acid, p- and o- p-coumaric acid and veratric acid. The highest content of p-hydroxybenzoic acid (26.3 mg/100 g d.w.) was found in *Inonotus obliquus*,

while protocatechuic acid (9.6 mg/100 g d.w.), caffeic acid (1.8 mg/100 g d.w.) and p-coumaric acid (3.8 mg/100 g d.w.) were found in *Sparassis crispa* [13].

The content of phenolic acids determined in edible mushrooms by Vaz et al., ranged from approximately 0.4 mg/100 g d.w. in *Armillaria mellea* to 8.1 mg/100 g d.w. in *Coprinus comatus*. In all the studied mushrooms, p-hydroxybenzoic acid (0.4 to 6.2 mg/100 g d.w.) was present, which was quantitatively dominant. The presence of p-coumaric acid (0.2 to 1.9 mg/100 g d.w. ) and protocatechuic acid has also been confirmed. [14]

Analysis of the six selected species of the genus *Cortinarius* showed that the richest in phenolic acids was *Cortinarius speciosissimus*. In this species determined p-hydroxybenzoic, p-coumaric, protocatechuic, and vanillic acids. The compound identified in all the analyzed species was p-hydroxybenzoic acid [15].

The analysis of the content of phenolic acids in fruiting bodies is an important direction of mycochemical research conducted in the Department of Pharmaceutical Botany at Jagiellonian University Medical College in Kraków. The objects of the studies are edible species, both wild-growing and from crops. The second group is inedible species classified as arboreal mushrooms. Qualitative and quantitative analyses confirmed the presence of a wide range of hydroxybenzoic acid and hydroxycinnamic acid derivatives. In methanolic extracts obtained from edible mushrooms such as *Agaricus bisporus*, *Armillaria mellea*, *Auricularia auricula-judae*, *Boletus edulis*, *Cantharellus cibarius*, *Imleria badia*, *Lactarius deliciosus*, *Leccinum scabrum*, *Pleurotus ostreatus*, *Suillus bovinus*, *Suillus luteus*, *Tricholoma equestre*, the presence of protocatechuic acid, p-hydroxybenzoic acid, syringic acid, and gallic acid has been confirmed [16].

The presence of p-hydroxybenzoic, protocatechuic, and vanillic acids was confirmed in the extracts of the arboreal species (*Fomitopsis pinicola*, *Geophyllum sepiarium*, *Laetiporus sulphureus*, *Dedalepsis confragosa*). The content of the individual compounds varied and ranged from 1.1 to 9.1 mg/100 g d.w. The predominant phenolic acid

was protocatechuic acid, the content of which ranged from 1.8 mg/100 g d.w. in *Laetiporus sulphureus* to 9.2 mg/100 g d.w. in *Fomitopsis pinicola*. There was less content of p-hydroxybenzoic acid (from 1.1 mg/100 g d.w. in *Fomitopsis pinicola* to 3.1 mg/100 g d.w. in *Geophyllum sepiarium*) and vanillic acid (from 1.2 mg/100 g d.w. in *Dedalepsis confragosa* up to 1.4 mg/100 g d.w. in *Fomitopsis pinicola*) [17].

Analysis of selected species of the genus *Phellinus* confirmed the presence of 3,4-dihydrophenylacetic acid, gallic acid, protocatechuic acid and, syringic acids. The total phenolic acid content ranged from 9.9 mg/100 g d.w. (*Phellinus igniarius*) to 32.6 mg/100 g d.w. (*Phellinus robustus*). The content of individual compounds ranged from 0.9 (syringic acid in *Phellinus robustus*) to 26.7 mg/100 g d.w. (3,4-dihydrophenylacetic acid in *Phellinus robustus*). Protocatechuic acid was determined in all analyzed species [18].

In fruiting bodies of another sample of medicinal mushrooms *Fomitopsis betulina* syringic acid, gallic acid, p-hydroxybenzoic and 3,4-dihydrophenylacetic acid were determined [19].

The concentration of phenolic compounds in fruiting bodies depends on many factors, i.a. environmental conditions, phase of development of fruiting bodies, storage, processing, UV radiation, infection by pathogens, injury, pollution and air temperature [20].

### **The importance of phenolic acids in mushroom fruiting bodies**

According to the knowledge, the biochemistry of fungi is more akin to animal chemistry than to plants. The presence of phenolic acids in fungi – characteristic plants compounds is an exception to the above-mentioned thesis. These compounds are important for the survival of the sporocarps, as evidenced by the various biogenetic pathways leading to their synthesis.

The biological activity of phenolic acids is determined by the number of hydroxyl groups

and their position in the aromatic ring [21].

Among the directions of biological activity, the antioxidant effect deserves special attention. The mechanism of this action is mainly based on the neutralization of free radicals and the chelation of metal ions. Phenolic acids with significant antioxidant activity include ferulic, chlorogenic, or caffeic acids. Extensive studies on the biological activity of this group of compounds have also confirmed their antitumor effect (ellagic, gallic, ferulic acids) immunostimulant effect (chlorogenic acid), anti-inflammatory effect (caffeic, ferulic acids) or cytotoxic activity [21,22].

Many phenolic compounds possess antibacterial activity or inhibit the growth of other microorganisms. The biosynthesis of protective compounds against pathogens by fungi is presumably due to the inability of these organisms to move. Presumably, like in plants, phenolic acids perform a defensive function against parasites and microbes.

Their insecticidal, antibacterial, anti-mold, and anti-microfungal properties are known. Besides, phenolic acids are substrates of polymeric structures such as tannins [4,21,22]. The antiviral effect of gallic acid [20] has also been confirmed [4,22].

Another role of phenolic acids in arboreal species of mushrooms is connected with the production of the enzymes. During wood decomposition processes, on which arboreal fungi are found, catalyzed by many enzymes, i.e. ligninase, hemicellulose, cellulose, oxidase, and peroxidase (lignin peroxidase – LIP, Mn- peroxidase – MnP) there is an increased production of free oxygen radicals, which induce the process of self-oxidation of fatty acids. Probably to avoid oxidative damage, arboreal fungi in the process of evolution have developed the ability to synthesize antioxidants, the main representatives of which are phenolic acids. Many phenolic compounds may play the role of natural substrates for enzymes such as polyphenolic oxidase and peroxidase, present in large quantities in fungi [4,20-22].

Table 1. Chemical structure of phenolic acids, derivatives of hydroxybenzoic and cinnamic acid

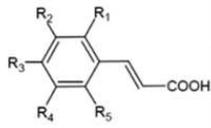
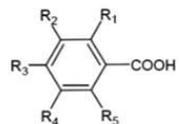
	Basic structure	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	Name of compounds
Derivatives of hydroxycinnamic acid		H	H	OH	H	H	<i>p</i> -Coumaric v acid
		H	OH	OH	H	H	Caffeic acid
		H	OCH <sub>3</sub>	OH	H	H	Ferulic acid
		H	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	H	Synapinic acid
	Basic structure	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	Name of compounds
Derivatives of hydroxybenzoic acid		OH	H	H	H	H	Salicylic acidity
		H	H	OH	H	H	<i>p</i> -Hydroxybenzoic
		H	OH	OH	H	H	Protocatechuic
		H	OCH <sub>3</sub>	OH	H	H	Vanillic
		H	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	H	Syryngic
		H	OH	OH	OH	H	Gallic
		H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	Veratric
		OH	H	H	OH	H	Gentisic

Table 2. The occurrence of phenolic acids and cinnamic acid in selected higher fungi

Phenolic acid	Species of higher fungi	References
<b>Protocatechiuc acid</b>	<i>Agaricus bisporus</i> , <i>Agaricus blazei</i> , <i>Auricularia polytricha</i> , <i>Calocybe gambosa</i> , <i>Cortinarius orellanus</i> , <i>Cortinarius rubellus</i> , <i>Flammulina velutipes</i> , <i>Ganoderma applanatum</i> , <i>Ganoderma lucidum</i> , <i>Gomphus clavatus</i> , <i>Helvella crispa</i> , <i>Hydnum repandum</i> , <i>Inonotus obliquus</i> , <i>Lactarius deliciosus</i> , <i>Lactarius sangifluus</i> , <i>Laetiporus sulphureus</i> , <i>Lentinus edodes</i> , <i>Lentinus sajor-caju</i> , <i>Lentinus squarrosuls</i> , <i>Lepista nuda</i> , <i>Macrolepiota procera</i> , <i>Meripilus giganteus</i> , <i>Morchella anguiticeps</i> , <i>Morchella conica</i> , <i>Panus tigrinus</i> , <i>Phellinus igniarius</i> , <i>Phellinus linteus</i> , <i>Pleurotus djamor</i> , <i>Pleurotus eryngii</i> , <i>Pleurotus ostreatus</i> , <i>Pleurotus pulmonarius</i> , <i>Ramaria botrytis</i> , <i>Russula brevipes</i> , <i>Sparassis crispa</i> , <i>Termitomyces heimii</i> , <i>Termitomyces mammiformis</i> , <i>Termitomyces microcarpus</i> , <i>Termitomyces shimperi</i> , <i>Termitomyces tylerance</i> , <i>Trametes versicolor</i>	[5,6,10,12 15]
<b>p-Hydroxybenzoic acid</b>	<i>Agaricus arvensis</i> , <i>Agaricus bisporus</i> , <i>Agaricus romagnesii</i> , <i>Agaricus silvicola</i> , <i>Amanita rubescens</i> , <i>Armillaria mellea</i> , <i>Calocybe gambosa</i> , <i>Clitocybe odora</i> , <i>Coprinus comatus</i> , <i>Cortinarius orellanus</i> , <i>Cortinarius rubellus</i> , <i>Grifola frondosa</i> , <i>Inonotus obliquus</i> , <i>Lactarius deliciosus</i> , <i>Lentinula edodes</i> , <i>Lepista nuda</i> , <i>Lycoperdon molle</i> , <i>Phellinus linteus</i> , <i>Ramaria botrytis</i> , <i>Sarcodon imbricatus</i> , <i>Sparassis crispa</i> , <i>Tricholoma acerbum</i> , <i>Tricholoma equestre</i>	[6,9–11,13–15]
<b>Vanillic acid</b>	<i>Agaricus bisporus</i> , <i>Auricularia polytricha</i> , <i>Boletus edulis*</i> , <i>Cortinarius orellanus</i> , <i>Cortinarius rubellus</i> , <i>Gomphus clavatus</i> , <i>Helvella crispa</i> , <i>Hydnum repandum</i> , <i>Inonotus obliquus</i> , <i>Lactarius deliciosus*</i> , <i>Lactarius sangifluus</i> , <i>Lentinus sajor-caju</i> , <i>Lentinus squarrosuls</i> , <i>Lycoperdon molle</i> , <i>Macrolepiota procera</i> , <i>Morchella anguiticeps*</i> , <i>Morchella conica</i> , <i>Pleurotus djamor</i> , <i>Pleurotus pulmonarius</i> , <i>Russula brevipes</i> , <i>Sparassis crispa</i> , <i>Termitomyces heimii</i> , <i>Termitomyces mammiformis*</i> , <i>Termitomyces microcarpus</i> , <i>Termitomyces shimperi</i> , <i>Termitomyces tylerance*</i> , <i>Tricholoma acerbum</i>	[9,10,12,13,15]

<b>Gallic acid</b>	<i>Agaricus bisporus, Agaricus blazei, Auricularia polytricha, Boletus edulis*, Cantharellus cibarius, Flamulina velutipes, Ganoderma applanatum, Ganoderma lucidum, Gomphus clavatus, Helvella crispa, Hydnum repandum, Lactarius deliciosus, Lactarius sangifluus, Laetiporus sulphureus, Lentinula edodes, Lentinus sajor-caju, Lentinus squarrosuls, Macrolepiota procera, Meripilus giganteus, Morchella anguiticeps, Morchella conica, Phellinus linteus, Pleurotus djamor, Pleurotus eryngii, Pleurotus ostreatus, Pleurotus pulmonarius, Russula brevipes, Sparassis crispa, Sparassis crispa, Termitomyces heimii, Termitomyces mammiformis, Termitomyces microcarpus, Termitomyces shimperi, Termitomyces tylerance, Trametes versicolor</i>	[5,12,13]
<b>p-Coumaric acid</b>	<i>Agaricus arvensis, Agaricus bisporus, Agaricus silvicola, Auricularia polytricha*, Boletus edulis, Calocybe gambosa, Cantharellus cibarius, Clitocybe odora, Coprinus comatus, Cortinarius speciosissimus, Fistulina hepatica, Ganoderma applanatum, Gomphus clavatus*, Helvella crispa*, Lactarius deliciosus*, Lactarius sangifluus, Lentinus sajor-caju, Lentinus squarrosuls, Lepista nuda, Macrolepiota procera, Morchella anguiticeps*, Morchella conica, Pleurotus djamor, Pleurotus pulmonarius*, Russula brevipes, Sparassis crispa, Termitomyces heimii, Termitomyces mammiformis*, Termitomyces microcarpus*, Termitomyces tylerance*</i>	[5,7-10,12-15]
<b>Caffeic acid</b>	<i>Agaricus bisporus, Auricularia polytricha*, Boletus edulis, Cantharellus cibarius, Fistulina hepatica, Flamulina velutipes, Ganoderma applanatum, Gomphus clavatus, Grifola frondosa, Helvella crispa, Lactarius deliciosus*, Lactarius sangifluus, Lentinus edodes, Lentinus sajor-caju, Lentinus squarrosuls, Macrolepiota procera, Morchella anguiticeps, Morchella conica, Phellinus linteus, Pleurotus djamor, Pleurotus pulmonarius*, Russula brevipes*, Sparassis crispa, Termitomyces heimii, Termitomyces mammiformis*, Termitomyces microcarpus, Termitomyces tylerance, Trametes versicolor</i>	[5-9,12,13]
<b>t-Cinnamic acid</b>	<i>Agaricus arvensis, Agaricus bisporus, Agaricus bisporus, Agaricus blazei, Agaricus</i>	[6,9,10,12-14]

<b><i>t</i>-Cinnamic acid</b>	<i>Agaricus arvensis, Agaricus bisporus, Agaricus bisporus, Agaricus blazei, Agaricus romagnesii, Agaricus silvicola, Armillaria mellea, Boletus edulis*, Calocybe gambosa, Cantharellus cibarius, Clitocybe odora, Coprinus comatus, Gomphus clavatus, Grifola frondosa, Helvella crispa*, Hydnum repandum, Lactarius deliciosus*, Lactarius sangifluus, Lentinula edodes, Lentinus sajor-caju*, Lentinus squarrosulus, Lycoperdon perlatum, Macrolepiota procera*, Pleurotus djamor, Pleurotus pulmonarius, Russula brevipes*, Sparassis crispa, Termitomyces heimii, Termitomyces mammiformis, Termitomyces microcarpus</i>	[6,9,10,12-14]
<b>Ferulic acid</b>	<i>Boletus edulis, Cantharellus cibarius, Flamulina velutipes, Gomphus clavatus, Helvella crispa*, Inonotus obliquus, Lactarius deliciosus*, Lactarius sangifluus, Lentinus sajor-caju*, Lentinus squarrosulus, Macrolepiota procera, Morchella anguiticeps*, Pleurotus djamor, Pleurotus pulmonarius*, Russula brevipes*, Sparassis crispa, Termitomyces heimii, Termitomyces mammiformis*, Termitomyces microcarpus, Termitomyces shimperi</i>	[12,13]
<b>Gentisic acid</b>	<i>Agaricus blazei, Auricularia polytricha, Boletus edulis*, Gomphus clavatus, Helvella crispa, Hydnum repandum, Lactarius deliciosus, Lactarius sangifluus, Lentinus sajor-caju*, Lentinus squarrosulus, Macrolepiota procera, Morchella anguiticeps, Morchella conica, Pleurotus djamor, Pleurotus pulmonarius, Russula brevipes, Sparassis crispa, Termitomyces heimii, Termitomyces mammiformis, Termitomyces microcarpus, Termitomyces shimperi, Termitomyces tylerance</i>	[12,13]
<b>Syringic acid</b>	<i>Agaricus blazei, Auricularia polytricha*, Boletus edulis*, Gomphus clavatus, Helvella crispa*, Hydnum repandum, Inonotus obliquus, Lactarius deliciosus*, Lactarius sangifluus, Lentinus sajor-caju, Macrolepiota procera, Morchella anguiticeps, Pleurotus djamor, Pleurotus pulmonarius*, Russula brevipes, Sparassis crispa, Termitomyces heimii*, Termitomyces mammiformis, Termitomyces microcarpus, Termitomyces tylerance,</i>	[12,13]
<b>Chlorogenic acid</b>	<i>Cantharellus cibarius, Flamulina velutipes, Phellinus linteus, Pleurotus ostreatus</i>	[8,13]
<b>Veratric acid</b>	<i>Sparassis crispa</i>	[13]
<b>Elagic acid</b>	<i>Fistulina hepatica</i>	[7]

\* – trace amounts  
 Own study

## Resumo

*Fenolaj acidoj konsistigas kemie diversspecan grupon de bioaktivaj komponaĵoj. La efiko de fenolaj acidoj rilatas al la ĉeesto de hidrosikarboksilaj grupoj kaj ilia pozicio sur la aroma ringo. Ĉi tiuj estas komponaĵoj ofte trovitaj en naturo - ili ĉeestas en multaj specioj de plantoj kaj fungoj. En la mondo de pli altaj fungoj (en senco pli komplikaj, ne mikroskopiaj), ili konsistigas la plej multan grupon inter fenolaj komponaĵoj. La plej oftaj fenolaj acidoj en la mondo de fungoj estas kafeika acido, galacido, genticacido, ferula acido, p-kumara acido, p-hidroksibenzoa acido, protokateĥua acido, vanila acido, kaj komponaĵo kun strukturo simila al fenolaj acidoj – t-cinamika acido. Fenolaj acidoj troveblaj en fruktkorpoj de fungoj montras, interalie, antioksidajn, antimikrobajn kaj kontraŭkancerajn proprecojn.*

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