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HPLC-DAD ANALYSIS, AND DETERMINATION OF ANTIOXIDANT, ANTICHOLINESTERASE AND ANTIDIABETIC ACTIVITIES OF *Polyporus squamosus* (Huds.) Fr.

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Abstract

Polyporus species is a valuable species of the Polyporaceae family with defined bioactive properties among medicinal mushrooms. This study was undertaken to investigate the antioxidant, anticholinesterase and antidiabetic activities of Polyporus squamosus (Huds.) Fr. with characterization of phenolic profile by HPLC-DAD. Fumaric acid (190.07±0.08 µg/g), catechin hydrate (46.96±0.02 µg/g), ferulic acid (21.98±0.03 µg/g), trans-cinnamic acid (16.66±0.02 µg/g), and protocatechuic acid (13.29±0.06 µg/g) were detected as the most abundant compounds in P. squamosus by HPLC-DAD. P. squamosus methanol extract exhibited higher antioxidant activity than the hexane extract in β-carotene linoleic acid (IC_{50}: 73.75±0.28 µg/mL), DPPH• scavenging (7.56±0.00% inhibition at 400 µg/mL), $ABTS^{•+}$ scavenging (IC_{50}: 154.30±0.55 µg/mL), and metal chelating (35.61±1.20% inhibition at 400 µg/mL) assays. P. squamosus hexane extract was determined as the stronger inhibitor than the methanol extract against all enzymes with inhibition values of 39.48±0.45% on AChE at 200 µg/mL, 28.02±1.34% on BChE at 200 µg/mL, 96.70±0.80% on α-amylase at 1000 µg/mL, and 39.93±0.52% on α-glucosidase at 500 µg/mL. The present study highlighted the importance of P. squamosus as a promising source of valuable therapeutic compounds that could be considered as an alternatives to synthetic drugs.

Keywords: *Polyporus squamosus*, Phenolic profile, HPLC-DAD, Antioxidant activity, Anticholinesterase activity, Antidiabetic activity

Polyporus squamosus (Huds.) Fr. MANTARININ HPLC-DAD ANALİZİ, ANTİOKSİDAN, ANTİKOLİNESTERAZ VE ANTİDİYABETİK AKTİVİTELERİ

Özet

Polyporus türleri, tıbbi mantarlar arasında tanımlanan biyoaktif özellikleri ile Polyporaceae familyasının değerli bir türüdür. Bu çalışma, Polyporus squamosus (Huds.) Fr. mantarının antioksidan, antikolinesteraz ve antidiyabetik aktiviteleri ile HPLC-DAD ile fenolik profilinin karakterizasyonu araştırmak için yapılmıştır. Fumarik asit (190,07±0,08 µg/g), kateşin hidrat (46,96±0,02 µg/g), ferulik asit (21,98±0,03 µg/g), trans-sinnamik asit (16,66±0,02 µg/g) ve protokateşik asit (13,29±0,06 µg/g) HPLC-DAD ile P. squamosus'ta en bol bulunan bileşikler olarak tespit edilmiştir. P. squamosus metanol ekstresi β-karoten linoleik asit (IC_{50}: 73,75±0,28 µg/mL), DPPH• giderim (400 µg/mL'de %7,56±0,00 inhibisyon), $ABTS^{•+}$ giderim (IC_{50}: 154,30±0,55 µg/mL) ve metal kelatlama (400 µg/mL'de %35,61±1,20 inhibisyon) yöntemlerinde hekzan ekstresinden daha yüksek antioksidan aktivite göstermiştir. P. squamosus hekzan ekstresi AChE enzimine karşı 200 µg/mL'de %39,48±0,45, BChE enzimine karşı 200 µg/mL'de %28,02±1,34, α-amilaz enzimine karşı 1000 µg/mL'de %96,70±0,80 ve α-glukozidaz enzimine karşı 500 µg/mL'de %39,93±0,52 inhibisyon değerleri ile metanol ekstresinden daha güçlü bir inhibitör olarak belirlenmiştir. Mevcut çalışma, P. squamosus'un sentetik ilaçlara alternatif olarak değerlendirilebilecek değerli terapötik bileşiklerin umut verici bir kaynağı olarak önemini vurgulamıştır.

Anahtar Kelimeler: *Polyporus squamosus*, Fenolik profil, HPLC-DAD, Antioksidan aktivite, Antikolinesteraz aktivite, Antidiyabetik aktivite

Cite

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1. Introduction

Mushrooms, which have survived on earth for centuries, are the indispensable part of both health and world cuisine. Mushrooms are used as a source for the preparation of both nutraceuticals and drugs. In addition to their essential amino acids, fatty acids, vitamins, iron, phosphorus, ascorbic acid, and low-fat composition with their special texture and aroma, mushrooms are

important components of nutrition and cuisine [1]. Various studies have described that mushrooms have antitumor, antiviral, antithrombotic, antiviral, antioxidant, antibacterial, antifungal, anti-parasitic, antiosteoporotic, anti-cancer, wound healing, immunomodulating, hepatoprotective, anti-diabetic, cardiovascular, immune-modulating, anti-hypercholesterolemia, and detoxification properties through functional components (polysaccharides, polysaccharide-protein complexes, steroids, peptides, terpenoids and phenolic compounds) [2,3]. Phenolic compounds are minor and unique metabolites of mushroom species. The phenolic compounds in mushrooms include phenolic acids, flavonoids, tannins, tocopherols, and other phenolic compounds. Within the scope of their advantages for human health, these bioactive components are more targeted as functional foods and nutraceutical agents to provide better health conditions. It has been proven that these phenolics in the mushrooms are the basis of many bioactivities [4]. Therefore, extraction of phenolic compounds is the primary step in the preparation of dietary supplements or nutraceuticals, pharmaceuticals, food ingredients, and cosmetics and to replace/complement synthetic or industrial products with bioactive compounds [5].

Polyporus genus (Polyporaceae family) is manifested as an invaluable member of the class of medicinal mushrooms with various activities such as anti-cancer, nephroprotective, anti-inflammatory, hepatoprotective, immune-enhancing, antioxidant, hair-growing, and antimicrobial activities [6]. *Polyporus squamosus* (Huds.) Fr., which is pronounced as ''dryad's saddle'' or ''pheasant's back mushroom'' among the people, is a fast-growing mushroom that can be seen on the stumps, dead and living stems of deciduous tree species. Especially in the early growth stage, it is as valuable as meat in terms of delicious and nutritive value and is often used as a spice [7]. Moreover, it was reported that the bioactive properties of *P. squamosus* consist of immunomodulating, antibacterial, antifungal, anti-biofilm, antioxidant, and anti-quorum sensing effects [8].

Diabetes mellitus is a life-threatening multifactorial metabolic disease characterized mainly by high blood glucose levels. According to the World Health Organization (WHO), diabetes mellitus is seen in approximately 422 million people worldwide and causes 1.6 million deaths. Again to WHO, it is estimated that there will be 570 million individuals diagnosed with diabetes mellitus in 2030 and 700 million in 2045 [9]. According to the International Diabetes Federation, 463 million people live with the diagnosis of diabetes mellitus [10]. Diabetes mellitus and its complications have significant effects on the health system and the country's economy in addition to human life. Anti-diabetic mechanism of action consists of six basic groups: change of glucose metabolism (α-amylase and α-glucosidase inhibition); hypolipidemic effect; pancreatic effect; antioxidative effect; treatment of diabetes complication; insulin-like effect. The most common of this list is the change of glucose metabolism with α-amylase and α-glucosidase [11]. Today, there are commercial drugs that have serious side effects and cannot change the complications of the disease in the control of diabetes [12]. In recent years, mushrooms have become an essential target for new sources of possible anti-diabetic effects, as they are recognized as a reservoir of natural bioactive compounds.

Alzheimer's disease (AD) is a disease that deforms brain cells and is mostly characterized by dementia. Advancing age, head traumas, genetic factors, environmental factors, infections, and vascular diseases are rated as risk factors for the disease. It is estimated that there are nearly 50 million AD patients worldwide, and this number will be twice every 5 years to reach 152 million in 2050. Currently, there are two categories of drugs (cholinesterase enzyme inhibitors and *N*-methyl-D-aspartate (NMDA) antagonists) approved for AD treatment. Although there are treatment methods that improve symptoms in treating AD, this disease is still in the category of incurable diseases that cannot be fully treated [13, 14]. Accordingly, natural sources represent an important potential for researchers in the treatment of AD, with more promising properties than conventional synthetic groups that can target or modulate multiple pathways [15]. The living organism has a defense system that includes a complex combination of non-enzymatic and natural enzymatic antioxidants that fight the damaging impacts of other oxidants and free radicals. Free radicals are an underlying factor in many diseases such as neural disorders, cardiovascular disease, AD, aging, diabetes mellitus, cancer, Parkinson's disease, atherosclerosis, ulcerative colitis, and alcohol-induced liver disease. Significant findings have revealed that the intake of dietary antioxidants or foods containing nutrients with possible antioxidant properties is an important force in the fight against free radicals. In addition, there is a common agreement among scientists that consuming antioxidants in different combinations rather than consuming them individually will be more effective in the long run [16, 17].

To date, there have been many reports documenting the efficacy of bioactive metabolites isolated and extracted from mushrooms, which are successful in the treatment of diabetes mellitus, AD, and oxidative-stress based diseases. Therefore, it was aimed to profile the phenolic and organic acid of *Polyporus squamosus* (Huds.) Fr. mushroom by HPLC-DAD and investigate antioxidant and enzyme inhibition activities.

2. Materials and Method

2.1. Mushroom Material and Extraction

Polyporus squamosus (Huds.) Fr. was harvested from Fethiye district of Muğla, Turkey in 2020, characterized at the Mushroom Application and Research Center in Muğla Sıtkı Koçman University. Powdered *P. squamosus* samples were first extracted with *n*-hexane at room temperature conditions. Subsequently, the residue (completely dried) was extracted with methanol at room

temperature conditions. The rotary evaporator was used for evaporating *n*-hexane and methanol solvents to get the mushroom extracts and both extracts were kept at +4°C for advance analysis.

2.2. Phenolic Composition

Phenolic composition of *P. squamosus* was identified using high performance liquid chromatography (HPLC) as explained by Barros et al. with slight changes [18, 19]. The detection was practiced by diode array detector (DAD) using 280 nm as the wavelength. UV data and retention times were compared with commercial standards for the characterization of the compounds. The calibration chart obtained because of injection of known concentrations of different standard compounds (fumaric acid, coumarin, catechin hydrate, gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillin, caffeic acid, *p*-coumaric acid, 6,7-dihydroxy coumarin, 2,4-dihydroxybenzoic acid, rosmarinic acid, ferulic acid, *trans*-cinnamic acid, ellagic acid, *trans*-2-hydroxy cinnamic acid) was used to identify and quantify the compounds. The analysis was repeated three times. The results were given as µg per g of dry weight (dw).

2.3. Antioxidant Activity

Five different assays comprising of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging, cupric reducing antioxidant capacity (CUPRAC), β-carotene linoleic acid, and metal chelating assays were used to test antioxidant activity [20]. BHA, α-tocopherol, and EDTA were used as the standards. The results were presented as inhibition percentages (%) and absorbance at 400 µg/mL concentration, IC_{50} (defined as the extract concentration needed to have 50% inhibition), and $A_{0.50}$ (defined as the extract absorption needed to have 50% inhibition) values.

2.4. Enzyme Inhibition Activity

Four different enzymes including acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-amylase, and α-glucosidase were used to test enzyme inhibition activity [21]. Acarbose and galantamine were used as the standards. The results were presented as inhibition percentages (%) (at 200 µg/mL concentration for AChE and BChE, at 1000 µg/mL concentration for α-amylase and at 500 µg/mL concentration for α-glucosidase).

2.5. Statistical Analysis

Data were recorded as the mean ±S.E. in the results shown as the mean of three repetitions. Significant differences between the means were observed according to Student's t test and were considered significant in case of p values <0.05.

3. Results and Discussion

3.1. Phenolic Composition

Among the alkaloids, flavonoids, terpenes, phenolics, steroids, saponins and glycosides categorized as primary components, phenolic compounds are the most noted and researched components. It is essential to identify phenolic compounds that act as a bridge between the food and pharmaceutical industries with their excellent health-protective effects such as stimulating the immune system, slowing, or inhibiting cancer formation, reducing oxidation, reducing inflammation, triggering apoptosis, and preventing DNA damage [22]. HPLC-DAD was used to screen 16 phenolic and organic acid compounds in *P. squamous*. The chromatograms of the standards and *P. squamous* are given in Figures 1 and 2. Table 1 lists the screened and identified compounds. The presence of 10 of 16 investigated compounds was characterized in *P. squamous. P. squamous* was found as rich in fumaric acid (190.07±0.08 µg/g), catechin hydrate (46.96±0.02 µg/g), ferulic acid (21.98±0.03 µg/g), *trans*-cinnamic acid (16.66±0.02 µg/g), and protocatechuic acid (13.29±0.06 µg/g). The low amounts of *p*-coumaric acid (0.01±0.01 µg/g) and *p*-hydroxybenzoic acid (0.01±0.01 µg/g) were also detected. Fumaric acid is the most depicted organic acid in mushrooms and its bioactivities such as analgesic, antioxidant, and anti-inflammatory have been evidenced [23]. Catechin is an important active ingredient with proven anti-cancer, anti-allergen, antioxidant, anti-inflammatory, and anti-viral effects [24]. The well-known bioactive properties of ferulic acid were revealed to consist of antioxidant, anti-diabetic, anti-inflammatory, anti-apoptotic, anti-platelet, vascular endothelial protection, and anti-cancer activities [25]. Many studies reported that protocatechuic acid is antioxidant, antiulcer, anti-aging, anti-cancer, and antibacterial compound [26]. *Trans*-cinnamic acid was stated as an antimicrobial, antioxidant, anti-cancer, antidiabetic, neuroprotective, and anti-inflammatory agent [27].

Table 1. Phenolic composition of *P. squamosus*[a]

Compounds	RT[b] (min)	Concentration (µg/g)
Fumaric acid	3.86	190.07±0.08
Gallic acid	5.66	2.51±0.01
Protocatechuic acid	8.88	13.29±0.06
p-Hydroxybenzoic acid	12.18	0.01±0.01
Catechin hydrate	13.22	46.96±0.02
6,7-Dihydroxy coumarin	14.60	nd
2,4-Dihydroxybenzoic acid	15.09	nd
Caffeic acid	15.42	6.29±0.01
Vanillin	16.33	nd
p-Coumaric acid	18.39	0.01±0.01
Ferulic acid	19.21	21.98±0.03
Coumarin	19.78	2.72±0.03
trans-2-Hydroxycinnamic acid	20.67	nd
Ellagic acid	21.41	nd
Rosmarinic acid	22.12	nd
trans-Cinnamic acid	22.78	16.66±0.02

[a] Values represent the means ±S.E. of three repeats ($p < 0.05$), [b] Retention time, nd: not detected.

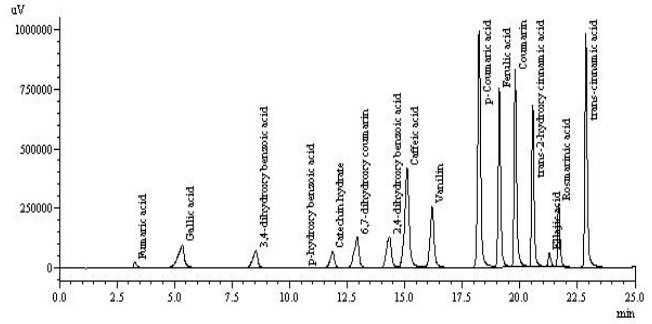

Figure 1. The HPLC-DAD chromatogram of standards

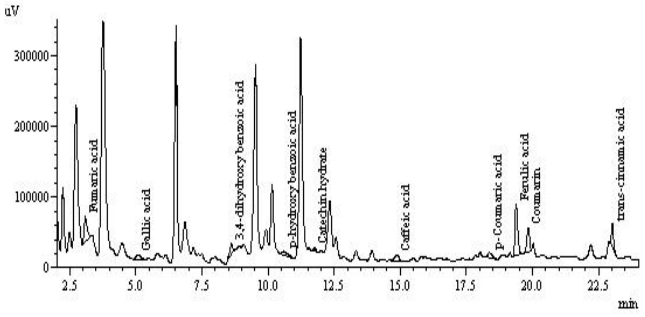

Figure 2. The HPLC-DAD chromatogram of *P. squamosus*

A rare number of studies have been found in the literature about the chemical characterization of *P. squamosus* collected from different localities in terms of phenolic and organic acid profiles. Considerable amounts of *p*-hydroxy benzoic acid (2.03±0.05 mg/100 g), *p*-coumaric acid (0.272±0.002 mg/100 g), cinnamic acid (0.037±0.001 mg/100 g) with low amounts of fumaric acid (0.0003±0.0001 g/100 g) were found in *P. squamosus* (from Romania) [8]. In another study, the levels of *p*-hydroxy benzoic acid, fumaric acid, and cinnamic acid were respectively recorded as 1.29±0.01 mg/100 g, 0.21±0.01 g/100 g, and 0.04±0.01 mg/100 g in *P. squamous* from Portugal and 0.63±0.03 mg/100 g, 0.41±0.01 g/100 g, and 0.13±0.01 mg/100 g in *P. squamous* from Serbia [28]. In the study on four different species of *Trametes* which are the members of the Polyporaceae family, *trans*-cinnamic acid (0.49±0.05 µg/g) was determined to be a major compound in *T. pubescens,* fumaric acid (4.51±0.10 µg/g) was in *T. bicolor*, catechin hydrate in *T. versicolor* (0.96±0.12 µg/g) and *T. suaveolens* (0.92±0.16 µg/g) [29]. Two *Trametes* species from the Polyporaceae family were investigated for phenolic compounds by Zengin et al. and catechin (84.75±2.40 µg/g), caffeic acid (37.29±0.72 µg/g), and chlorogenic acid (33.90±1.44 µg/g) were detected in *T. gibbosa* methanol extract while catechin (128.47±2.93 µg/g), protocatechuic (33.15±1.17 µg/g), *p*-hydroxybenzoic (78.74±2.93 µg/g), chlorogenic (62.16±2.05 µg/g), caffeic (87.03±2.93 µg/g), benzoic (1263.99±20.51 µg/g), and rosmarinic (207.21±20.51 µg/g) acids were identified in *T. hirsuta* methanol extract [30]. The results here show similarities and differences with prior literature results. There is still no fully suitable extraction technique for obtaining phenolic compounds from the mushrooms. Therefore, different extraction techniques (conventional and unconventional techniques) and conditions (duration of extraction period, light, temperature, pH, solvent/substrate ratio, material size,) which are particularly preferred even in the same mushroom samples, are considered the main effects of these differences [31].

3.2. Antioxidant Activity

The negative impacts of oxidative stress on human health form a global problem. The human body provides additional reactive oxygen species compared to enzymatic and non-enzymatic antioxidants under stress, resulting in cell damage and health problems. The state of antioxidant deficiency, which inhibits reactive free radicals, enables the development of degenerative diseases, including cancers, neurodegenerative diseases, cardiovascular diseases, inflammatory diseases and AD. Supplementing the diet with antioxidant compounds found in natural sources is an alternative solution to this problem [32]. Antioxidant activities of the mushroom extracts were evaluated based on five different *in vitro* assays namely DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging, CUPRAC, metal chelating and β-carotene linoleic acid assays. The results were presented in Table 2. *P. squamosus* methanol extract possessed higher antioxidant activity than the hexane extract in all assays excluding the CUPRAC assay. IC_{50} values were found as 73.75±0.28 µg/mL for the methanol extract and 314.20±0.73 µg/mL for the hexane extract in the β-carotene linoleic acid assay. The methanol extract inhibited 7.56±0.00% of DPPH$^{\bullet}$ and 87.23±1.09% of ABTS$^{\bullet+}$ at 400 µg/mL. When the hexane extract was inactive in the metal chelating assay, the methanol extract displayed a 35.61±1.20% inhibition value at 400 µg/mL. Also, the $A_{0.50}$ value was calculated as 265.38±0.46 µg/mL for the hexane extract in the CUPRAC assay. Characterization by HPLC-DAD revealed that *P. squamosus* is substantial in fumaric, ferulic, *trans*-cinnamic, protocatechuic acids and catechin hydrate which have notable biological properties. It can be thought that the higher antioxidant properties of *P. squamosus*, especially the methanol extract, are associated with these identified phenolic compounds.

It was seen in previous studies that the antioxidant activities of *P. squamosus* originating from different locations were determined. As similar to our findings, *P. squamosus* (from Romania) methanol extract was specified as antioxidant active in ferricyanide/Prussian blue (EC_{50}: 2.35±0.02 mg/mL), DPPH$^{\bullet}$ scavenging (EC_{50}: 8.2±0.1 mg/mL), β-carotene-linoleic acid (EC_{50}: 1.41±0.01 mg/mL), and lipid peroxidation inhibition (EC_{50}: 0.22±0.01 mg/mL) assays [8]. The inhibition values of *P. squamosus* (from Black Sea Region-Turkey) methanol extract were estimated as 82.8% at 180 µg/mL for DPPH$^{\bullet}$ scavenging assay; 98.4% at 100 µg/mL for β-carotene-linoleic acid assay; 78% at 50 µg/mL for superoxide anion scavenging assay; 74.2% at 100 µg/mL for metal chelating assay [33]. Akata et al. stated that *P.*

squamosus (from Anatolia-Turkey) scavenged 95.35±0.10% of DPPH• at 3.46 mg/mL [34]. In a different report, antioxidant activity of *P. squamosus* (from Serbia and Portugal) methanol extracts was investigated. In this study *P. squamosus* methanol extract from Portugal was

Table 2. Antioxidant activities of *P. squamosus* extracts[a]

		P. squamosus		Standards		
		Hexane extract	**Methanol extract**	**α-Tocopherol**	**BHA**	**EDTA**
β-carotene linoleic acid	**Inhibition[b]**	51.57±1.77	85.31±1.99	90.45±0.90	92.95±0.46	NT[e]
	IC_{50}[c]	314.20±0.73	73.75±0.28	2.10±0.08	1.34±0.04	NT[e]
DPPH•	**Inhibition[b]**	5.73±0.68	7.56±0.00	87.30±0.55	87.16±0.63	NT[e]
$ABTS^{•+}$	**Inhibition[b]**	7.03±0.79	87.23±1.09	91.79±0.96	91.10±0.72	NT[e]
	IC_{50}[c]	>400	154.30±0.55	38.74±1.02	11.97±0.90	NT[e]
CUPRAC	**Absorbance[b]**	0.66±0.02	0.19±0.02	1.81±0.10	3.14±0.01	NT[e]
	$A_{0.50}$[c]	265.38±0.46	>400	89.47±0.95	24.34±0.13	NT[e]
Metal chelating	**Inhibition[b]**	NA[d]	35.61±1.20	NT[e]	NT[e]	95.20±0.14

[a] Values represent the means ±S.E. of three parallel sample repeats (p <0.05), [b] Results were expressed at 400 µg/mL concentration, [c] Results were expressed as µg/mL, [d] Not active, [e] Not tested.

recorded as more antioxidant than *P. squamosus* methanol extract from Serbia in ferricyanide/Prussian blue (EC_{50}: 1.27±0.07, 3.53±0.03 mg/mL), DPPH• scavenging (EC_{50}: 8.30±0.12, 13.57±0.19 mg/mL), β-carotene-linoleic acid (EC_{50}: 3.60±0.05, 8.41±0.11 mg/mL), and lipid peroxidation inhibition (EC_{50}: 0.86±0.02, 2.03±0.01 mg/mL) assays [28]. *P. squamosus* (from Serbia) ethanol extract was described as antioxidant active in CUPRAC (15.481±0.132 µg TE/1 mg), total reducing power (0.249±0.007 mg AAE/1 mg), DPPH• scavenging (EC_{50}: ~22 mg/mL), $ABTS^{•+}$ scavenging (EC_{50}: ~10 mg/mL), and ferric-reducing antioxidant power (23.340±0.046 µmol Fe/1 mg) assays [35]. Antioxidant activity of nine different *Polyporus* species (*P. sulphureus, P. gilvus, P. pinicola, P. annosus, P. radiatus, P. fomentarius, P. badius, P. volvatus, P. stevenii*) was studied by β-carotene-linoleic acid (antioxidant activity coefficient: not calculated-3.50±0.56 at 5000 µg/mL), DPPH• scavenging (3.40±0.73-36.94±0.62% at 500 µg/mL), ferrous chelating (not active-23.22±0.04% at 5000 µg/mL), and ferric-reducing antioxidant power (absorbance: 0.089±0.02-0.295±0.01) assays [36].

3.3. Enzyme Inhibition Activity

Enzyme inhibitors occupy an important place in human medicine, constituting an average of half of all marketed drugs. For instance, α-amylase and α-glucosidase inhibitors have been demonstrated as the target mechanism of drug-active substances used in the treatment of diabetes mellitus, and cholinesterase inhibitors in AD treatment. At this stage, the negative effects of synthetic enzyme inhibitors, which are used, accelerated the studies on the discovery of more effective and reliable inhibitors from natural sources [37]. *P. squamosus* hexane and methanol extracts were screened for α-amylase, α-glucosidase, AChE and BChE inhibition activities and the results were expressed in Table 3. *P. squamosus* hexane extract (39.48±0.45%) exhibited two times more inhibition activity than the methanol extract (19.99±0.02%) against AChE at 200 µg/mL. Against BChE, the hexane extract (28.02±1.34%) was found five times better inhibitor than the methanol extract (5.66±1.44%) at 200 µg/mL. The hexane extract (96.70±0.80%) exhibited an excellent inhibitory

Table 3. Enzyme inhibition activities of *P. squamosus* extracts[a]

	P. squamosus		Standards	
	Hexane extract	**Methanol extract**	**Galantamine**	**Acarbose**
AChE[b]	39.48±0.45	19.99±0.02	80.41±0,98	NT[e]
BChE[b]	28.02±1.34	5.66±1.44	82.23±2.67	NT[e]
α-Amylase[c]	96.70±0.80	43.34±0.12	NT[e]	96.60±0.08
α-Glucosidase[d]	39.93±0.52	20.00±0.18	NT[e]	67.01±2.28

[a] Values represent the means ±S.E. of three parallel repeats (p < 0.05), [b] Inhibition % at 200 µg/mL concentration, [c] Inhibition % at 1000 µg/mL concentration, [d] Inhibition % at 500 µg/mL concentration, [e] NT: not tested.

character, showing better α-amylase inhibition activity than acarbose (96.60±0.08%) at 1000 µg/mL. In addition, the hexane extract showed significant α-glucosidase inhibition with an inhibition value of 39.93±0.52% half that of acarbose (67.01±2.28%) at 500 µg/mL. *P. squamosus* hexane extract was determined as the better inhibitor than the methanol extract against all enzymes. In line with the obtained results, the hexane extract (9.14±0.07 mg GALAE/g for BChE, 0.73±0.02 mmol ACAE/g for α-amylase) of *Tricholosporum goniospermum* mushroom was defined as the better enzyme inhibitor than the methanol extract (5.07±0.02 mg GALAE/g for BChE, 0.21±0.01 mmol ACAE/g for α-amylase) [38]. In our earlier study, we displayed that the hexane extracts of *Schizophyllum commune, Omphalotus olearius,* and *Hydnum repandum* mushrooms were more strong

inhibitors in comparison of the methanol extracts in AChE (29.32±0.58-71.58±0.28% for the hexane extracts, 3.96±0.23-12.89±0.26% at 200 µg/mL); BChE (50.41±0.42-67.30±0.15% for the hexane extracts, 16.01±0.51-53.81±0.79% at 200 µg/mL), α-amylase (83.09±0.64-95.75±0.16% for the hexane extracts, 23.99±0.82-29.21±0.13% for the methanol extracts at 1000 µg/mL), and α-glucosidase (23.49±0.32-49.99±0.64% for the hexane extracts, 3.41±0.12-43.54±1.09% for the methanol extracts at 500 µg/mL) inhibition assays [39]. The vast majority of the hexane extracts from mushrooms have been verified to be rich in fatty acids. In a metabolomic study presented by Snowden et al. with 43 individuals, it was emphasized that there was a relationship between the dysregulation of fatty acid metabolism and AD [40]. Although fatty acids are not structurally similar to carbohydrates, they were reported as inhibitors that compete with digestive enzymes. It has been suggested that they can act as an inhibitor by acting on the inhibition of α-amylase and α-glucosidase, especially with the double bonds they contain [41]. The better enzyme inhibition activity of the hexane extract can be attributed to these described effects of fatty acids.

Herein, enzyme inhibition activities of *P. squamosus* were presented for the first time. In previous studies, enzyme inhibition activity studies of different mushroom species belonging to *Polyporus* genus and Polyporaceae family could be reached. Supporting our results, inhibition values of the methanol extracts of AChE at 500 µg/mL were calculated as 24.82±1.39% for *P. gilvus*, 37.61±0.48% for *P. sulphureus,* 27.27±0.95% for *P. annosus*, 14.02±1.50% for *P. radiatus,* 31.44±1.77% for *P. pinicola,* 23.12±0.50% for *P. volvatus,* 15.50±1.82% for *P. fomentarius,* 19.72±1.02% for *P. stevenii*, and 21.10±0.54% for *P. badius* [36]. Anti-diabetic properties of the hexane and methanol extracts of four *Trametes* (*T. pubescens*, *T. bicolor*, *T. versicolor, T. suaveolens,*), a *Fomes* (*F. fomentarius*), and a *Funalia* (*F. trogii*) mushroom species belonging to Polyporaceae family have been stated. In this study, α-amylase inhibition activities of the hexane extracts were recorded in the range of 25.47±0.91-94.52±1.07%, while the methanol extracts were in the range of 39.39±0.31-79.56±0.10% at 1000 µg/mL. α-Glucosidase inhibition values were measured as not active-43.60±0.30% for the hexane extracts and 0.34±0.03-70.39±0.69% for the methanol extracts at 500 µg/mL [42]. Enzyme inhibition activity values of the methanol extracts of two *Trametes* species (*T. gibbosa* and *T. hirsuta*) (the member of Polyporaceae family) were found as 1.87±0.02 mg GALAEs/g, 1.71±0.04 mg GALAEs/g for AChE; 1.60±0.06 mg GALAEs/g, 1.90±0.05 mg GALAEs/g for BChE; 0.05±0.01 mmol ACEs/g, 0.16±0.06 mmol ACEs/g for α-amylase; 0.93±0.08 mmol ACEs/g, 1.18±0.01 mmol ACEs/g for α-glucosidase, respectively [30].

4. Conclusion

In the current study, the phenolic and organic acid profile, antioxidant, anticholinesterase and antidiabetic activities of *P. squamosus* were examined. HPLC-DAD characterization revealed phenolic and organic compounds with the predominance of fumaric acid, catechin hydrate, ferulic acid, *trans*-cinnamic acid, and protocatechuic acid in *P. squamosus.* The methanol extract of *P. squamosus* was found to be more active at varying rates in all antioxidant activity assays (excluding CUPRAC assay). When both extracts showed inhibitory activity against all studied enzymes, *P. squamosus* hexane extract exhibited better enzyme inhibition activity than methanol extract. More importantly, *P. squamosus* hexane extract was observed as the stronger α-amylase inhibitor than acarbose. In conclusion, enzyme inhibition activities of *P. squamosus* extracts were revealed for the first time. The findings presented here suggest that *P. squamosus* can be regarded as a source of antioxidants and enzyme inhibitors (especially α-amylase inhibitors). Although further studies are required to fully elucidate the effect of *P. squamosus* in this area, the first step required in the literature may have been completed with this study.

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