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Nutritional Composition, Antioxidant Activity, Cytotoxicity, and Enzymatic Potential of *Ficus nitida*-Associated *Tomophagus colossus*

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Abstract: A fruiting body of a basidiomycete fungus was discovered growing on chopped *Ficus nitida* tree trunks in the student housing on the Assiut University campus during the course of this inquiry and a normal collecting operation in the Assiut Governorate, Egypt. Following the growth of the basidioma's inner tissue on PDA, fungal mycelial growth was achieved. Internal transcribed spacer region (ITS) sequencing has allowed for the identification of the fungus as *Tomophagus colossus*. On the dry weight basis, chemical analysis of *T. colossus* AUMC 14536 basidioma revealed that it contains 28.81% carbohydrates, 25.34% crude fats, 23.44% crude fibers, 20.64% crude proteins, and 3.02% ash, in addition to potassium, phosphorus, calcium, selenium, iron, and zinc (133.59, 114.46, 6.27, 3.08, 1.28, and 0.73 mg/100 g dry weight, respectively). The total phenolic compounds (39.26 mg/g) and total flavonoids (5.62 mg/g) were also evaluated. The basidioma extract's antioxidant activity was assessed as %DPPH radical scavenging activity with an IC50 of 4.15 µg/mL compared with a 1.89 µg/mL IC50 of ascorbic acid. In solid-state fermentation (SSF), the fungus could ferment broad bean straw, palm leaf hay, rice husk, rice straw, sugarcane bagasse, and wheat bran to produce endoglucanase, exoglucanase, laccase, pectinase, and xylanase in substantial amounts. Specific activity exhibited the highest values for endoglucanase (81.48 U/mg), exoglucanase (114.35 U/mg), pectinase (81.94 U/mg), and xylanase (70.18 U/mg) on the rice husk, while the peak of laccase activity (94.27 U/mg) was gained on bean straw. This is the first assessment of the organism's nutritional value, amino acid content, antioxidant activity, and enzymatic capabilities in Egypt.

Keywords: amino acids; antioxidant; biodiversity; cytotoxicity; *Ficus*; ITS; phenolic; phylogeny; polyporaceae

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1. Introduction

The family Polyporaceae Fr. ex Corda includes polypore fungi that have considerable economic significance. Many species cause significant annual crop losses in many tropical countries [1], and some are of vital importance for medical uses, especially in East Asia [2,3]. *Ganoderma* species are a major group of Polyporaceae [1–3], which develop double-walled basidiospores that differentiate them from other members of this family. Eventually, the term "*Ganoderma*" was used to describe the Costa Rican species *Tomophagus colossus* (Fr.) [4], formerly known as *Polyporus colossus* Fr. [5]. Many scientists [6–10] recognized *Tomophagus colossus* as a synonym for *Ganoderma colossus* (Fr.) C.F. Baker, but many others [6–9] rejected

the genus *Tomophagus*. The exceptionally dense and pale context of the genus *Tomophagus*, which dries to a soft and light consistency, led to its recognition as distinct from *Ganoderma*. Additionally, recent molecular phylogenetic analyses using internal transcribed spacers (ITS) of ribosomal DNA repeats [11] and nearly complete mitochondrial small-subunit ribosomal DNA sequences [12] led to the recognition of *Tomophagus* as a distinct genus.

Although rare, reports of this species have been found in all of the tropics, with the exception of East Africa [8,13]. The first record of *T. colossus* in Vietnam dates back to Patouillard [14]. Patouillard mistakenly identified the species as *Ganoderma obokense*, which was subsequently synonymized with *G. colossus* [6–8,15]. Vietnam is the only place where this endangered species has been found again [4]. In Egypt, the Suez Canal University's Faculty of Agriculture's ornamental farm was the source of the first isolation of *T. colossus* [16].

The members of Polyporaceae have been shown to be pharmacologically active, and their medicinal use has been studied [17,18]. Several new lanostan triterpenes and lactones (colossolactones) from cultivated *T. colossus* Murrill have been identified in recent studies [19,20]. As a consequence, the current study was created to provide *T. colossus*, which has been connected, for the first time, with the *Ficus nitida* tree in Assiut, Egypt. The fungal basidioma underwent chemical investigation, including the assessments of macro- and micronutrients' content, antioxidant activity, amino acid content, and cytotoxicity, as well as the exploitation of some agricultural wastes for enzymes synthesis. Finding this species in Egypt for the first time may have implications for the creation of new bioactive compounds for medical applications and/or extracellular cocktail enzymes that may find application in numerous other industrial domains.

2. Materials and Methods

2.1. Collection and Isolation of Fungal Material

In close proximity to the student residence buildings (55QG + QHF) at Assiut University, Assiut Governorate, Egypt, fruiting bodies on *Ficus nitida* were found. After being severed, the fungal basidiomata were promptly transported to the Assiut University Mycological Center's mycological laboratory. A tiny portion of the inner tissue of the basidioma was excised under sterile conditions and plated on Petri dishes containing potato dextrose agar (PDA) [21]. The plates were then left to develop fungal growth in an incubation period of 10 days at 25 °C. The fungus's pure culture was isolated on PDA plates and preserved as AUMC 14536, frozen mycelia stored at −86 °C and lyophilized cultures, in the culture collection of the Assiut University Mycological Center (AUMC).

2.2. Molecular Identification of the Fungal Sample

The DNA extraction from the fungus was performed following the method described by Moubasher et al. [22], in which 0.2 g of 7-day-old fungal mycelia grown on PDA was grinded and transferred to a 1.5 mL microfuge tube. The isolation of the DNA was performed by CTAB method (800 µL CTAB buffer composed of 3% CTAB, 1.4 M/L NaCl, 0.2% Mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl pH 8.0, and 1% PVP-40). The PCR reaction was carried out using the universal primers ITS1 and ITS4 [23] and SolGent EF-Taq, as described by Al-Bedak and Moubasher [24] and Al-Bedak et al. [25].

2.3. Alignments and Phylogenetic Analyses

DNASTAR (version 5.05) was used to produce the contiguous sequence of *Tomophagus* sp. In this study. From GenBank, sequences of the closest species in the genera Ganoderma and *Tomophagus*, as well as those of the accessible-type specimens, were retrieved. As an outgroup, *Favolus subtropicus* BJFC Cui4292 was employed. Using MAFFT (version 6.861b) and the default settings, the sequences of *Tomophagus* sp. AUMC 14536 and those retrieved from GenBank were aligned together [26]. Alignment gaps and parsimony uninformative characters were optimized by BMGE [27]. Maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic analyses were performed using MEGA X version

10.2.6 [28]. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications [29]. The best optimal model of nucleotide substitution for the ML analyses was determined using Akaike information criterion (AIC), as implemented in Modeltest 3.7 [30]. The phylogenetic tree was drawn and visualized using MEGA X (version 10.2.6) [28], and the resulting phylogenetic tree was edited and saved as TIF file [25].

2.4. Macro- and Micronutrient Analyses of Tomophagus colossus Basidioma

All chemical analyses were performed at the Central Laboratories Unit, Faculty of Agriculture, Assiut University, Assiut, Egypt. Prior to chemical analyses, *T. colossus* basidioma was oven-dried at 50 ± 1 °C for 36 h and then grinded to fine powder. Nutrient composition of the *T. colossus* basidioma was determined using the standard methods of the association of official analytical chemists' procedures (AOAC). All values were expressed in mg/100 g dry weight. Triplicate samples were used for moisture content in a hot-air-circulating oven. Ash was determined by incineration (550 °C) of the known weight of the sample in a muffle furnace (Method No 930.05). Crude fat was determined by exhaustively extracting a known weight of the sample in petroleum ether (boiling point, 40 °C to 60 °C) using a Soxhlet extractor (Method No 930.09). Protein (N × 6.25) was determined by the Kjeldahl method (Method No 978.04). Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide (Method No. 930.10). Mineral content was determined using the atomic absorption amanitas (Perkin–Elmer, Model 3300, Encino, CA, USA). Total phenolic compounds was determined by the Folin–Ciocalteau reagent method using Gallic acid as standard [31,32]. The total flavonoids were determined using the technique described by [33]. The carbohydrate content (%) was determined by difference. The addition of all the percentages of moisture content (MC), fat content (FC), ash content (AC), crude fiber (CF), and crude protein (CP) was subtracted from 100% according to Equation (1)

$$\%\text{Carbohydrate} = 100 - (\%\text{MC} + \%\text{FC} + \%\text{AC} + \%\text{CF} + \%\text{CP}) \quad (1)$$

2.5. Antioxidant Activity of Tomophagus colossus's Basidioma

This test was carried out at Nawah Center for Scientific Services (www.nawah-scientific.com, accessed on 16 September 2023), Al-Moqattam, Cairo, Egypt. The antioxidant activity was determined by the colorimetric technique [34,35] using the radical scavenging activity of a freshly made methanol solution containing 0.004% (*w*/*v*) of 2, 2′-Diphenyl-1-picrylhydrazyl (DPPH). A total of 1 mL of this solution was added to 3 mL of various concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) of *T. colossus* extract in ethanol. The mixture was vigorously mixed and left to stand for 30 min at room temperature (30 °C). UV–VIS Milton Roy spectrophotometer (Milton Roy, Spectronic 1201; Markham, ON, Canada) was used to determine the absorbance at 517 nm. The experiment was carried out in triplicate, with ascorbic acid serving as the reference chemical. Using a log dosage inhibition curve, the sample's IC 50 value—the amount of sample needed to block 50% of the DPPH free radical—was determined. Higher levels of free radical activity were indicated by the reaction mixture's lower absorbance. Estimates were made of the DPPH radical's percentage of inhibition [36] according to Equation (2).

$$\%\ \text{Inhibition} = \frac{[\text{Ac} - \text{At}]}{\text{Ac}} \times 100 \quad (2)$$

where Ac = absorbance of the control at zero time, and At = absorbance of the antioxidant at 1 h.

2.6. Amino Acids Content of T. colossus Basidioma

2.6.1. Preparation of Standard Stock Solution

A stock solution of 17 amino acids, namely aspartic acid, glutamic acid, threonine, serine, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyro-

sine, phenylalanine, histidine, lysine, and arginine, was prepared at a concentration of 2.5 µmol/mL, except Cystine, which was prepared at 1.25 µmol/mL.

2.6.2. Sample Hydrolysis

Glass ampoules were filled with approximately 30 mg of the defatted material. Nitrogen gas was introduced to the samples along with 7.0 mL of 6 M HCl to remove any remaining oxygen. The glass ampoules were sealed using a Bunsen flame and baked for 22 h at 105 ± 5 °C. Humins were eliminated by filtering the contents after the ampoule was allowed to cool. After that, the filtrate was vacuum-sealed and dried at 40 °C in a rotating evaporator. Before being used, the residue was dissolved in 5 mL of pH 2.0 acetate buffer and kept in a deep freezer.

2.6.3. Analysis of Amino Acids Content

About 2.5 g of the basidioma sample were weighed into the extraction thimble, and fat was extracted with a chloroform/methanol (2:1, *v*/*v*) mixture using Soxhlet apparatus (AOAC, 2005). The extraction lasted for 5–6 h. The stock solution was diluted by 60 µL in 1.5 mL vial with buffer and filtered by 0.22 µm syringe filter. Afterwards, Sykam Amino Acid Analyzer (Sykam GmbH, Eresing, Germany) subjected 100 µL of this solution to analysis. The amino acid analyzer was equipped with Solvent Delivery System S 2100 (Quaternary pump with flow rate of 0.01–10.00 mL/min and maximum pressure up to 400 bar), Autosampler S 5200, Amino Acid Reaction Module S4300 (with built-in dual-filter photometer between 440 and 570 nm with constant signal output and signal summary option) and Refrigerated Reagent Organizer S 4130.

2.7. Cytotoxicity Test

This test was conducted at Nawah Scientific Inc., Moqattam, Cairo, Egypt. Oral epithelial cells (OECs) were used in this test, and the methodology described by [37,38] was followed. The cells were kept alive in DMEM medium at 37 °C in a humidified atmosphere of 5% (*v*/*v*) CO_2. The DMEM was supplemented with 100 mg/mL streptomycin, 100 U/mL penicillin, and 10% heat-inactivated fetal bovine serum. The sulforhodamine B (SRB) assay was used for cell density determination, based on the measurement of cellular protein content [39]. Aliquots of 100 µL cell suspension (contained 5×10^3 cells/mL) were grown in complete medium in 96-well plates for 24 h. To treat the cells, 100 µL of media with various concentrations of *T. colossus* fruiting body's extract was utilized. After 72 h of extract exposure, cells were fixed by replacing the medium with 150 µL of 10% TCA and incubated at 4 °C for 60 min. After removing the TCA solution, the cells were washed five times with distilled water. Aliquots of a 70 µL LSRB solution (0.4% *w*/*v*) were added, and the mixture was incubated in a dark atmosphere for 10 min at room temperature. The plates were then washed three times with 1.0% acetic acid before being allowed to air dry overnight. To dissolve the protein-bound SRB dye, 150 µL of 10 mM TRIS was added. The absorbance was then measured at 540 nm using a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany) [37].

2.8. Cell Viability

Cell viability was calculated as a ratio to the reference values. The scores were compared using the intraclass correlation coefficient (ICC) and limits of agreement statistics [40,41]. As a descriptive measure of agreement, the data on limits of agreement were also employed. The dose–response curve was used to obtain the IC50, and Equation (3) was used to determine the relative viability percentage [42].

$$\text{Relative viability } (\%) = \frac{\text{Absorbance of the treated cells}}{\text{Absorbance of the control cells}} \times 100 \quad (3)$$

2.9. Solid-State Fermentation of Some Lignocellulosic Wastes by T. colossus AUMC 14536

2.9.1. Substrate Pretreatment

Six different agricultural wastes, including bean straw (BS), palm leaf hay (PLH), rice husk (RH), rice straw (RS), sugarcane bagasse (SB), and wheat bran (WB), were chosen for solid-state fermentation (SSF). Every substrate was purchased from a public market in the governorates of Assiut and New Valley. The samples were cleaned with distilled water before being used. They were then uniformly weighted after being oven dried at 50 °C.

2.9.2. Enzyme Production in SSF

Each 250 mL Erlenmeyer flask in the triplicate set included 5 g of agricultural waste. Each was given 10 mL of the fermentation medium, which contains the following ingredients (g/L): oat spelt xylan, 1.0; $NaNO_3$, 2.0; K_2HPO_4, 1.0; KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0.01; and $CuSO_4$, 0.005. At the end, the pH was set to 7.0. The flasks were injected with 1.0 mL of spore suspension containing 1.5×10^8 spores derived from a 7-day-old culture of *Tomophagus* isolate AUMC 14536 after being autoclaved (121 °C for 20 min). The inoculated flasks underwent a 14-day static incubation period at 30 °C. After the incubation, centrifugation (10,000 rpm for 10 min at 4 °C) produced the cell-free supernatants, which were then used as crude enzymes.

2.9.3. Enzyme Assay and Protein Estimation

Using the substrates carboxymethyl cellulose (CMC), microcrystalline cellulose (MCC), pectin, and oat spelts xylan, the estimation of endoglucanase, exoglucanase, pectinase, and xylanase was performed. Prepared in a 50 mM Na-citrate buffer (pH 5.0), the reaction mixture contained 0.5 mL of crude enzyme and 0.5 mL of the aforementioned substrates at a 1.0% concentration. The reaction was carried out at 50 °C in a water bath for 20 min and stopped by introducing 2 mL of 3, 5, Dinitrosalicylic acid (DNS) reagent. The quantity of reducing sugars released was measured using glucose or xylose calibration curves [43]. When measuring the activity of the laccase enzyme at 450 nm, the reddish brown color that resulted from laccase's oxidation of guaiacol was employed [44]. The reaction mixture contained 1.0 mL of 2 mM guaiacol, 1.0 mL of the fungal supernatant, and 3.0 mL of 10 mM sodium acetate buffer (pH 5), and the reaction was carried out at 30 °C for 15 min [45]. Protein content was estimated by Lowry's method, using bovine serum albumin as standard [46]. Calculations of sugar concentration and enzyme activity were conducted according to Al-Bedak et al. [25] and Al-Kolaibe et al. [47].

3. Results

3.1. Molecular Identification of the Tomophagus Isolate

Based on a megablast search in the NCBI database using ITS sequence of *Tomophagus* isolate AUMC 14536, the closest hits are *Tomophagus colossus* cultivar Sai Gon ((GenBank accession number MG650109; identities = 614/614 (100%); Gaps = 0/614 (0%)) and *T. colossus* isolate A164FB2 ((GenBank accession number OQ558872; identities = 613/614 (99.83%); Gaps = 0/614 (0%)). The taxonomic status of *Tomophagus* sp. in this investigation was ascertained using a phylogenetic analysis of the ITS dataset. There were 24 species in the final batch of ITS data. Out of the 614 characters in the maximum-parsimony dataset, 509 could be aligned without any doubt, 151 were variable characters that were parsimony-uninformative, and 101 were counted as parsimony-informative. For nucleotide substitution, the Kimura 2-parameter and Gamma distribution (K2 + G) model was ideal. Four trees were produced via maximum-parsimony analysis, and the most parsimonious one was given; it had a tree length of 432 steps, a consistency index of 0.548387, a retention index of 0.736752, and a composite index of 0.404025. Figure 1 displays the single tree that the maximum-likelihood analysis produced (log likelihood = 2964.62).

The relationships between the *Tomophagus* sp. AUMC 14536 and other *Ganoderma* and *Tomophagus* species were described by the phylogenetic result. A very strong bootstrap support value of 94% ML/99% MP is endorsed by the strain found within the *T. colossus*

clade at the same branch as *T. colossus* SG 812 (100% similarity), *T. colossus* URM 83330 (99.84% similarity), *T. colossus* SCUF21M (99.83%), and *T. colossus* UMNFL 110 (RAB) (99.51% similarity). This led to the identification of the strain under study as *T. colossus* (Figure 1).

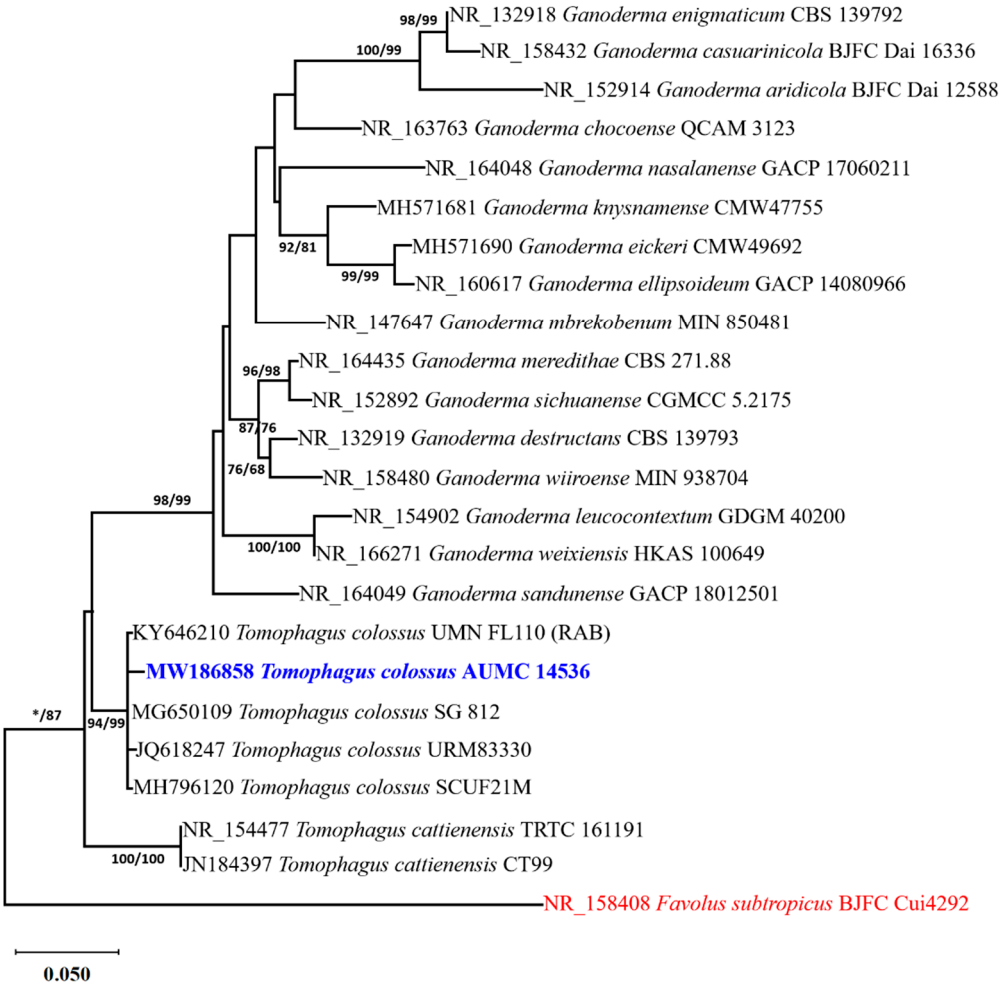

Figure 1. The most parsimonious phylogenetic tree generated from ML/MP analysis using a heuristic search (1000 replications) of ITS sequence of *T. colossus* AUMC 14536 (in blue color) compared with other closely similar ITS sequences belonging to *Tomophagus* and *Ganoderma* in GenBank. Bootstrap support values for ML/MP $\geq$ 50% are indicated near the respective nodes. Bootstrap support values less than 50% are signed with (*). The tree is rooted to *Favolus subtropicus* BJFC Cui4292 as outgroup (in red color).

3.2. Brief Description of T. colossus AUMC 14536

Pileus thick, 8–15 (−25) cm, spongy, floccose, light in weight when dried, sessile, or with a very short cylindrical stipe. Pilear surface glabrous, opaque in appearance, yellowish in young specimens, brownish in older basidiocarps, thin, readily broken when cracked or pressed (Figure 2A–C). Chlamydospores dark-brown, thick-walled, 10–12 × 7–10 µm (Figure 2D). Basidiospores abundant, verrucose, ovate, truncate or rounded at the apex, yellowish, thick-walled, 13–18 × 8–12 µm (Figure 2E). Colonies on PDA attaining a diameter of 75 mm after 7 days at 28.0 ± 2.0 °C, centrally brown, floccose; margin entire, wide, white; reverse brown beneath the colony center, pale outwards (Figure 2F). Chlamydospores formed on PDA culture, globose, double-walled, pigmented, ornamented with abundant papillate projections on the surface (Figure 2G–I).

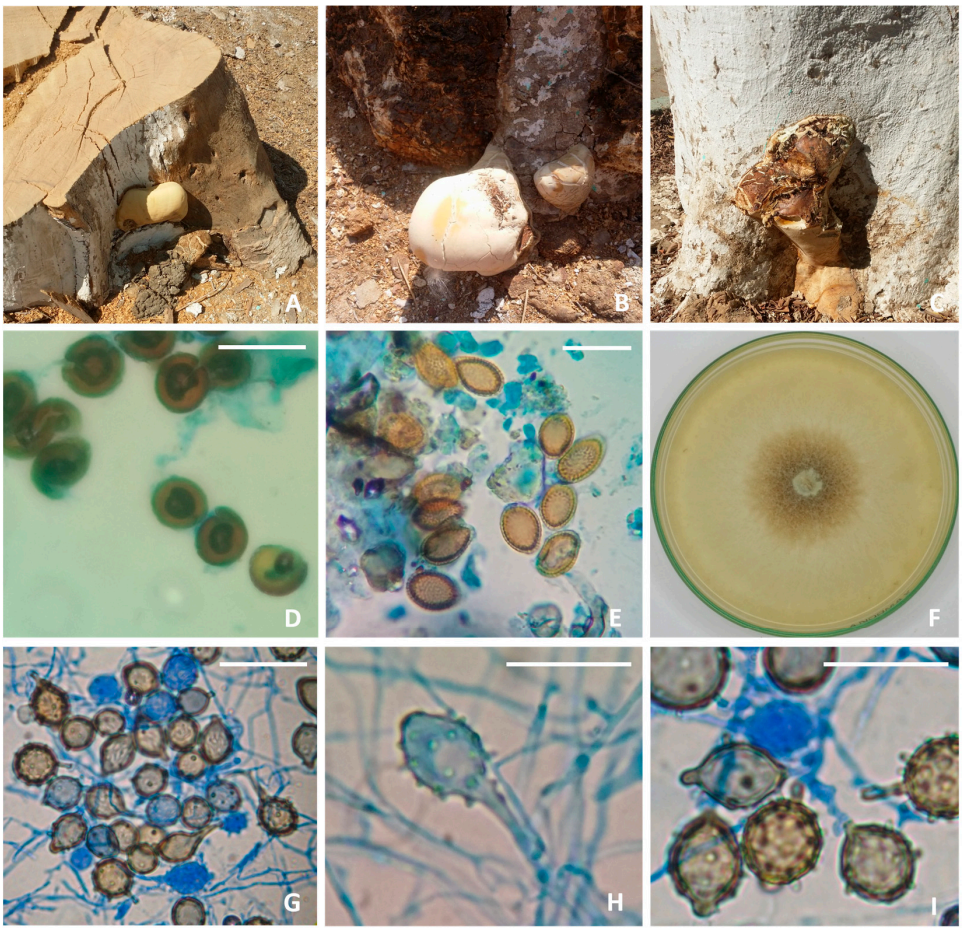

Figure 2. *T. colossus* AUMC 14536 (**A**–**C**), *Ficus nitida*-associated basidioma (**D**), dark-colored, thick-walled chlamydospores observed in the context (**E**), verrucose basidiospores (**F**), seven-day-old colony on PDA at 25 °C (**G**–**I**), globose, double-walled, pigmented, ornamented chlamydospores with abundant papillate projections (scale bars = 20 µm).

3.3. Chemical Analyses of T. colossus's Basidioma

Tomophagus colossus AUMC 14536 basidioma's chemical analysis showed that, based on dry weight, crude fats and carbohydrates (28.81 and 25.34%, respectively) make up the majority of the basidioma, followed by crude fibers (23.44%) and crude proteins (20.64%), as well as ash content (3.02%) (Table 1). We assessed potassium, phosphorus, calcium, iron, zinc, and selenium. The highest quantities of calcium (6.27 mg/100 g) were followed by potassium (133.59 mg/100 g) and phosphorus (114.46 mg/100 g). Iron and selenium levels were moderate (3.08 and 1.28 mg/100 g, respectively) at the same time. Moreover, it contains the least zinc (0.73 mg/100 g). The concentrations of total flavonoids and total phenolic compounds were 562 and 3926 mg/100 g, respectively (Table 1).

Table 1. Chemical Composition of *T. colossus* AUMC 14536's Basidioma.

Nutritional Component	Value (%)	Nutritional Component	Value (mg/100 g)
Carbohydrates	28.81	K	133.59
Crude Fibers	23.44	P	114.46
Crude Proteins	20.64	Ca	6.27
Crude Fats	25.34	Se	3.08
Ash	3.02	Fe	1.28
		Zn	0.73
		Total phenolic compounds	3926
		Total flavonoids	562

3.4. Antioxidant Activity of T. colossus AUMC 14536 Biomass

A DPPH test was used to further investigate the biomass of *T. colossus* AUMC 14536's capacity to scavenge hydrogen, and the free radical scavenging activity was reported as an IC50 value. The results indicate that DPPH activity increased with increasing sample concentration (Figure 3A). The extract of *T. colossus* AUMC 14536 basidioma had an IC50 value of 4.15 µg/mL, which was higher than the ascorbic acid activity value of 1.89 µg/mL (Figure 3B).

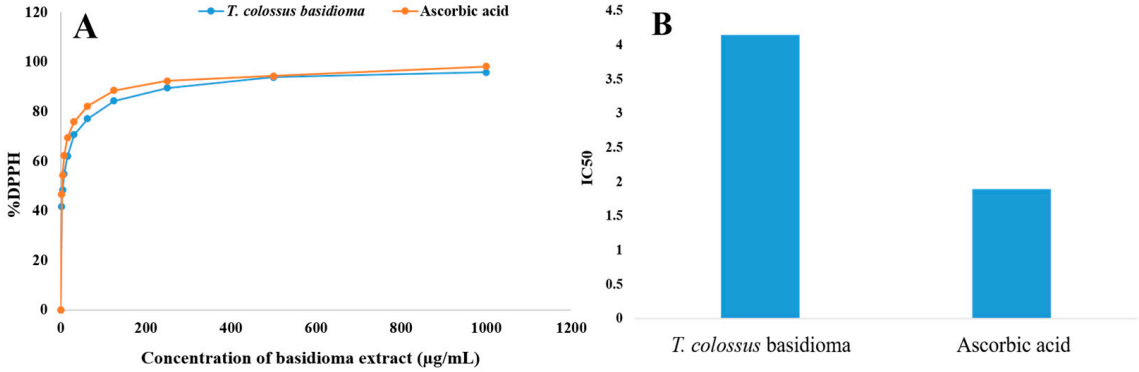

Figure 3. (**A**) Antioxidant activity (% DPPH) and (**B**) IC50 of the *T. colossus* basidioma extract compared with standard ascorbic acid.

3.5. Analysis of Amino Acids Content

When the *T. colossus* AUMC 14536 basidioma extract was analyzed for amino acid content, 16 amino acids were identified (Figure 4). It was shown that arginine (14.396 mg/100 mg dry weight), glutamic acid (6.801), alanine (5.556), histidine (4.558), and aspartic acid (2.727) were the most common. From 0.282 (methionine) to 1.945 (leucine), the remaining amino acids are listed in Table 2.

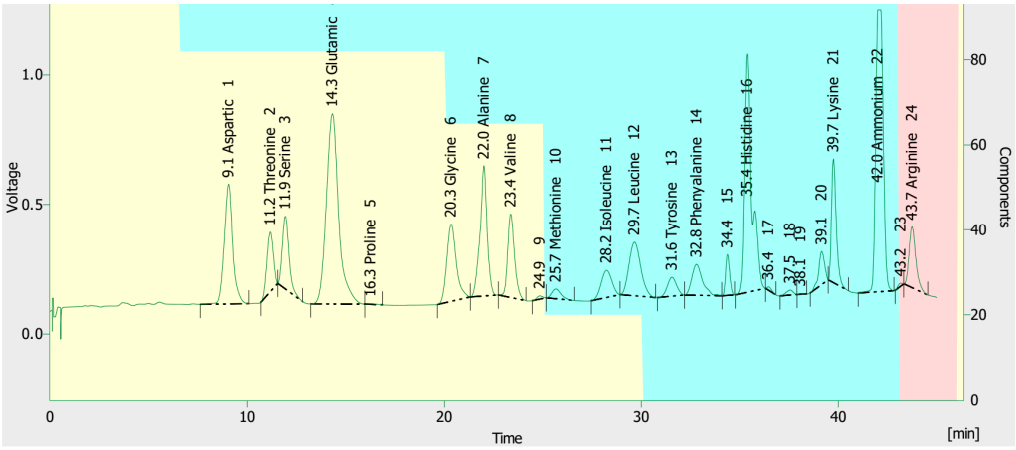

Figure 4. Amino acid profile of the *T. colossus* AUMC 14536's basidioma.

Table 2. Amino acids profile of *T. colossus* AUMC 14536's basidioma.

Retention Time (min)	Area (mV.s)	Compound Name	Amount (mg/100 g Dry wt.)
9.061	13,936.801	Aspartic acid	2.727
11.171	5248.426	Threonine	1.371
11.933	6686.706	Serine	1.390
14.325	31,964.8	Glutamic acid	6.801
16.272	76.739	Proline	0.691

Table 2. *Cont.*

Retention Time (min)	Area (mV.s)	Compound Name	Amount (mg/100 g Dry wt.)
20.347	10,087.029	Glycine	1.256
22.013	12,613.589	Alanine	5.556
23.376	7872.286	Valine	1.388
25.669	1234.684	Methionine	0.282
28.229	3966.965	Isoleucine	0.932
29.651	8677.939	Leucine	1.945
31.560	2391.457	Tyrosine	0.732
32.808	4518.379	Phenylalanine	1.241
35.373	23,137.48	Histidine	4.558
39.749	8056.973	Lysine	1.714
43.747	5677.821	Arginine	14.396
Total	174,933.787	16 amino acids	46.98

3.6. Cytotoxicity

The percentage of cell viability was used to illustrate the cytotoxic effects of *T. colossus* AUMC 14536's basidioma biomass on the OECs. The SRB assays' negative control reactions had intraclass correlation coefficient values (ICC) of 0.997, 0.985, 0.974, 0.968, and 0.960 at different concentrations of 0.01, 0.1, 1.0, 10.0, and 100 µg/mL, respectively. The IC50 was found to be 4.15 µg/mL, and the cell viability varied across all tested concentrations, ranging from 95.997% to 99.714 (Figure 5).

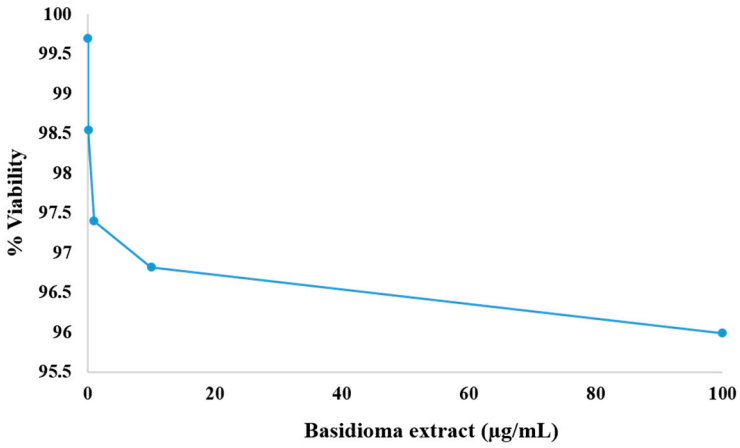

Figure 5. Dose–response curve for evaluation of cytotoxicity of *T. colossus* AUMC 14536's basidioma on OECs using SRB assays.

3.7. Cell Wall Hydrolyzing Enzymatic Potential of T. colossus AUMC 14536 in SSF

The lignocellulosic wastes utilized in SSF could potentially be fermented by *T. colossus* AUMC 14536, which could result in the production of large quantities of all the enzymes that were identified (endoglucanase, exoglucanase, laccase, pectinase, and xylanase). The highest specific activity was obtained by the rice husk waste for endoglucanase (81.48 U/mg), exoglucanase (114.35 U/mg), pectinase (81.94 U/mg), and xylanase (70.18 U/mg). Laccase production was boosted by bean straw and wheat bran, with maximal specific activities of 94.27 and 91.7 U/mg, respectively (Figure 6).

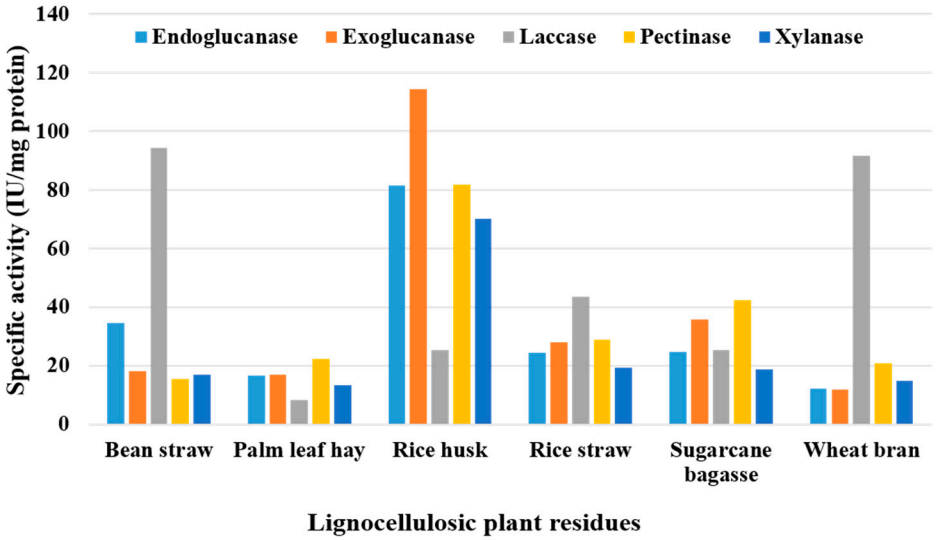

Figure 6. Specific activity of endoglucanase, exoglucanase, laccase, pectinase, and xylanase produced by *T. colossus* AUMC 14536 in SSF at 30 °C.

4. Discussion

The members of the Polyporaceae are identified by their characteristic double-walled basidiospores, which were clearly observed in *T. colossus* in this study. The existence of chlamydospores is a significant feature for species identification, as evidenced by molecular phylogenetic research. Chlamydospores are found in the basidiomata of just a few *Ganoderma* species, including *G. subamboinense* (Henn.) Bazzalo and J.E. Wright and *G. weberianum* (Bres. and Henn. ex Sacc.) Steyaert [12,48]. The macro- and micromorphological characteristics of *Ganoderma* are very variable, with over 250 species reported [8]. As a result, it is the most difficult genus to classify among order Polyporales [11,49]. The variety of *Ganoderma* morphological features encouraged several taxonomists to look into chemical and molecular methods for differentiating *Ganoderma* species. Based on morphological features and molecular phylogeny, the strain AUMC 14536 was identified as *T. colossus* in this study, which was associated with the *Ficus nitida* tree for the first time in Egypt and worldwide. *Tomophagus colossus* has been isolated from the Faculty of Agriculture Ornamental farm at Suez Canal University, Egypt [16], but this is the first time that its nutritional value, amino acids content, antioxidant activity, and cytotoxicity, as well as its enzymatic capabilities, have been assessed.

High levels of carbohydrates, fats, fibers, and proteins were found in the chemical analysis of *T. colossus* basidioma used in this study. Additionally, high levels of potassium, phosphorus, calcium, selenium, iron, and zinc were found, suggesting that *T. colossus* biomass has a high nutritional value as a nutritional supplement. Polypore fungi such as *Ganoderma* have a long history of usage in traditional medicine in several Asian countries to promote health and longevity. It has also been widely employed in a variety of medical applications and nutritional supplements to prevent and treat a wide range of illnesses [50]. Regarding this, Triterpenoids (Colossolactones A, B, C, D, E, F, and G) and colossolactones I, II, III, IV, V, VI, and VIII, as well as Ganomycin B, Ganomycin I, farnesyl hydroquinone, Ganomycin I, Ganomycin B, schisanlactone A, Colossolactone VII, Ergosterol, and Ganorbiformin A, were extracted from Vietnamese *T. colossus* [19,20,51,52]. Conversely, polysaccharides, triterpenoids, nucleosides, sterols, fatty acids, protein, and alkaloids are only a few of the bioactive compounds discovered and extracted from many Polypore fungi [53–64].

Considerable levels of flavonoids (562 mg/100 g) and phenolic compounds (3926 mg/100 g) were detected in the biomass of *T. colossus* basidioma in this investigation, as well as strong antioxidant activity with IC50 of 4.15 µg/mL. Regarding this, *Hericium erinaceus*, *Ganoderma lucidum*, and *Agrocybe aegerita* fruiting bodies have total phenolic content values of 17.10,

28.11, and 16.05 mg/g, respectively. However, the total flavonoid content in *Hericium erinaceus*, *Ganoderma lucidum*, and *Agrocybe aegerita* exhibits values of 445.6, 627.7, and 393.9 µg/g, respectively [65]. Both *Ganoderma carnosum* Pat. and *Ganoderma pfeifferi* Bres. yielded significant amounts of flavonoids (10.06 ± 0.51 and 2.71 ± 0.24 mg/g) and phenolic compounds (43.28 ± 3.83 and 22.81 ± 0.68 mg/g) [66]. However, the existence of flavonoids in some mushroom species is uncertain, because these species lack the chalcone isomerase enzymes necessary for the biosynthesis of flavonoids [67]. Despite not being the primary component of edible and medicinal mushrooms, phenolic compounds have been shown to exhibit a number of advantageous properties, including antioxidant [68,69] and enzyme inhibition [70], which appears to be closely associated with the anti-inflammatory qualities of mushrooms [71].

Enzymatic potential by mushrooms and filamentous fungi has been shown to be the most efficient, cost-effective, environmentally friendly, and, in most situations, high-yielding method [72–74]. In the present study, *T. colossus* AUMC 14536 showed good growth on all agricultural wastes (BS, PLH, RH, RS, SB, and WB) used in SSF, and produced high levels of endoglucanase, exoglucanase, laccase, pectinase, and xylanase enzymes. These findings bode well for the development of a practical application for the treatment of lingo-cellulosic plant wastes, as well as for the bioremediation of damaged ecosystems produced by the burning of such residues. Regarding this, no research on *T. colossus* enzyme synthesis in SSF has been disclosed. Some *Ganoderma* strains isolated from the base of the trees and decayed wood in Havana, Cuba [75], and Pakistan [76] have been used to produce laccases and peroxidases. In liquid media and solid-state cultures incorporating wood chips from *Eucalyptus globulus* or *Drimys winteri*, some strains of *Ganoderma australe* (Fr.) Pat. (A464 and A272) generated laccase and manganese peroxidase activity [77]. Two strains of *Ganoderma multipileum* Ding Hou produced a maximum laccase activity of 1355.5 ± 8.8 U/L utilizing guaiacol as substrate [78]. Two strains of *Ganoderma* (GASI3.4, and CCB209) produced lignin peroxidase, manganese peroxidase, and laccase on wheat bran [79].

5. Conclusions

In addition to using *T. colossus* for enzyme synthesis and lignocellulosic residue consumption, this work is a first for Egypt, since it is the first to isolate the fungus in relation to *Ficus nitida* trees. It was discovered that the basidiomata biomass had strong antioxidant activity, along with high concentrations of minerals, phenolic compounds, total lipids, total proteins, total fibers, carbohydrates, and amino acid content. Due to its enzymatic properties, this species is proposed to be accepted here for potential use in the manufacturing of animal feed, as well as in the prevention and treatment of various medical conditions. This requires special cultivation techniques, extracts, and commercial formulations. Although *T. colossus* seems promising, more investigation and credible scientific evidence are needed to confirm *Tomophagus*'s safe and effective use as a dietary supplement.

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