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Evaluation of antioxidant, antimicrobial and tyrosinase inhibitory activities of extracts from *Tricholosporum goniospermum*, an edible wild mushroom

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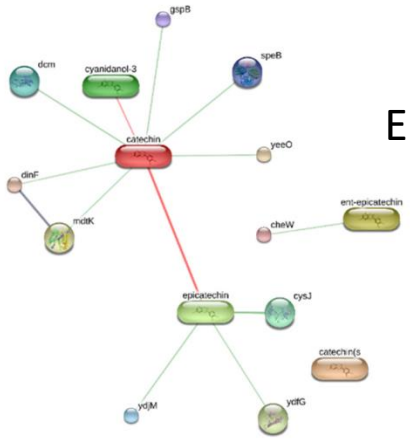
Graphical Abstract

Antimicrobial effects

Phytochemical analysis



E. coli

T. goniospermum Parts	Solvents	TPC (mg GAE/g)	DPPH (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)
Fruiting bodies	n-hex	10.91 ± 0.56	9.35 ± 0.16	53.92 ± 1.31	14.86 ± 1.21
	EA	70.51 ± 0.06	88.82 ± 1.47	307.71 ± 3.83	134.06 ± 1.50
	MeOH	14.87 ± 0.79	17.69 ± 0.95	131.52 ± 0.67	20.54 ± 0.77
Mycelia	n-hex	9.63 ± 0.22	7.53 ± 0.70	54.35 ± 0.92	15.15 ± 1.62
	EA	33.74 ± 0.80	29.93 ± 3.54	155.31 ± 1.85	74.26 ± 1.79
	MeOH	7.39 ± 0.14	7.74 ± 0.69	129.60 ± 0.56	12.94 ± 1.33

GAE: Gallic acid equivalent; TE: Trolox equivalent; Values are reported as mean ± S.D.

Abstract: *Tricholosporum goniospermum* is an excellent edible mushroom whose compounds and biological properties are still unknown. In this study, n-hexane, ethyl acetate and methanol extracts from fruiting bodies and liquid-cultured mycelia were compared for the analysis of phenolic compounds, the evaluation of scavenger and reducing activities, and the enzyme inhibition of α-amylase, acetylcholinesterase, butyrylcholinesterase and tyrosinase. Additionally, *T. goniospermum* extracts were evaluated for antibacterial and antimycotic activities against Gram+ and Gram− bacteria, and clinical yeast and fungal dermatophytes. Finally, based on the extract content in phenolic compounds, *in silico* studies, including the docking approach, were conducted to predict the putative targets (namely tyrosinase, lanosterol-14-α-demethylase, the multidrug efflux system transporters of *E. coli* (mdtK) and *P. aeruginosa* (pmpM), and *S. aureus* β-lactamase (ORF259)) underlying the observed bio-pharmacological and microbiological effects. The mycelia methanolic extract was the richest in gallic acid, whereas fruit ethyl acetate extract was the sole to show levels of catechin. Specifically, docking runs demonstrated an affinity of catechin towards all docked proteins, in the micromolar range. These *in silico* data are consistent, at least in part, with the highest activity of ethyl acetate extract as an antimicrobial and anti-tyrosinase agent. The ethyl acetate extracts were also noted as being the most active on α-amylase. Concluding, among tested extracts, the ethyl acetate extract showed the highest efficacy as both antimicrobial and anti-tyrosinase agent, thus suggesting innovative pharmacological applications of *T. goniospermum*.

Keywords: *Coronilla minima;* resveratrol; antiproliferative effects; antimicrobial effects.

Introduction

Fungi represent some of the principal components of biodiversity in all terrestrial habitats .They are important decomposers and recyclers of organic materials; they positively or negatively interact with plant roots in the rhizosphere or with above-ground plant components. For centuries, mushrooms, the fruiting bodies of macroscopic filamentous fungi that grow above the ground, have been a part of the human diet and used as both food and medicine. They contain minerals, vitamins and nutritive compounds such as proteins and polysaccharides and have a low fat content. Mushrooms are also appreciated as a delicacy. They have many flavors and nutrient characteristics that make them an ideal addition to many dishes. Their texture and umami or savory flavor properties make them a suitable substitute for meat. While the usage of medicinal mushrooms has a long tradition in Eastern countries, in the Western world it has increased only slightly over the last few decades. Mushrooms have been found to have potent biological activities such as anti-bacteria, anti-fungal, antitumor, anti-inflammatory, anti-hepatotoxic, cardio-tonic, cholesterol level lowering, antiviral and immune-modulatory activities. The enormous structural diversity of natural compounds originated from mushrooms offers opportunities for discovering new drugs. Antimicrobial resistance (AMR) presents one of the biggest challenges to global public health, and it was estimated to have claimed 700,000 lives globally in 2014. Furthermore, it has been predicted that its attributable mortality will hit 10 million by 2050 if measures are not taken to tackle it. Thus, it has become critical to safeguard the integrity of the antimicrobials currently in use, considering that the discovery of novel antimicrobials has stalled over recent decades. Factors such as inappropriate use of antibiotics, inadequate infection prevention and control programs, limited laboratory capacity, poor surveillance, population growth and migration, as well as inadequate sanitation have compounded the problem of AMR . Over the past decades, fungal infections have become a major problem in clinical practice, with immunocompromised patients being easily susceptible. Notably, systemic fungal infections are usually associated with high mortalities. Infections by multidrug resistant isolates of Trichophyton rubrum, Candida spp.,Staphylococcus epidermidis, S. aureus, Streptococcus spp.,Enterococcus sp. And Escherichia coli, among others, became more and more frequent, stimulating the search for new antibiotics with novel mechanisms of action. Considering the current problem of resistance to microbial drugs and the growing concern about opportunistic infections, there is an urgent need for alternative antimicrobial drugs that could be found in plants and mushrooms. Over the past two decades, the health-promoting effect of mushrooms has attracted a lot of attention due to the wide range of secondary metabolites present in fruiting bodies and submerged culture. In particular, about 270 species are now considered as potential therapeutic or preventative agents used to ensure human health. To the best of our knowledge, the antioxidant and antimicrobial activities obtained from wild edible fungal extracts against pathogenic fungi and bacteria have been rarely explored. Tricholosporum goniospermum (Bres.) Guzmán ex T.J. Baroni (Tricholomataceae, Agaricales, Basidiomycota) is a wild mushroom mainly known from northern Europe and north-central Italy. It is considered a saprotroph that colonizes small soil patches near deciduous trees. Although it is considered an excellent edible mushroom, the biological properties of its fruiting bodies and mycelia extracts have not yet been studied. It is apparent that, depending on the extraction solvent, the extracts from the same mushroom will differ in the composition of bioactive compounds. Therefore, on the basis of these considerations, our present study aims to shed more light on this poorly understood mushroom.

Results and discussion

The HPLC-fluorimetric analysis focused on selected phenolic compounds, namely gallic acid, catechin, epicatechin and resveratrol. The results indicated that the T. goniospermun methanolic extract from mycelia was the richest in gallic acid, whereas the T. Goniospermum ethyl acetate from the fruiting body displayed the best qualitative profile, alongside low levels of catechin . Conversely, the T. goniospermumn-hexane extract from mycelia displayed the poorest quantitative profiles with regards to the selected phenolic compounds. The results of the HPLC-fluorimetric analysis were consistent with the colorimetric evaluations of total phenols (expressed as mg of gallic acid per g of dry extract) previously described, although a punctual description of the T. goniospermum phenolic profile is still lacking. According to colorimetric analyses, the ethyl acetate extract seems to be the most effective in terms of scavenger/reducing and enzyme inhibitory activities. Specifically, the anti-tyrosinase effect of this extract was significantly higher when compared to the other extracts, thus suggesting applications in skin disorders characterized by an increased tyrosinase activity. Therefore, a docking analysis was conducted on catechin, in order to investigate the mechanism of action of the extracts. The docking runs showed a good affinity of catechin towards the selected target proteins.

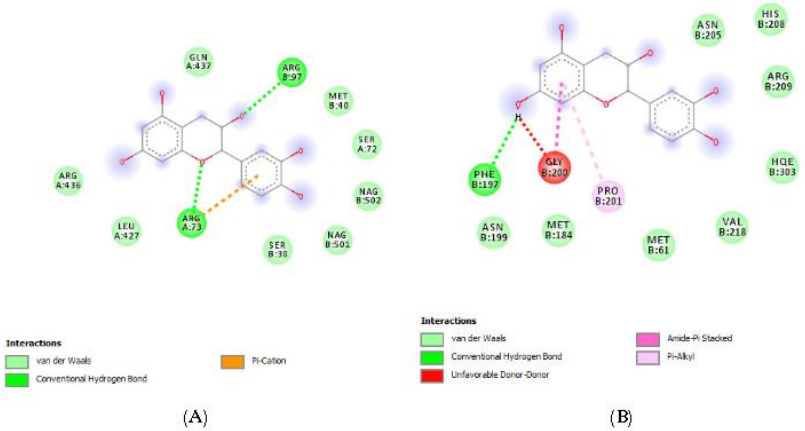
T. goniospermum Parts	Solvents	Gallic Acid	Catechin	Epicatechin	Resveratrol
		µg/mL	µg/mL	µg/mL	µg/mL
Fruiting bodies	n-hex	0.96 ± 0.5	0.00	0.00	0.00
	EA	0.09 ± 0.01	0.03 ± 0.01	0.00	0.00
	MeOH	0.65 ± 0.03	0.00	0.00	0.00
Mycelia	n-hex	0.95 ± 0.3	0.00	0.00	0.00
	EA	2.18 ± 0.11	0.00	0.00	0.00
	MeOH	3.95 ± 0.13	0.00	0.00	0.00

T. goniospermum Parts	Solvents	AChE Inhibition	BChE Inhibition	Tyrosinase Inhibition	Amylase Inhibition
		(mg GALAE/g)	(mg GALAE/g)	(mg KAE)	(mmol ACAE/g)
Fruiting bodies	n-hex	NA	5.48 ± 0.03	83.80 ± 1.45	0.55 ± 0.01
	EA	NA	26.78 ± 0.21	554.30 ± 9.41	2.97 ± 0.10
	MeOH	NA	5.07 ± 0.02	48.48 ± 0.07	0.21 ± 0.01
Mycelia	n-hex	NA	9.14 ± 0.07	127.76 ± 0.73	0.73 ± 0.02
	EA	NA	25.21 ± 0.24	412.81 ± 1.39	2.25 ± 0.07
	MeOH	NA	2.61 ± 0.01	28.17 ± 0.39	0.17 ± 0.01

GALAE: Galatamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; NA: not active. Values are reported as mean ± S.D.

T. goniospermum Parts	Solvents	TPC	DPPH	CUPRAC	FRAP
		(mg GAE/g)	(mg TE/g)	(mg TE/g)	(mg TE/g)
Fruiting bodies	n-hex	10.91 ± 0.56	9.35 ± 0.16	53.92 ± 1.31	14.86 ± 1.21
	EA	70.51 ± 0.06	88.82 ± 1.47	307.71 ± 3.83	134.06 ± 1.50
	MeOH	14.87 ± 0.79	17.69 ± 0.95	131.52 ± 0.67	20.54 ± 0.77
Mycelia	n-hex	9.63 ± 0.22	7.53 ± 0.70	54.35 ± 0.92	15.15 ± 1.62
	EA	33.74 ± 0.80	29.93 ± 3.54	155.31 ± 1.85	74.26 ± 1.79
	MeOH	7.39 ± 0.14	7.74 ± 0.69	129.60 ± 0.56	12.94 ± 1.33

GAE: Gallic acid equivalent; TE: Trolox equivalent; Values are reported as mean ± S.D.



Putative interaction between catechin and human tyrosinase-related protein 1 (PDB: 5M8P).The free energy of binding (ΔG) and affinity (Ki) are−6.7 kcal/mol and 12.4µM, respectively (A).Putative interaction between catechin and tyrosinase from Bacillus megaterium (PDB: 5I3B). The free energy of binding (ΔG) and affinity (Ki) are−6.6 kcal/mol and 14.7µM, respectively (B).

Results and discussion

All mushroom extracts showed antimicrobial activity within the concentration range tested, but with a wide variability in terms of potency and selectivity. Regarding bacteria, the strongest inhibition was observed for EA and MeOH mycelia extracts (MIC 0.099 mg/mL against E. coli),while the methanol extract obtained by fruiting bodies resulted in actively reducing B. Cereus growth. Conversely, the n-hex fruiting bodies extract seemed to be the least effective against all the tested microorganisms. All bacterial strains were sensible to EA extracts from mycelia with MIC values lower than 0.315. The same extracts obtained from fruiting bodies gave similar results, but with slightly higher MIC values against P. aeruginosa (0.396 mg mL−1).All tested extract results were actively inhibitory of fungal growth, but a huge variability was recorded between the most active (mycelia-EA on C. albicans YEPGA 6379: MIC 0.051) and the lesser ones (fruiting bodies-n-hex on A. curreyi, A. insingulare, A. quadrifidum: MIC>1.0; fruiting bodies-MeOH on A. insingulare:>1.0). Regarding dermatophytes, A. crocatum (CCF 5300) and A. gypseum(CCF 6261) were the most sensitive fungal species to mushroom extracts, with MIC ranges of 0.031–0.46 mg mL−1.The ethyl acetate extracts of mycelia were particularly active, showing the highest antifungal activity among all the tested strains. All of the ten tested fungal strains showed an increased susceptibility toward the mushroom mycelia extract. Griseofulvin showed good activity in vitro against A. gypseum (CCF 6261)(MIC: 1–2µg ml−1). It was not possible to determine whether the isolates were resistant to the griseofulvin as no breakpoints have yet been established.

Bacterial Strain (ID)	Extract Typology	Minimum Inhibitory Concentration (MIC)			
		n-hex (mg mL^{-1}) *	EA (mg mL^{-1}) *	MeOH (mg mL^{-1}) *	Ciprofloxacin (µg mL^{-1}) **
Gram					
E. coli (ATCC 10536)	mycelia	0.157 (0.125–0.25)	0.099 (0.0625–0.125)	0.099 (0.0625–0.125)	<0.12
	fruiting bodies	0.198 (0.125–0.25)	0.157 (0.125–0.25)	0.198 (0.125–0.250)	
E. coli (PeruMycA 2)	mycelia	0.315 (0.25–0.5)	0.157 (0.125–025)	0.198 (0.125–0.25)	1.23 (1.95–0.98)
	fruiting bodies	0.396 (0.25–0.5)	0.315 (0.25–0.5)	0.315 (0.25–0.5)	
E. coli (PeruMycA 3)	mycelia	0.396 (0.25–0.5)	0.157 (0.125–0.250)	0.198 (0.125–0.250)	0.62 (0.98–0.49)
	fruiting bodies	0.315 (0.125–0.25)	0.198 (0.125–0.250)	0.315 (0.25–0.5)	
P. aeruginosa (PeruMyc 5)	mycelia	0.63 (0.5–1)	0.315 (0.25–0.5)	0.396 (0.25–0.5)	1.23 (1.95–0.98)
	fruiting bodies	0.79 (0.05–1)	0.396 (0.25–0.5)	0.63 (0.5–1)	
S. typhy (PeruMyc 7)	mycelia	0.79 (0.5–1)	0.315 (0.25–0.5)	0.62 (0.5–1)	0.38 (0.49–0.24)
	fruiting bodies	>1	0.62 (0.5–1)	0.79 (0.5–1)	
Gram+					
B. cereus (PeruMycA 4)	mycelia	0.198 (0.125–0.5)	0.099 (0.0625–0.125)	0.157 (0.125–0.250)	<0.12
	fruiting bodies	0.314 (0.25–0.5)	0.157 (0.125–0.250)	0.099 (0.0625–0.125)	
B. subtilis (PeruMyc 6)	mycelia	0.315 (0.125–0.25)	0.078 (0.062–0.125)	0.198 (0.125–0.25)	<0.12
	fruiting bodies	0.396 (0.25–0.5)	0.157 (0.125–0.25)	0.314 (0.25–0.5)	
S. aureus (ATCC 6538)	mycelia	0.396 (0.25–0.5)	0.157 (0.125–0.25)	0.198 (0.125–0.25)	0.62 (0.98–0.49)
	fruiting bodies	0.63 (0.5–1)	0.198 (0.125–0.25)	0.315 (0.25–0.5)	

* MIC values are reported as the geometric means of three independent replicates (n – 3); MIC ranges are reported within brackets. ** MIC values and ranges for fluconazole are expressed as µg mL^{-1}. In case no growth was observed at the lowest concentration tested, MIC values are reported as < (lowest concentration tested).

Dermatophytes (ID Strain)	Extract Typology	Minimum Inhibitory Concentration (MIC)			
		n-hex (mg mL^{-1}) *	EA (mg mL^{-1}) *	MeOH (mg mL^{-1}) *	Griseofulvin (µg mL^{-1}) **
A. crocatum (CCF 5300)	mycelia	0.25 (0.125–0.25)	0.084 (0.031–0.062)	0.157 (0.125–0.25)	>8
	fruiting bodies	0.314 (0.25–0.5)	0.46 (0.62–0.125)	0.198 (0.125–0.25)	
A. curreyi (CCF 5207)	mycelia	0.794 (0.5–1)	0.157 (0.125–0.25)	0.363 (0.62–0.125)	>8
	fruiting bodies	>1	0.315 (0.25–0.5)	0.39 (0.031–0.62)	
A. gypseum (CCF 6261)	mycelia	0.397 (0.25–0.5)	0.099 (0.62–0.125)	0.049 (0.031–0.062)	1.587 (1–2)
	fruiting bodies	0.397 (0.25–0.5)	0.181 (0.031–0.062)	0.25 (0.125–0.5)	
A. insingulare (CCF 5417)	mycelia	0.794 (0.5–1)	0.194 (0.125–0.5)	0.794 (0.5–1)	>8
	fruiting bodies	>1	0.623 (0.5–1)	>1	
A. quadrifidum (CCF 5792)	mycelia	0.623 (0.5–1)	0.198 (0.125–0.25)	0.315 (0.25–0.5)	>8
	fruiting bodies	>1	0.623 (0.5–1)	0.794 (0.5–1)	
T. mentagrophytes (CCF 4823)	mycelia	0.315 (0.25–0.5)	0.157 (0.125–0.25)	0.315 (0.25–0.5)	2.52 (2–4)
	fruiting bodies	0.623 (0.5–1)	0.194 (0.125–0.25)	0.397 (0.5–1)	
T. mentagrophytes (CCF 5930)	mycelia	0.794 (0.5–1)	0.57 (0.125–0.25)	0.63 (0.5–1)	3.174 (2–4)
	fruiting bodies	>1	0.198 (0.125–0.25)	>1	
T. rubrum (CCF 4933)	mycelia	0.397 (0.25–0.5)	0.049 (0.031–062)	0.039 (0.031–0.062)	1.26 (1–2)
	fruiting bodies	0.794 (0.5–1)	0.198 (0.125–0.25)	0.315 (0.25–0.5)	
T. rubrum (CCF 4879)	mycelia	0.397 (0.25–0.5)	0.049 (0.031–0.062)	0.315 (0.25–0.5)	3.175 (2–4)
	fruiting bodies	0.794 (0.5–1)	0.157 (0.125–0.25)	0.623 (0.5–1)	
T. tonsurans (CCF 4834)	mycelia	0.397 (0.25–0.5)	0.039 (0.031–0.062)	0.099 (0.062–0.125)	0.198 (0.125–0.25)
	fruiting bodies	0.623 (0.5–1)	0.314 (0.25–0.5)	0.397 (0.25–0.5)	

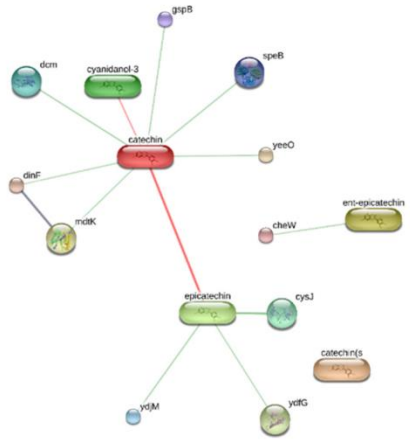
* MIC values are reported as the geometric means of three independent replicates (n – 3); MIC ranges are reported within brackets. ** MIC values and ranges for griseofulvin are expressed as µg mL^{-1}. In case no growth was observed at the lowest concentration tested, MIC values are reported as < (lowest concentration tested).

Yeasts (ID)	Extract Typology	Minimum Inhibitory Concentration (MIC)			
		n-hex (mg mL^{-1}) *	EA (mg mL^{-1}) *	MeOH (mg mL^{-1}) *	Fluconazole (µg mL^{-1}) **
C. albicans (YEPGA 6183)	mycelia	0.314 (0.25–0.5)	0.099 (0.0625–0.150)	0.157 (0.125–0.250)	2
	fruiting bodies	0.396 (0.25–0.5)	0.198 (0.125–0.250)	0.314 (0.25–0.5)	
C. tropicalis (YEPGA 6184)	mycelia	0.157 (0.125–0.25)	0.099 (0.0625–0.125)	0.099 (0.0625–0.125)	2
	fruiting bodies	0.198 (0.125–0.25)	0.157 (0.125–0.250)	0.198 (0.125–0.250)	
C. albicans (YEPGA 6379)	mycelia	0.099 (0.0625–0.125)	0.051 (0.031–0.0625)	0.099 (0.0625–0.125)	1
	fruiting bodies	0.157 (0.125–0.25)	0.198 (0.125–0.250)	0.198 (0.125–0.250)	
C. parapsilopsis (YEPGA 6551)	mycelia	0.314 (0.25–0.5)	0.079 (0.125–0.0625)	0.157 (0.125–0.25)	4
	fruiting bodies	0.396 (0.25–0.5)	0.099 (0.0625–0.125)	0.198 (0.125–0.250)	

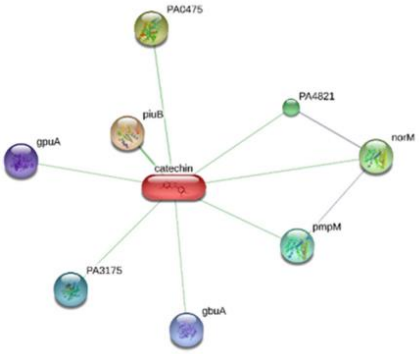
* MIC values are reported as the geometric means of three independent replicates (n – 3); MIC ranges are reported within brackets. ** MIC values and ranges for fluconazole are expressed as µg mL^{-1}. In case no growth was observed at the lowest concentration tested, MIC values are reported as < (lowest concentration tested).

Results and discussion

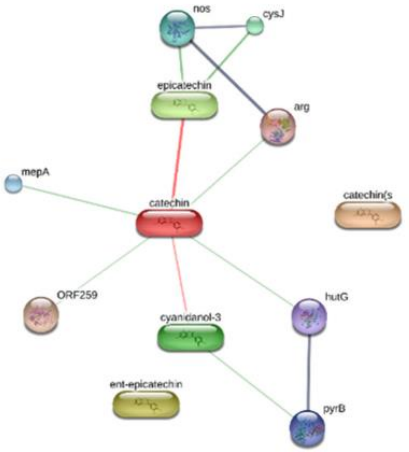
A bioinformatics analysis was also conducted for unraveling microbial proteins that could be targets of the catechin. The STITCH bioinformatics platform identified multidrug efflux system transporters of E. Coli (mdtK) and P. Aeruginosa (pmpM), and S. aureus β-lactamase (ORF259) as putative targets of the catechin.



E. coli



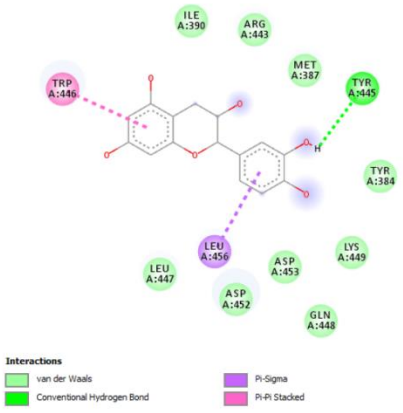
P. aeruginosa



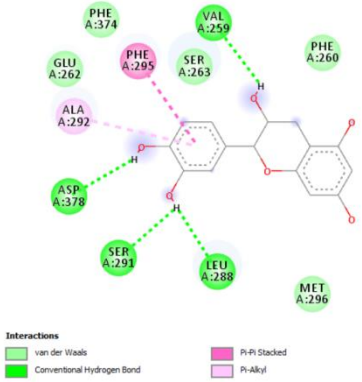
S. aureus

Results and discussion

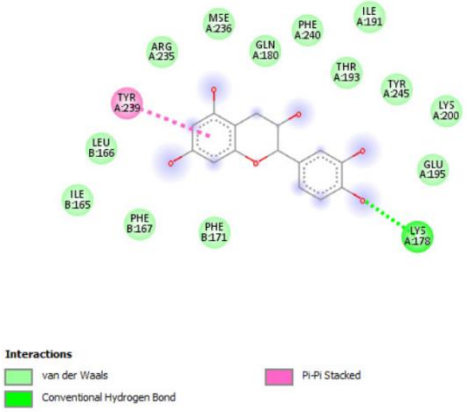
Meanwhile, the docking analysis showed the micromolar (1.7-6.3 µM) binding affinities of catechin towards these microbial proteins, further supporting the observed antibacterial activities exerted by the ethyl acetate extract.



E. coli



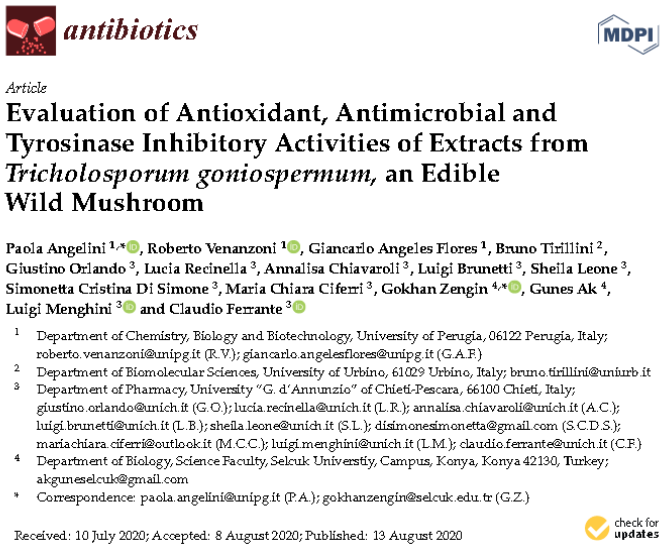
P. aeruginosa



S. aureus

Conclusions

In conclusion, all mushroom extracts tested in this study had potent antimicrobial activities. Particularly, among the tested extracts, the ethyl acetate extract showed the highest efficacy in all proposed experimental paradigms, which could be related, albeit partially, to the content of catechin. The bioinformatics analyses also suggested interactions between this compound and specific microbial proteins involved in the resistance to chemotherapeutic drugs, thus suggesting innovative pharmacological applications of T. Goniospermum extracts.



antibiotics

MDPI

Article

Evaluation of Antioxidant, Antimicrobial and Tyrosinase Inhibitory Activities of Extracts from *Tricholosporum goniospermum*, an Edible Wild Mushroom

Paola Angelini [1,*], Roberto Venanzoni [1], Giancarlo Angeles Flores [1], Bruno Tirillini [2], Giustino Orlando [3], Lucia Recinella [3], Annalisa Chiavaroli [3], Luigi Brunetti [3], Sheila Leone [3], Simonetta Cristina Di Simone [3], Maria Chiara Ciferri [3], Gokhan Zengin [4,*], Gunes Ak [4], Luigi Menghini [3] and Claudio Ferrante [3]

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