ORIGINAL ARTICLE

Tyrosinase inhibitory potential of phytochemicals and mycomolecules

Kiran Kumar Nannapaneni, Krishnamoorthy Akkanna Subbiah, Kamalakannan Ayyanar, Amirtham Damodarasamy

Department of Plant Pathology, Mushroom Research Laboratory, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Abstract

Aim: The aim of this study is to delve the tyrosinase inhibitory potential of various mycomolecules and phytochemicals. **Material and Methods:** Sporocarps of *Volvariella volvacea* strain CBE TNAU 1505 were produced. The extract was collected and centrifuged at 15,000 rpm for 30 min at 4°C. The supernatant was used as enzyme source. Methanol, acetone, and water fractions of phytochemicals from Adhatoda *vasica, Morinda citrifolia, Aloe vera, Glycyrrhiza glabra, Piper betle, Citrus limon, Azadirachta indica, Morus alba, Vitex negundo, Curcuma longa* and mycomolecules from *Ganoderma lucidum, Coriolus versicolor*, *Pisolithus albus*, *Lycoperdon esculentum, Schizophyllum commune, Ophiocordyceps neovolkiana, Ophiocordyceps sinensis,* and *Lentinus edodes* were extracted and tested for tyrosinase inhibitory activity. **Results and Discussion:** Among the fractions, tyrosinase inhibitory activity of methanolic fractions was always found to be better, when used at 500 µg ml^{-1},irrespective of the sample tested. Among phytochemicals, *G. glabra* exhibited the maximum tyrosinase inhibition of 65.6%. *C. limon* showed the minimum tyrosinase inhibitory activity of 16.3%. The mycomolecules extracted from *G. lucidum* registered the maximum tyrosinase inhibition of 53.3%. The tyrosinase inhibitory activity of mycomolecules was found to be positively correlated with their antioxidant activities. Tyrosinase inhibition may be attributed to the presence of melanogenic inhibitors, namely, isoflavones, chalcone derivatives, stilbenes in phytochemicals and flavonoids, polyphenols and triterpenoids in mycomolecules. **Conclusion:** Methanolic fractions of *G. glabra* and *G. lucidum* have inhibited the tyrosinase activity to the maximum extent. Future research for exploiting more benign mycomolecules and phytochemicals in this regard is most warranted.

Key words: Antioxidant, browning, melanin, phenols, quinones, shelf life

INTRODUCTION

Species of *Volvariella volvacea* (Bull. Ex Fr.) Sing. are perishable and tend to lose their appearance due to short shelf life, which is an impediment to the distribution and marketing of the fresh mushrooms. Extension of the quality and shelf life is therefore a scientific, technical, and economical challenge. Albeit there are several approaches for extending the shelf life of mushrooms, an alternative safe, cheaper, and eco-friendly approach is of utmost importance.[1] Mushroom browning occurs as a result of two distinct mechanisms of phenol oxidation, that is, by the activation of tyrosinase that belongs to polyphenol oxidase family and by spontaneous oxidation.[2] Tyrosinase (E.C.1.14.18.1), a multifunctional, glycosylated cytosolic copper-containing monooxygenase, is the principal enzyme for the synthesis of melanin pigment from tyrosine, which is responsible for enzymatic browning during development and postharvest storage.[3] Furthermore, to their intended use in cosmetic products, tyrosinase inhibitors such as traditional herbal medicines, vegetable oils, and kojic acid have been reported to have great reducing power. This kind of tyrosinase inhibitory potential has been greatly explored for controlling browning of vegetables and fruits.[4-7] Mulberroside F, purified from the leaves of *Morus alba*, showed the antidiphenolase activity of mushroom tyrosinase which is 4.5-fold higher than that of kojic acid and exhibited an inhibitory effect on melanin formation within melanoma cells.[8] Acetonic, methanol,

Address for correspondence:
Kiran Kumar Nannapaneni, Mushroom Research Laboratory, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.
E-mail: nannapanenic@gmail.com

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and hot water extracts of *Lentinus lepideus* and *Pleurotus eryngii* are proved to have tyrosinase inhibitory activity.[9,10] Keeping this limited available literature as background, the present study is thus aimed to probe the tyrosinase inhibitory potential of various mycomolecules and phytochemicals.

MATERIAL AND METHODS

V. volvacea strain CBE TNAU 1505 obtained from the germplasm bank of Mushroom Research Laboratory, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, was used in this experiment. It was maintained on 90 mm Petri dishes with potato dextrose agar medium at 32±2°C.

Spawn Preparation and Crop Rising

Paddy straw-based spawn was prepared by following the method suggested by Krishnamoorthy *et al*.[11] Fresh, good quality paddy straw bits (1-2 inches) were soaked in water for 6 h and dried under shade up to 65% moisture content. The paddy straw substrate was supplemented with horse gram powder at 2% (dry weight basis), and the contents were packed in polypropylene bags and autoclaved at 1.46 Kg/cm^2 for 90 min. On the next day, 90 mm mycelial mats of 7-day-old culture were propagated and the bags were incubated at room temperature. 15-day-old spawn with well-developed chlamydospores were used to seed the beds. For bed preparation, circular compact bed method was followed,[12] in which golden yellow, well-dried, good quality paddy straw was made into small twists of 2.5 m length and 5-8 cm diameter and each twist weighing 1.25 kg. After draining the excess water, the twists were presoaked in cold water for about 24 h and steam sterilized at 1.46 kg/cm^2 for 1 h. Later, the paddy straw twists were shade dried to get 65-75% moisture. The twists were compactly placed clockwise in a circular fashion as close as possible on a wooden plank to make the first layer. Eight paddy straw spawn bits each weighing 25 g were placed at the periphery of the first layer of the bed leaving equal distance between them. Over the spawn bits, 20 g of presterilized horse gram powder was sprinkled. The second layer was formed over the first layer following the same procedure, but the twist was placed compactly in the counterclockwise direction. Similarly, the third and fourth layers of bed were formed. The size of the bed measured 30 cm diameter and 20 cm height. Total weight of each bed was 5 kg on dry weight basis. The perfectly prepared bed was pressed tightly and placed in a poly house for cropping. Appropriate room temperature inside the cropping room ranged from 32°C to 35°C, and the relative humidity of 80-85% was maintained to prevent desiccation of young buttons. The commercially marketable eggs were collected and instantaneously macerated with phosphate buffer (pH 6.8) at 1:1 (w/v) ratio in the prechilled pestle and mortar. The extract was collected and centrifuged at 15,000 rpm for 30 min at 4°C. The supernatant was used as enzyme source.

Phytochemical Extraction

The medicinal plant parts, namely, leaves of *Adhatoda vasica*, *Aloe vera*, *Piper betle*, *Azadirachta indica*, and *M. alba*; fruits of *Morinda citrifolia*; bark of *Glycyrrhiza glabra*; peel of *Citrus limon*; roots of *Vitex negundo*; and rhizome of *Curcuma longa* were obtained from the Department of Medicinal plants, Tamil Nadu Agricultural University, Coimbatore. The plant parts were macerated finely in pestle and mortar and extracted by refluxing with 80% methanol, 80% acetone, and water separately at 1:3 ratio and then filtered using Whatman No. 1. filter paper. The procedure was repeated two times and the filtrates were pooled. The extracts were air dried by pouring in Petri dishes at 30-35°C. The dry deposition was scrapped with sterile blade, and the condensate obtained was weighed and recorded.

Mycomolecules Extraction

Cultures of *Ganoderma lucidum, Coriolus versicolor, Lycoperdon esculentum, Ophiocordyceps neovolkiana, Ophiocordyceps sinensis, Pisolithus albus, Schizophyllum commune*, and *Lentinus edodes* used in the study were obtained from the germplasm bank of Mushroom Research Laboratory, TNAU, Coimbatore. The 15-day-old mycelial mats cultured in potato dextrose broth were powdered with liquid nitrogen. A 5 g of powdered samples was extracted separately with 100 ml of 80% acetone, 80% methanol, and water with continuous stirring at 150 rpm for 24 h at 25°C to obtain acetonic, methanolic, and water extracts. The mixture was filtered through Whatman No.1 filter paper. The residues were reextracted with acetone, methanol, and water. The pooled extracts obtained with each solvent separately were air dried by pouring in Petri dishes at 30-35°C, and dried powder was obtained by scrapping condensate with sterile blade and weighed.

Determination of Tyrosinase Inhibition

Tyrosinase inhibitory activity was determined using modified dopachrome method with L- DOPA as substrate.[13] A 96-well microtiter plate was used to measure the absorbance at 475 nm with 700 nm as reference. Extract fractions were dissolved in 50% DMSO for making up the concentrations to 100, 250, and 500 µg ml^{-1}. Each well contained 40 µl of extract sample with 80 µl of phosphate buffer (0.1 M, pH 6.8), 40 µl of freshly extracted enzyme source, and 40 µl of L-DOPA (2.5 mM). The mixture was incubated for 10 min at 37°C, and absorbance was measured at 475 nm using a UVM 340 microplate reader. Each sample was accompanied by a blank containing all components except L- DOPA. L-ascorbic acid was used as positive control. The results were compared with

a control comprising 50% DMSO in place of the sample. The percentage of tyrosinase inhibition was calculated as follows:

$$\text{Tyrosinase inhibition} = [(A_{control}-A_{sample})/A_{control}] \times 100$$

where, A is the absorbance of control and sample, respectively.

2,2-Diphenyl-1-Picrylhydrazyl. (DPPH) Radical-scavenging Assay

The capacity to scavenge the stable free radical DPPH was monitored according to the method suggested by Hatano *et al*.[14] The methanolic, acetonic, and water extracts separately at concentration of 500 µg ml^{-1} (0.3 ml) were mixed with 2.7 ml of methanolic solution containing DPPH radicals (6×10^{-5} mol l^{-1}). The mixture was shaken vigorously and left undisturbed for 60 min in the dark (until stable absorption values were obtained A_S). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The radical-scavenging activity (RSA) was calculated as percentage of DPPH discoloration using the equation:

$$\text{Per cent RSA} = [(A_{DPPH}-A_S)/A_{DPPH}] \times 100$$

where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

Statistical Analysis

Statistical software AGRES (Developed by the Department of Physical Science, TNAU, Coimbatore) was used for the analysis of data. The determination of tyrosinase inhibition and antioxidant properties was carried out in triplicate and the results presented are mean values ± standard deviations. In case of zero values, data were log transformed (X+0.5) before statistical analysis.

RESULTS

Screening the Tyrosinase Inhibitory Potential of Phytochemicals

Medicinal plant extracts were evaluated for their tyrosinase inhibitory activity along with control (Ascorbic acid), and the results are presented in Table 1. Compared to control (67.58%), methanolic and acetonic extracts of *G. glabra* when used at 500 µg ml^{-1} exhibited the maximum tyrosinase inhibitory activity (65.67 and 53.59%, respectively). This was followed by methanolic and acetonic extracts of *M. alba* (60.0 and 46.94%) and *M. citrifolia* (54.28 and 43.65%) when used at 500 µg ml^{-1}. Methanolic and acetonic extracts of *C. limon* showed the minimum tyrosinase inhibitory activity (16.36% and 9.17%). Water extracts of *M. citrifolia* had shown the maximum tyrosinase inhibitory activity of 35.30%, followed by the water extract of *G. glabra* (29.71%) and *A. vera* (25.48%), whereas water extract of *C. longa* showed the minimum tyrosinase inhibitory activity of 7.91%.

Screening the Tyrosinase Inhibitory Potential of Mycomolecules

Mycelial extracts of medicinal mushrooms were evaluated for their tyrosinase inhibitory potential keeping ascorbic acid as control. The results revealed that ascorbic acid had exhibited the maximum tyrosinase inhibitory activity (67.58%). Among the mushrooms fungi, *G. lucidum* was significantly different and exhibited the maximum tyrosinase inhibitory activity when extracted with methanol, acetone, and water (53.3, 40.44, and 29.3%, respectively). This was followed by methanolic extract of *O. neovolkiana* (47.71%) and *C. versicolor* (45.68%). Extracts of *S. commune* recorded the minimum tyrosinase inhibitory activity when eluted with all the three solvents (24.65, 19.22, and 11.57%). Condensate concentration of 500 µg ml^{-1} and 100 µg ml^{-1} exhibited the highest and the lowest tyrosinase inhibitory activities [Table 2]. As tyrosinase inhibitory potential is known to be expressed in relation to antioxidant properties in all living systems, further experiments were conducted to test the antioxidant activities of mycomolecules.

Antioxidant Activities of Mycomolecules

Methanolic, acetonic, and water extracts of medicinal mushroom fungi evaluated in the previous experiment were used to test their antioxidant activities, and the results are presented in Table 3. Methanolic, acetonic, and water extracts of *G. lucidum* (79.08, 61.17, and 38.46%) were found to be significantly superior for their antioxidant activities as compared to other mushroom fungi. This was followed by methanolic, acetonic, and water extracts of *O. neovolkiana* (56.67, 34.6, and 23.77%), *L. edodes* (54.21, 46.02, and 21.50%), and *C. versicolor* (51.07, 37.69, and 24.16%), respectively. All the three extracts of *S. commune* registered comparatively less antioxidant activities (18.41, 11.61, and 5.39%).

DISCUSSION

Melanin formation due to tyrosinase activity is inhibited by certain molecules acquainted as tyrosinase inhibitors by means of competitive, uncompetitive, mixed (competitive or un competitive), and non-competitive types. Competitive inhibition is by combining with a free enzyme in a manner that precludes substrate binding whereas an uncompetitive inhibition is by binding only to the enzyme-substrate complex. Non-competitive inhibition involves binding to a free enzyme and an enzyme-substrate complex with

Table 1: Tyrosinase inhibitory potential of phytochemicals

Medicinal plants	Plant part used	Tyrosinase inhibition (%)*								
		Methanolic extract			Acetonic extract			Water extract		
		100 µg/ml	250 µg/ml	500 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml
A. vasica	Leaves	29.80^{c} (33.08)	40.46^{bc} (39.50)	53.42^{c} (46.96)	19.34^{d} (26.08)	25.70^{d} (30.46)	34.01^{f} (35.67)	4.46^{d} (12.19)	11.69^{d} (19.99)	19.75^{e} (26.38)
M. citrifolia	Fruits	21.26^{f} (27.45)	35.23^{d} (36.40)	54.28^{c} (47.45)	16.19^{e} (23.72)	30.46^{c} (33.49)	43.65^{cd} (41.35)	10.36^{bc} (18.77)	22.11^{b} (28.04)	35.30^{b} (36.45)
A. vera	Leaves	23.76^{e} (29.17)	39.17^{cd} (38.74)	49.05^{c} (44.45)	16.43^{e} (23.91)	28.93^{c} (32.53)	32.56^{f} (34.79)	9.33^{c} (17.78)	17.30^{c} (24.57)	25.48^{d} (30.31)
G. glabra	Bark	31.91^{b} (34.39)	52.19^{a} (46.25)	65.67^{ab} (54.13)	26.63^{b} (31.06)	39.08^{b} (38.69)	53.59^{b} (47.05)	11.00^{b} (19.36)	21.44^{b} (27.58)	29.71^{c} (33.02)
P. betle	Leaves	14.34^{g} (22.25)	27.04^{e} (31.33)	35.01^{d} (36.27)	19.45^{d} (26.16)	30.41^{c} (33.46)	41.84^{de} (40.30)	3.86d*e* (11.33)	6.50^{f} (14.77)	14.12^{f} (22.07)
C. limon	Peel	4.10^{j} (11.68)	9.15^{h} (17.60)	16.36^{f} (23.85)	1.41^{g} (6.81)	5.79^{f} (13.92)	9.17^{i} (17.62)	9.88^{c} (18.32)	17.92^{c} (25.04)	28.34^{cd} (32.16)
A. indica	Leaves	14.60^{g} (22.46)	22.86^{f} (28.56)	28.23^{e} (32.09)	9.65^{f} (18.09)	14.97^{e} (22.76)	21.83^{g} (27.85)	2.26^{f} (8.64)	5.93^{f} (14.09)	11.52^{g} (19.84)
M. alba	Leaves	28.40^{d} (32.20)	43.83^{b} (41.45)	60.80^{b} (51.23)	23.66^{c} (29.10)	35.94^{b} (36.83)	46.94^{c} (43.24)	3.41^{e} (10.64)	8.91^{e} (17.36)	14.14^{f} (22.08)
V. negundo	Roots	12.26^{h} (20.49)	21.37^{f} (27.53)	37.66^{d} (37.85)	9.26^{f} (17.71)	13.52^{e} (21.57)	17.23^{h} (24.52)	4.51^{d} (12.26)	10.48^{d} (18.88)	15.76^{f} (23.39)
C. longa	Rhizome	9.50^{i} (17.95)	14.54^{g} (22.41)	23.86^{e} (29.23)	8.04^{f} (16.47)	23.91^{d} (29.27)	39.15^{e} (38.73)	1.22^{g} (6.34)	3.58^{g} (10.90)	7.91^{h} (16.33)
L- ascorbic acid	-	41.62^{a} (40.17)	54.02^{a} (47.30)	67.58^{a} (55.29)	41.62^{a} (40.17)	54.02^{a} (47.30)	67.58^{a} (55.29)	41.62^{a} (40.17)	54.02^{a} (47.30)	67.58^{a} (55.29)
CD (*P*=0.05)		1.57	2.53	3.38	1.64	1.90	2.46	1.02	1.43	1.91

*Data in parenthesis are arcsine transformed values. Means in a column followed by the same letter are not significantly different at *P*=0.05 by one way ANOVA. *C. longa*: *Curcuma longa*, *V. negundo*: *Vitex negundo*, *M. alba*: *Morus alba*, *A. indica*: *Azadirachta indica, C. limon: Citrus limon, P. betle: Piper betle, G. glabra: Glycyrrhiza glabra, A. vera: Aloe vera, M. citrifolia: Morinda citrifolia, A. vasica: Adathoda vasica*

the same equilibrium constant.[15] Kojic acid, a fungal metabolite, inhibits the tyrosinase by showing competitive inhibitory effect on monophenolase and mixed inhibitory effect on diphenolase activity.[16] Methimazole inhibits mushroom tyrosinase by chelating copper at the active site of the enzyme and by conjugating with o-quinones.[17] As the egg stage is the commercial marketable stage with more enzymatic activity, sporocarps in egg stage were used for the enzyme source. In the present inquest, tyrosinase inhibitory prospect of phytochemicals and mycomolecules were concerted, and the results are presented in Tables 1 and 2. Among the different compounds, ascorbic acid showed the maximum tyrosinase inhibitory activity, on account of its capacity to reduce back, o-dopaquinone to dopa, thereupon avoiding dopachrome and melanin formation as pellucidly betokened by Chang.[18] Amid the phytochemicals, methanolic fraction of *G. glabra* expositioned maximum tyrosinase inhibitory potential of 65.6%. Perhaps, this may be attributed to the fact that isoflavonoids such as glabridin and glyasperin C are known to have melanogenesis inhibitory activity with non-competitive mode.[19,20] In accordance to Fu *et al.*,[21] chalcone derivatives including licuraside, isoliquiritin, and licochalcone A isolated from *Glycyrrhiza* roots also competitively inhibited the monophenolase activity of mushroom tyrosinase. Concomitant results have been reported by Vaibhav and Lakshaman[22] in testing the tyrosinase inhibitory activity of selected Indian herbs, of which methanolic extract of *G. glabra* showed more than 50% inhibition. Besides, tyrosinase inhibitory potential of the methanolic fraction of *M. alba* was also recorded to about 43.8%. This may be attributed to the presence of mulberroside F (moracin M-6, 3'-di-*O*-beta-D-glucopyranoside) in the leaves of *Morus sp* which might have shown antidiphenolase activity of mushroom tyrosinase and exhibited an inhibitory effect on melanin formation.[8] Oxyresveratrol (2, 4, 3, 5-tetrahydroxy-*trans*-stilbene) isolated from *M. alba* acts non-competitively on both monophenolase and diphenolase activity of mushroom tyrosinase.[23]

Among the mycomolecules tested, methanolic fraction of *G. lucidum* showed the maximum tyrosinase inhibition of 53.3%. Zhang *et al.*[24] prodigiously isolated methyl lucidenate F, a triterpenoid from *G. lucidum,* showed

Table 2: Tyrosinase inhibitory potential of mycomolecules

Fungi	Tyrosinase inhibition (%)*								
	Methanolic extract			Acetonic extract			Water extract		
	100 µg/ml	250 µg/ml	500 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml
G. lucidum	21.15^{c} (27.38)	41.98^{b} (40.38)	53.3^{b} (46.89)	19.34^{c} (26.08)	31.95^{b} (34.41)	40.44^{b} (39.48)	11.97^{b} (20.24)	20.5^{b} (26.92)	29.3^{b} (32.77)
C. versicolor	21.56^{c} (27.66)	33.12^{cd} (35.13)	45.68^{c} (42.52)	17.62^{c} (24.81)	28.97^{b} (32.56)	36.07^{c} (36.91)	7.71^{c} (16.12)	13.6^{bc} (21.64)	23.72^{c} (29.14)
P. albus	18.88^{c} (25.75)	28.9^{d} (32.51)	33.43^{d} (35.32)	12.4^{d} (20.61)	23.44^{c} (28.95)	29.33^{e} (32.79)	6.5^{d} (14.77)	16.71^{bc} (24.12)	22.94^{c} (28.61)
O. neovolkiana	28.84^{b} (32.48)	36.14^{c} (36.95)	47.71^{bc} (43.68)	22.69^{b} (28.44)	29.57^{b} (32.94)	33.99^{cd} (35.66)	12.11^{b} (20.36)	18.99^{b} (25.83)	26.45^{bc} (30.95)
O. sinensis	19.39^{c} (56.12)	28.9^{d} (32.51)	32.72^{d} (34.89)	10.47^{de} (18.87)	17.59^{d} (24.79)	25.38^{f} (30.25)	5.83^{d} (13.97)	11.24^{cd} (19.58)	15.14^{d} (22.89)
L. edodes	25.61^{b} (30.40)	33.12^{cd} (35.13)	41.85^{c} (40.30)	17.62^{c} (24.81)	25.19^{c} (30.12)	31.27^{de} (34.00)	12.41^{b} (20.62)	20.84^{b} (27.16)	26.07^{bc} (30.70)
S. commune	12^{d} (20.26)	18.42^{e} (25.41)	24.65^{e} (29.76)	9.66^{e} (18.10)	14.21^{e} (22.14)	19.22^{g} (26.00)	2.07^{f} (8.27)	7.64^{d} (16.04)	11.57^{e} (19.88)
L. esculentum	19.76^{c} (26.39)	22.53^{e} (28.33)	29.49^{de} (32.89)	11.88^{d} (20.16)	18.9^{d} (25.76)	24.39^{f} (29.59)	4.69^{e} (12.50)	10.43^{cd} (18.84)	15.04^{d} (22.81)
L- Ascorbic acid	41.62^{a} (40.17)	54.02^{a} (47.30)	67.58^{a} (55.29)	41.62^{a} (40.17)	54.02^{a} (47.30)	67.58^{a} (55.29)	41.62^{a} (40.17)	54.02^{a} (47.30)	67.58^{a} (55.29)
CD (*P*=0.05)	2.21	3.22	3.57	1.93	2.21	2.05	1.16	5.79	2.72

*Data in parenthesis are arcsine transformed values. Means in a column followed by the same letter are not significantly different at *P*=0.05 by one way ANOVA. *L. esculentum: Lycoperdon esculentum, O. sinensis: Ophiocordyceps sinensis, O. neovolkiana: Ophiocordyceps neovolkiana, S. commune: Schizophyllum commune, L. edodes: Lentinus edodes, P. albus: Pisolithus albus, C. versicolor: Coriolus versicolor, G. lucidum: Ganoderma lucidum*

Table 3: Antioxidant activities of mycomolecules

Fungi	Methanolic extract	Acetonic extract	Water extract
G. lucidum	79.08^{a} (62.78)	61.17^{a} (51.45)	38.46^{a} (38.32)
C. versicolor	51.07^{bc} (45.61)	37.69^{c} (37.87)	24.16^{b} (29.44)
P. albus	43.56^{c} (41.29)	25.50^{e} (30.32)	19.61^{de} (26.28)
S. commune	18.41^{e} (25.40)	11.61^{f} (19.92)	5.39^{f} (13.42)
L. esculentum	32.85^{d} (34.97)	24.58^{e} (29.72)	16.49^{e} (23.95)
O. neovolkiana	56.67^{b} (48.83)	34.96^{cd} (36.24)	23.77^{bc} (29.17)
O. sinensis	33.47^{d} (35.34)	29.18^{de} (32.69)	19.97^{cde} (26.54)
L. edodes	54.21^{b} (47.41)	46.02^{b} (42.71)	21.50^{bcd} (27.62)
CD (*P*=0.05)	8.53	6.34	3.99

*Data in parenthesis are arcsine transformed values. Means in a column followed by the same letter are not significantly different at *P*=0.05 by one way ANOVA. *L. edodes: Lentinus edodes, O. sinensis: Ophiocordyceps sinensis, O. neovolkiana: Ophiocordyceps neovolkiana, L. esculentum: Lycoperdon esculentum, S. commune: Schizophyllum commune, P. albus: Pisolithus albus, C. versicolor: Coriolus versicolor, G. lucidum: Ganoderma lucidum*

tyrosinase inhibitory activity with uncompetitive mode. *O. neovolkiana* also exhibited tyrosinase inhibition of 47.7%. From this, it is hypothesized that phenols in the mycelial extract might have inhibited the tyrosinase enzyme.[25] Mushrooms contain various polyphenols' and flavonoid compounds that are recognized as good antioxidants.[26] It is pertinent to note that the antioxidant activity of methanol, acetone, and water fractions of mycomolecules determined in the present study showed a positive trend with tyrosinase inhibition potential. As ascertained by Yoon *et al.*,[10] the antioxidant activity may also be one of the important tyrosinase inhibition mechanisms. The tyrosinase inhibition ability might depend on the hydroxyl groups of phenolic compounds that could form a hydrogen bond to the active site of enzyme leading to lower enzymatic activity.[27] Gallic acid, (-)-epicatechin, procyanidin B2 and (-)-epicatechin-3-gallate, flavonoids, isoflavonoids and phenols in mushrooms are cogently proved to have effective tyrosinase inhibitory conditioning.[6,28] It was alike recorded that the tyrosinase inhibitory activity of the methanol, acetone, and water fractions had escalated with the uptick in concentration of the respective fraction. Corresponding outcome on tyrosinase inhibition was attained with the acetonic, methanolic, and hot water extracts of *P. eryngii*.[9] Data in the present disquisition will furtherance our understanding on the tyrosinase inhibitory potential. Moreover, research by exploiting more innocuous

mycomolecules and phytochemicals may lead to accrued tyrosinase inhibition, thereby proliferating the commercial marketability of perishable mushrooms.

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