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Research Article

PHYTOCHEMICAL CHARACTERIZATION, *IN VITRO* AND *IN SILICO* STUDIES ON THERAPEUTIC POTENTIAL OF EDIBLE AND WILD MUSHROOMS

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ABSTRACT

Objective: The present study evaluates the *in vitro* and *in silico* analyses of mushrooms such as *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma wiiroense,* and *Pleurotus tuber-regium.*

Methods: The quantitative and qualitative analysis of the mushrooms was analyzed and their antioxidant, antimicrobial, and anti-inflammatory assays were done. In addition, molecular docking of their bioactive compounds was docked against the targeted proteins.

Results: The study found that the ethanolic extract of *Pleurotus ostreatus* contained high amounts of carbohydrates and the ethanolic extract of *G. wiiroense* contained high amounts of phenolics, and flavonoids. The hot water extract of *A. bisporus* showed antioxidant activity in phosphomolybdenum assay, while *G. wiiroense* showed the highest antioxidant activity in superoxide radical scavenging assay. The ethanol extract of the latter also showed maximum 2,2-Diphenyl-picrylhydrazyl radical scavenging and ferric-reducing antioxidant power (reducing activity). *A. bisporus* hot water extract showed the highest inhibitory activity against *Escherichia coli*, while *G. wiiroense* showed the highest anti-inflammatory activity. *In silico* analysis revealed that chlorogenic and ganodermic acids had high binding affinity toward protein targets.

Conclusion: The study compared the biological activities of commercial edible and wild mushrooms extracts, finding that active compounds from both mushrooms were effective against diseases such as cancer, tuberculosis, and rheumatoid arthritis through computational approaches.

Keywords: Mushroom, Antioxidant, Antimicrobial, Anti-inflammatory, Molecular docking.

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INTRODUCTION

Mushrooms are organisms that lack chlorophyll and get their nutrients from other sources. They have fruiting bodies that may be eaten or are not suitable for consumption. Mushrooms are considered to be the primary untapped source of nutritious meals. These foods have been included in the diet of Romans and Greeks from ancient times. The Romans revered mushrooms as divine sustenance, while the Chinese saw them as a life-prolonging elixir. Various civilizations have used them over extended periods of time [1]. Out of the 2000 species of mushrooms, only a few number have been identified as suitable for food. There are around 33 types of mushrooms that are grown for food worldwide. However, only three types, namely the white button mushroom (*Agaricus bisporus* L.), oyster mushroom (*Pleurotus ostreatus* L.), and paddy straw mushroom (*Volvariella volvacea* L.), are frequently farmed [2]. Edible mushrooms are often used as a primary resource for the production of nutraceuticals and pharmaceuticals that include antitumor, antioxidant, and antibacterial characteristics. Mushrooms not only have medicinal benefits but they also have a low fat, high protein, and low-calorie content [3]. The mushroom proteins include all the necessary amino acids required by humans, as well as many dietary elements including iron, phosphorus, and vitamins such as ascorbic acid, thiamine, riboflavin, niacin, and ergosterol [4]. *Ganoderma* P. Karst, belonging to the *Ganodermataceae* family in the *Polyporales* order, is a very varied and significant collection of untamed fungus often referred to as "Reishi" mushrooms [1]. *Ganoderma* species are significant plant infections that have economic importance since they cause illnesses in crops such as rubber and tea. Mushroom fruiting bodies are widely used in traditional medicine and have a longstanding history of usage in Asian nations such as China, Japan, and Korea. Wild mushrooms contain physiologically active chemicals that function as antioxidants, protecting the body from illnesses caused by oxidative stress. *Ganoderma* species are known to generate a variety of compounds including steroids, lignins, lectins, ganomycins, vitamins, nucleosides, nucleotides, alkaloids, amino acids, and triterpenes. These compounds include β-glucan, which is renowned for its potential to boost the immune system. Indeed, it is among the most potent immune system supplements. The chemicals mentioned exhibit anticancer, antibacterial, immunomodulatory, anti-inflammatory, antiviral, and antioxidant properties [5].

A computational technique is a dependable strategy for identifying active phytocompounds from diverse datasets. At present, this method is being used in the field of drug discovery research. The current work throws the light on the comparative examination of commercially cultivated mushrooms and mushrooms found in the wild, specifically focusing on wild species, in India. This study goes over and compares their antioxidant, antibacterial, and anti-inflammatory characteristics. The research also performs molecular docking analyses on bioactive chemicals, possibly assisting in the identification of new drugs for skin cancer and rheumatoid arthritis. Further investigation into the potential of mushrooms is dependent on this research.

METHODS

Collection of samples

The edible commercial mushrooms were bought from the local market in Vadavalli, Coimbatore, Tamil Nadu, and the wild mushrooms were collected from Marudhamalai Hills, Coimbatore, Tamil Nadu. The collection site was at an altitude of 450 m Mean Sea Level, with coordinates of 76° 51′ 46.8″ E longitude and 11° 2′ 38.4″ N latitude.

The identification of the mushrooms was carried out by the use of macroscopic and microscopic studies, as described by [6,7] in department of Botany, Bharathiar University, Coimbatore and in addition, we did molecular identification of the wild mushrooms using DNA sequencing method in Rajiv Gandhi Centre for Biotechnology, Kerala [8].

Preparation of powder and extracts

The mushrooms were powdered after being cleaned and allowed to air dry. A mushroom sample weighing about 10 g was measured and then dissolved in 100 mL of hot water and 100 mL of ethanol separately. The solutions were then held at a temperature of 25°C for a duration of 72 h. Afterward, the solutions were centrifuged at a speed of 3000 rpm for 15 min. The resulting extract was filtered and then allowed to evaporate. Finally, the extract was stored at a temperature of 4°C for future use.

Qualitative and quantitative analyses

A preliminary phytochemical examination was performed on the extracts. Active substances such as carbohydrates (Benedict's test), proteins (Ninhydrin test), starch (Iodine test), alkaloids (Mayer's test), phenolics (Ferric chloride test), flavonoids (Alkaline Reagent test), and steroids (Salkowski test) were checked for their existence. The quantification of carbohydrates and starch was conducted using the Anthrone technique. The Lowry technique was used to measure protein, while a colorimetric approach was utilized to evaluate flavonoids. The quantification of phenolics was performed using the Folin–Ciocalteu technique. Furthermore, the extracts were analyzed for total ash, lipid, and crude fiber content using the procedures described by [9-11].

Screening of antioxidants

Phosphomolybdenum assay

A mushroom sample weighing about 100 mg was mixed well with 80% ethanol and then subjected to centrifugation at 3000 rpm for a duration of 10 min. The resulting liquid portion, known as the supernatant, was carefully collected and diluted with distilled water to a final volume of 10 mL. Subsamples were made in addition to a standard solution of ascorbic acid. 1 mL of reaction mixture, consisting of 0.6 M H_2SO_4, 28 mM sodium metaphosphate, and 4 mM ammonium molybdate, was added to all the tubes, including the blank. The tubes were then vortexed and placed in a water bath at 95°C for 90 min. The spectrophotometer measured the absorbance at a wavelength of 695 nm. The absorbance value was then represented as a milligram of ascorbic acid equivalents per gram of the sample, as described by [12].

Superoxide radical scavenging activity

About 100 mg of mushroom sample was homogenized using 75% ethanol and centrifuged at 3000 rpm for 10 min, and the supernatant was collected and made up to 10 mL using ethanol. Aliquots were prepared and 3 mL of reagent mixture was added including the blank and the standard (rutin). The mixture was vortexed well and the absorbance was read at 590 nm using a spectrophotometer [13].

$$\text{Scavenging activity}(\%)=\left[\frac{(\text{Control OD - Sample OD})}{\text{Control OD}}\right]\times 100$$

2,2-Diphenyl-picrylhydrazyl radical ($DPPH^+$) scavenging activity

Various concentrations of the extracts (hot water and ethanol) and the standard (rutin) were taken and made up to equal volume using methanol. Then, 5 mL of 0.1 mM methanolic solution of DPPH was added and incubated for 20 min at 27°C. Methanol alone served as the blank, while 100 µL of methanol along with 5 mL of DPPH solution served as the negative control. The reduction of purple color to pale yellow color confirmed the scavenging activity. The percentage inhibition was found as follows: [14].

$$\text{Percentage Inhibition}(\%)=\left[\frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}}\right]\times 100$$

Ferric-reducing antioxidant power (FRAP) assay

Different concentrations of the extracts (hot water and ethanol) and the standard were taken and made up to equal volume using distilled water. Aliquots were prepared from both the extracts and 900 µL of FRAP reagent (A-20 mM TPTZ in 40 mM HCl, B-20 mM $FeCl_3$, C-25 mL of 0.2 M acetate buffer (pH 3.6) using 0.2 M acetic acid and 0.2 M sodium acetate) was added in all the test tubes including blank. The FRAP reagent was mixed in the order C, A, and B. The resulting solution was vortexed and incubated at 37°C for 30 min. The absorbance was read at 593 nm using a spectrophotometer and it was expressed as mmol Fe(II) equivalent/mg extract [15].

Antimicrobial activity

A slightly modified Mueller–Hinton broth was used to test the antimicrobial potential. Four different strains, namely *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa,* were used. The strains were inoculated in the Nutrient Broth (NB) media and incubated at 37°C for 24 h. The procedure was carried out by adding 5 mL of NB media in test tubes followed by the addition of 20 µL of broth culture of different strains. The samples of different concentrations (12.5, 25, 50, 100, and 200 µL) were added and incubated in the shaker overnight. The antimicrobial test used streptomycin at a concentration of 30 µg/mL as the positive control. The turbidity formed on the broth media was read at 660 nm using an ultraviolet (UV)-Vis spectrophotometer [16].

Anti-inflammatory activity

The *in vitro* anti-inflammatory activity of the samples was assessed using the membrane stabilization technique [17]. Aliquots were prepared and the sodium salt of diclofenac was used as the reference component. The Alsever's solution was formulated by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in distilled water. The resulting solution was then sterilized using an autoclave. The aseptic solution was combined with an equivalent amount of obtained goat blood (readily obtainable) and subjected to centrifugation at a speed of 3000 rpm for a duration of 10 minutes. The liquid portion was extracted and rinsed three times with a 0.9% isosaline solution at a pH of 7.2. The red blood cell (RBC) suspension was made by combining an equivalent amount of 10% isosaline solution. The reaction mixture was formulated by combining 1 mL of phosphate buffer (pH 7.4), 2 mL of hyposaline (0.45%), 1 mL of sample or standard solution, diclofenac (1 mg/mL), and 0.5 mL of RBC suspension. A control was prepared using a reaction mixture without the sample, and a blank was prepared using a reaction mixture without the phosphate buffer. The test mixture was subjected to incubation at a temperature of 37°C for a duration of 30 min, followed by another round of centrifugation. The concentration of hemoglobin in the liquid remaining after centrifugation was determined using a spectrophotometer at a wavelength of 560 nm. The calculation of percentage inhibition was performed using the following method:

$$\text{Percentage Inhibition}(\%)=\left[\frac{\left(\begin{array}{l}\text{Absorbance of control} - \\ \text{Absorbance of treated sample}\end{array}\right)}{\text{Absorbance of control}}\right]\times 100$$

Determination of compounds by high-performance liquid chromatography (HPLC)

HPLC was performed on a Shimadzu preparative and analytical HPLC system with a binary solvent pump and a photodiode array detector.

This technique was used to separate molecules based on their structure and composition. The analytical procedure was performed by applying the chromatographic conditions. Water (solvent A) and water: acetonitrile:acetic acid in 1:1:1 ratio (solvent B) were used as mobile phases. Gradient elution was used for the analysis of the sample. The run was started with solvent B and increased to 35% in 25 min, 50% in 45 min, and 100% in 60–65 min at 200–400 nm. The flow rate was maintained at 1 mL/min and 20 µL of sample was injected for the analysis. Standard solutions were injected separately to obtain the retention time for each compound and were calculated by comparing the sample with the standard chromatogram.

Drug-like properties of the ligands

Lipinski's rule of five factors, molecular weight <500 Da, no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, and LogP less than five indicate a molecule's druglikeness. Lipinski's rule of five was used to calculate the properties of active chemicals based on the SwissADME prediction [18].

Visualization and analysis of molecular docking

AutoDock 4.2 was used to dock the protein and ligand together. The protein was treated by eliminating irrelevant complex molecules, removing water, adding hydrogen, and providing Kollman charges. The ligand's Gasteiger charges were assigned, and rotatable bonds were detected. The structures of the proteins and ligands were stored in the PDBQT file format. Autogrid was applied to the whole molecule, with the default grid spacing of 0.375 Å and the grid size adjusted in the X, Y, and Z dimensions. For docking, the Lamarckian Genetic Algorithm (GA 4.2) was employed, which returns the best ten estimated scores of binding free energies for each trial. The protein–ligand interactions were analyzed, and the visualization was accomplished with Discovery

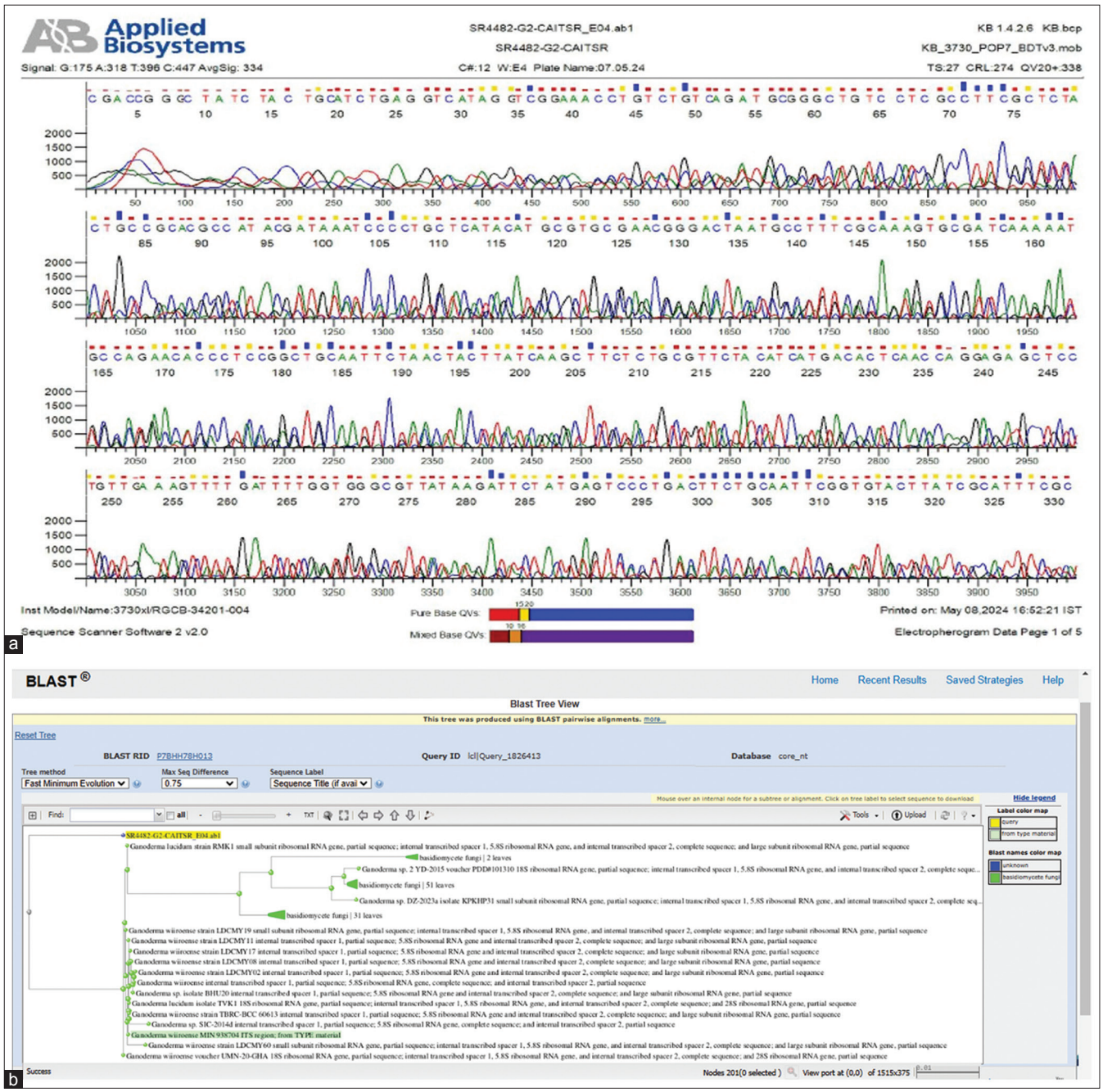

Fig. 1: The molecular identification of Wild mushrooms *Ganoderma wiiroense* (a and b), The molecular identification of wild mushrooms and *Pleurotus tuber-regium* (c and d)

Fig. 1: ***(Continued)***

Studio. Discovery Studio Visualizer was also used to produce the docked positions and 2D interaction plots. For the current studies, we chosen 2AXJ (rheumatoid arthritis), 5OTF (Skin Cancer), and 4KXR (Tuberculosis) as protein targets for respective diseases and the mushrooms compounds obtained from the HPLC analysis results were chosen as ligands for molecular docking.

RESULTS

Sample identification

The commercial mushrooms are identified as *A. bisporus* (J.E. Lange) Imbach and *P. ostreatus* (Jacq.) P. Kumm and the wild mushrooms are identified as *Pleurotus tuber-regium* (Fr.) Singerand and *Ganoderma wiiroense* E.C. Otto, Blanchette, C.W. Barnes & Held. (Fig. 1a and b shows the molecular identification of mushrooms).

Preliminary assays

The preliminary qualitative tests on hot water and ethanolic extracts of *A. bisporus, P. ostreatus, P. tuber-regium,* and *G. wiiroense* confirmed the presence of bioactive compounds. The phytochemical study demonstrated the presence of carbohydrates, proteins, starch, phenolics, steroids, alkaloids, and flavonoids in all of the chosen mushroom extracts. Commercially cultivated edible mushrooms often

have higher levels of phytochemicals in comparison to wild mushrooms. Table 1 shows the results of preliminary qualitative tests.

Quantitative analysis

The quantitative analysis of primary and secondary metabolites revealed that the amount of carbohydrates (40.02 mg glucose equivalent/g), starch (32.20 mg glucose equivalent/g), ash content (17 mg/100g), and fiber content (28%) obtained from ethanolic extract of *P. ostreatus* was the highest. Furthermore, protein (77.33 mg BSA equivalent/g) was high in ethanolic extract of *A. bisporus* and phenolics (37.96 mg gallic acid equivalent/g) and flavonoid (19.6 mg rutin equivalent/g) was high in ethanolic extract of *G. wiiroense*, whereas lipid content (10%) was high in both *A. bisporus* and *G. wiiroense* as shown in Fig. 2. From the above quantitative results, commercially edible mushrooms have a high amount of major primary and secondary compounds compare with wild mushrooms.

***In vitro* antioxidant activities of wild and commercial mushrooms**

Antioxidants are first-line defence against the damage caused by free radicals. They act as health-promoting agents. The DPPH assay is often used to assess the antioxidant capacity of plant extracts/compounds by quantifying their capacity to function as scavengers of free radicals. The IC_{50} value, which represents the substrate concentration at which 50% of DPPH is lost, is used for the interpretation of the test findings. The ethanol extract of G1 exhibited the highest IC_{50} values for scavenging DPPH radicals, with a value of 37.77 µg/mL. Its scavenging capacity was found to be greater than that of ascorbic acid, which had a value of 40.77 µg/mL (Table 2). The order of the DPPH scavenging activity was G1 > AB > P1 > PO. The phosphomolybdenum an antioxidant activity indicates that the hot water extract of *A. bisporus* has a significant level of antioxidant activity, measuring 93.42 mg of Ascorbic acid equivalents per g (Fig. 3). Table 3 displays the IC_{50} values of the chosen mushroom extracts for their ability to scavenge superoxide oxide. The hot water extract of G1 demonstrated potent scavenging action against the superoxide oxide radical, with an IC_{50} value of 54.75 µg/mL. In comparison, the standard rutin exhibited an IC_{50} value of 68.51 µg/mL. The order of superoxide oxide scavenging activity for the chosen mushroom extracts was G1 > PO > P1 > AB. In the FRAP assay (Table 4), an antioxidant activity indicates that the ethanol extract of *G. wiiroense* has a significant level of antioxidant activity, measuring 25.27 mg of mmol Fe (II) equivalent/mg.

***In vitro* anti-inflammatory activity of wild and commercial mushrooms**

The membrane stabilization method was used to determine the *in vitro* anti-inflammatory activity of the mushroom samples. The maximum IC_{50} value for anti-inflammatory activity was exhibited by *P. ostreatus* at 53.74 µg/mL, which was followed by P1. The IC_{50} values for standard diclofenac in 5 mg/mL was 56.42 µg/mL, respectively, as shown in Fig. 4.

Table 1: Qualitative test analysis

Phytochemical constituents	Tests	*Agaricus bisporus*	*Pleurotus ostreatus*	*Ganoderma wiiroense*	*Pleurotus tuber-regium*
Carbohydrates	Benedict's test	++	++	+	+
Proteins	Ninhydrin test	++	+	+	++
Starch	Iodine test	+	++	+	+
Phenolics	Ferric chloride test	+	+	++	+
Steroids	Salkowski test	+	+	+	+
Alkaloids	Mayer's test	++	++	+	++
Flavonoids	Alkaline reagent test	++	+	++	+

+Present, ++Strongly present

Table 2: 2,2-Diphenyl-picrylhydrazyl radical ($DPPH^{+}$) scavenging activity

Sample		% inhibition					IC_{50} value
Mushroom	Extract	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL	
Agaricus bisporus (AB)	Hot water	7.25±0.7	26.27±0.54	47.69±1.57	58.44±1.07	80.03±0.55	45.71
	Ethanol	25.25±0.35	38.9±0.78	54.01±1.36	66.38±1.25	89.67±0.95	53.79
Pleurotus ostreatus (PO)	Hot water	12.62±0.39	23.12±0.99	28.92±1.26	63.22±1.03	81.14±0.76	69.29
	Ethanol	15.10±0.96	24.48±1.03	43.17±1.13	58.95±0.76	78.32±1.09	67.49
Pleurotus tuber-regium (P1)	Hot water	18.68±0.32	32.50±1.16	53.32±0.87	59.47±0.76	86.17±0.83	60.01
	Ethanol	18.25±0.78	38.39±1.86	43.43±1.34	63.82±0.66	69.96±0.77	65.02
G. wiiroense (G1)	Hot water	36.09±0.55	44.96±0.23	57.5±0.3	74.31±0.74	89.84±0.78	44.58
	Ethanol	32.25±1.98	53.15±0.66	73.03±0.24	83.19±0.77	96.92±1.01	37.77
Standard	Ascorbic acid	24.57±1.11	50.46±0.26	78.69±0.53	86.36±0.04	91.53±0.6	40.77

*All values are mean±SD of three determinations. SD: Standard deviation

Table 3: Superoxide radical scavenging activity

Sample		% inhibition					IC_{50} value
Mushroom	Extract	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL	
Agaricus bisporus (AB)	Hot water	4.01±1.56	17.77±0.72	39.44±1.87	65.25±0.03	78.21±0.92	69.27
	Ethanol	2.86±0.94	24.42±0.61	46.1±1.2	58.83±0.48	80.84±0.57	67.81
Pleurotus ostreatus (PO)	Hot water	13.46±1.22	35.89±0.27	50.85±0.87	57.26±1.28	75.96±0.63	64.53
	Ethanol	22±0.32	26.2±0.71	52.67±0.16	64.90±1.05	77.5±0.78	61.78
Pleurotus tuber-regium (P1)	Hot water	24.73±0.16	35.84±0.66	53.68±0.73	58.22±1.03	66.45±0.26	64.18
	Ethanol	25.32±0.65	26.49±1.13	51.28±0.25	55.92±0.86	68.10±0.19	67.94
G. wiiroense (G1)	Hot water	11.92±1.56	47.82±0.21	57.79±0.12	72.13±0.61	81.88±0.61	54.75
	Ethanol	17.66±1.23	42.08±0.86	50.8±0.01	64.56±0.55	86.35±0.11	57.15
Standard	Rutin	9.6±0.56	31.2±0.33	40.8±0.54	64±0.16	71.2±1.17	68.51

*All values are mean±SD of three determinations. SD: Standard deviation

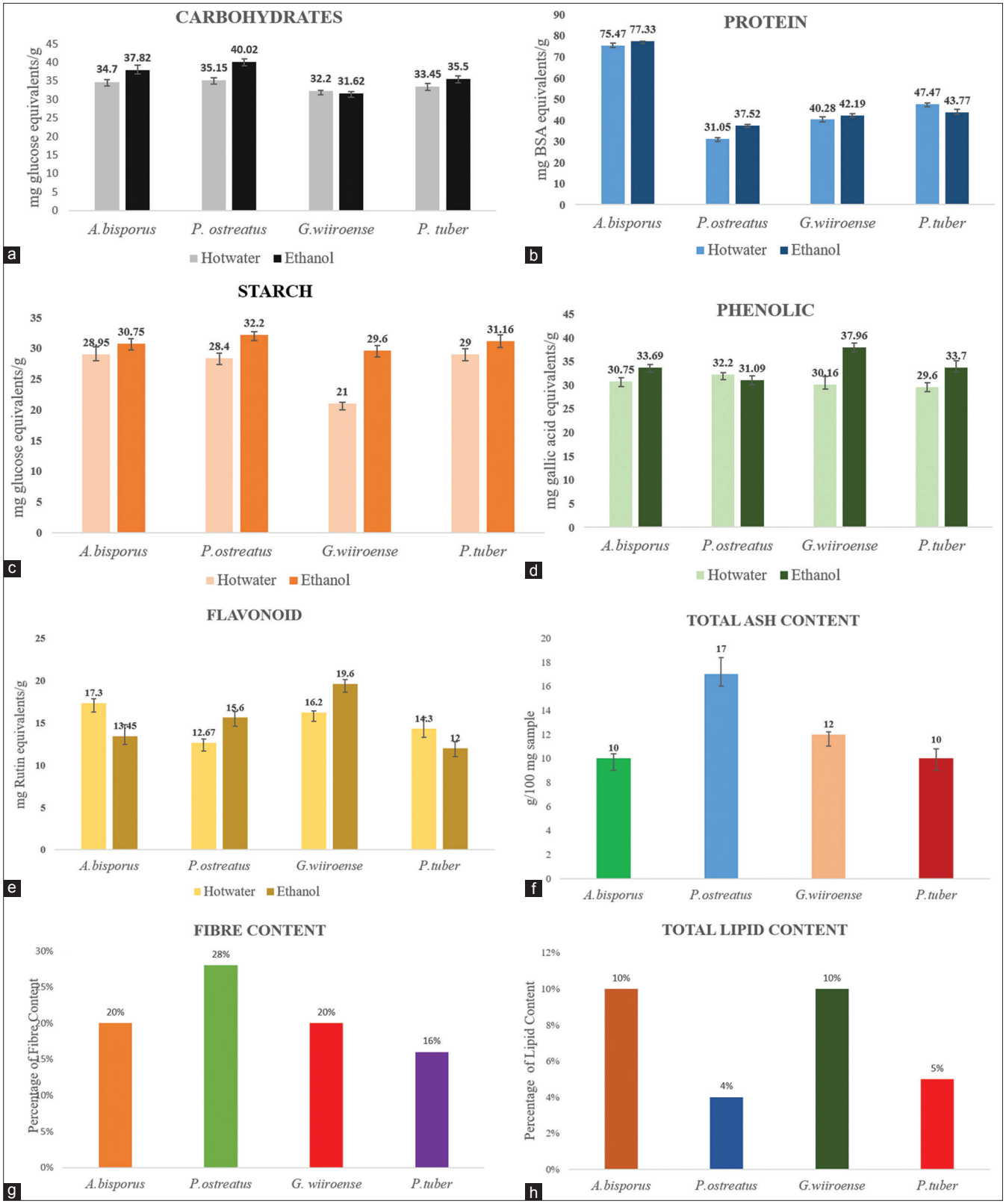

Fig. 2: Quantitative analysis of commercial and wild mushrooms – (a) carbohydrate estimation, (b) protein estimation, (c) starch estimation, (d) Phenolic estimation, (e) flavonoid estimation, (f) total ash content estimation, (g) fiber content estimation, and (h) total lipid estimation

Antimicrobial activity

The antimicrobial activity of the samples obtained from two different extracts was detected using minimum inhibitory concentration assay. Two Gram-negative strains of *E. coli* (MTCC 732) and *P. aeruginosa* (MTCC 424) and two Gram-positive bacteria of *B. subtilis* (MTCC 1133) and *S. aureus* (MTCC 3160) were taken. Comparing the four

mushroom species, the maximum inhibitory activity was shown in the hot water extract of *A. bisporus* in *E. coli* (87.28±0.12%) and positive control Streptomycin shows maximum activity against *P. aeruginosa* (39.48±0.84%) (Table 5).

HPLC analysis of mushroom extracts

The results of HPLC analysis of *A. bisporus*, *P. ostreatus*, *G. wiiroense,* and *P. tuber-regium* extracts at 254 nm show the presence of various constituents (Fig. 5 and Table 6). From that, ascorbic acid, mannitol, and glutamic acid are primary metabolites and cinnamic acid, ascorbic acid, rutin, gallic acid, ganodermic acid, quercetin, lovastatin, cholorogenic acid, and *P*-coumaric acid are belongs to secondary metabolites which are obtained and quantified from the HPLC analysis.

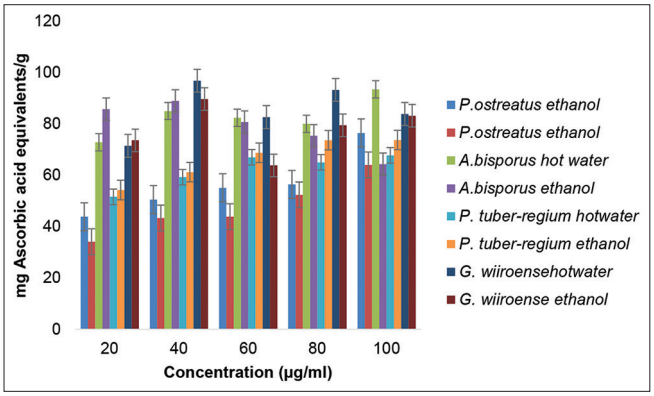

Fig. 3: Phosphomolybdenum assay

In silico studies of wild and commercial mushrooms

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies

The ADMET profile is critical in the pharmacy sector and is commonly utilized in CADD to decrease unwanted effects. (Table 7) shows Lipinski's rule of five for docking compounds calculated using the SWISSADME model. The majority of the drugs do not exceed the five characteristics, although chlorogenic acid has one violation and ganodermic acid has two violations in Lipinski's rule of five, indicating their druglikeness. Chlorogenic acid is a prevalent polyphenol chemical found in many foods consumed by humans. It is a kind of phenolic secondary metabolite generated by certain plants and is a significant constituent of coffee, biological characteristics, such as antimicrobial, antioxidative, and anticarcinogenic effects. Chlorogenic acid's responsibilities and uses, especially in regard to glucose and lipid metabolism, have recently been emphasized. It has substantial efficacy in preventing cardiovascular diseases, type 2 diabetes, and diseases associated with inflammation and Ganodermic acid, derived from the fungus Ganoderma species, acts as a membrane-active substance, and has various impacts on human platelet function. Hence, for further *in silico* studies, we took these two compounds.

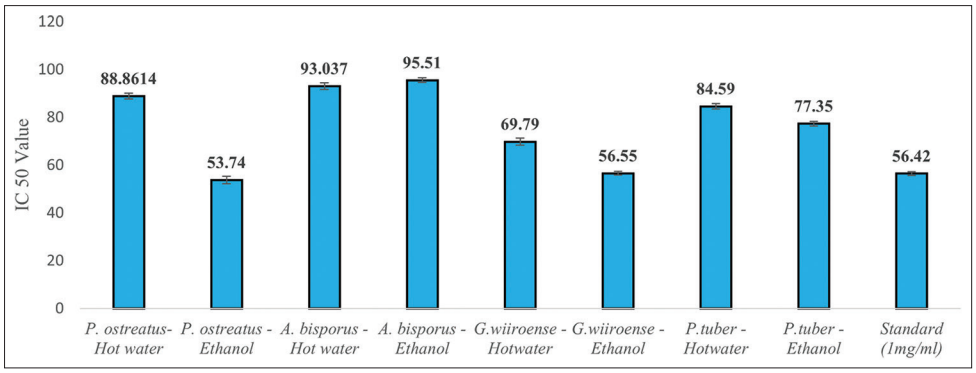

Fig. 4: _In vitro_ anti-inflammatory activity of wild and commercial mushroom

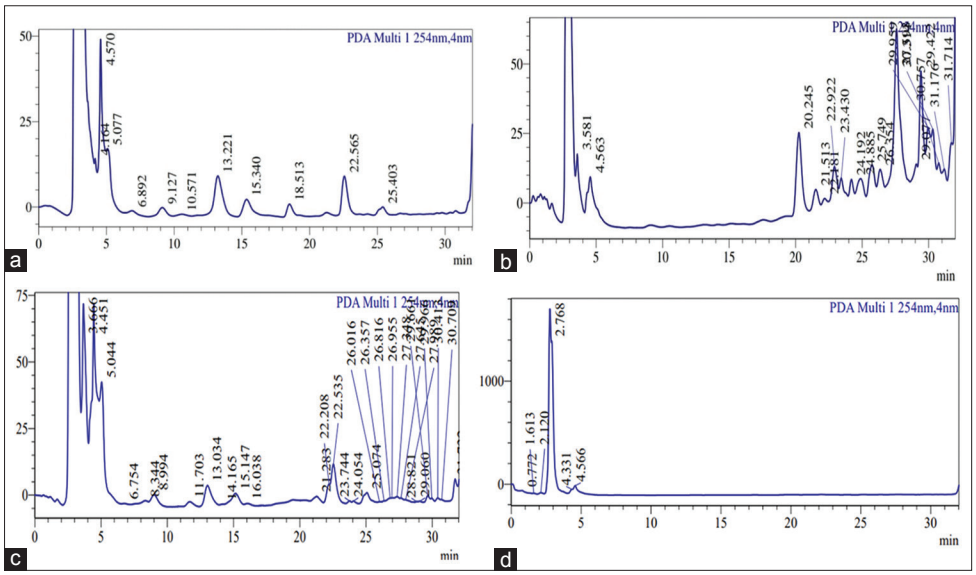

Fig. 5: High-performance liquid chromatography analysis of ethanolic extract of (a) *Agaricus bisporus*; (b) *Ganoderma wiiroense*; (c) *Pleurotus ostreatus;* and (d) *Pleurotus tuber-regium*

Table 4: Ferric-reducing antioxidant power assay

Sample		% inhibition					IC_{50} value
Mushroom	**Extract**	**20 µg/mL**	**40 µg/mL**	**60 µg/mL**	**80 µg/mL**	**100 µg/mL**	
Agaricus bisporus (AB)	Hot water	42.12±0.63	55.77±1.8	66.91±1.12	87.18±0.64	97.89±0.56	32.05
	Ethanol	43.27±0.28	50.63±0.41	56.61±0.47	61.76±0.81	66.28±0.94	40.00
Pleurotus ostreatus (PO)	Hot water	8.08±0.12	29.09±0.67	54.72±0.66	70.16±1.54	93.27±1.12	58.98
	Ethanol	3.25±1.26	25.21±0.97	41.49±0.87	54.41±1.25	83.82±0.93	68.78
Pleurotus tuber-regium (P1)	Hot water	5.77±0.58	17.12±0.78	44.95±0.68	55.77±0.87	87.9±1.02	67.62
	Ethanol	9.13±0.87	21.11±0.77	30.25±0.73	48.10±0.77	69.95±0.33	55.33
Ganoderma wiiroense (W1)	Hot water	47.58±0.09	51.68±0.89	56.19±0.53	59.87±0.37	63.23±0.27	31.05
	Ethanol	47.79±0.51	56.4±0.32	61.76±0.21	68.8±1.41	77.41±0.18	25.27
Standard	$FeSO_4.7H_2O$	3.36±0.11	23.1±1.18	45.79±0.73	50.42±0.06	61.55±0.44	60.91

*All values are mean±SD of three determinations. SD: Standard deviation

Table 5: Antimicrobial activity of mushroom extracts (% inhibition values)

Mushroom	Extract	Concentration (µL/mg)	*Escherichia coli*	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Pleurotus ostreatus	Hot water	12.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		25	06.21±0.03	10.81±0.09	16.21±0.13	11.11±0.22
		50	11.39±0.26	18.65±0.39	11.93±0.98	18.44±0.78
		100	15.42±0.62	26.77±0.23	28.37±0.64	22.10±0.51
		200	23.28±0.23	32.36±0.09	32.06±0.12	18.01±0.63
	Ethanol	12.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		25	06.21±0.03	15.81±0.09	06.21±0.23	13.11±0.22
		50	14±0.79	18.46±1.36	12.43±0.83	20.39±0.99
		100	32.36±0.22	30.46±0.91	36.99±1.21	32.54±0.68
		200	45.86±0.89	48.46±0.46	42.77±0.63	45.64±0.33
Agaricus bisporus	Hot water	12.5	29.88±0.85	29.45±0.19	13.26±0.57	10.77±0.39
		25	33.19±0.89	36.79±0.31	4.47±0.12	32.93±0.96
		50	40.53±0.15	39.44±0.59	61.99±0.7	40.88±0.42
		100	63.18±0.31	59.42±0.69	8.34±0.2	9.46±0.23
		200	87.28±0.12	76.45±0.57	12.51±0.65	34.13±0.81
	Ethanol	12.5	6.86±0.98	15.61±0.59	41.92±0.57	35.85±0.16
		25	16.25±0.6	19.21±0.6	47.28±0.49	36.40±0.18
		50	19.28±0.97	29.86±0.53	52.4±0.78	40.24±0.71
		100	39.70±0.89	31.95±0.54	54.22±0.36	56.79±0.81
		200	43.62±0.98	40.09±0.55	59.2±0.81	75.32±0.37
Pleurotus tuber-regium	Hot water	12.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		25	08.21±0.43	04.01±0.17	06.21±0.43	0.81±0.12
		50	15.32±1.02	18.11±0.88	14.40±1.32	10.89±0.26
		100	21.49±0.27	21.02±0.69	23.18±1.12	15.70±0.86
		200	30.04±0.86	28.49±0.67	32.18±1.22	19.30±0.79
	Ethanol	12.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		25	08.21±0.53	14.01±0.17	0.31±0.43	0.91±0.34
		50	16.73±0.73	21.11±1.03	26.51±0.57	19.18±0.83
		100	24.80±0.68	36.54±0.75	39.52±0.43	28.89±0.19
		200	39.17±0.35	42.19±0.57	42.55±0.12	39.16±0.26
Ganoderma wiiroense	Hot water	12.5	8.89±0.91	45.05±0.69	20.86±0.24	8.35±0.87
		25	29.67±0.87	46.38±0.79	22.95±0.64	42.69±0.21
		50	44.98±0.35	51.88±0.86	32.33±0.69	42.69±0.39
		100	52.74±0.3	82.16±0.14	55.73±0.92	60.82±0.46
		200	74.45±0.76	64.22±0.49	37.85±0.33	80.06±0.96
	Ethanol	12.5	3.59±0.35	12.56±0.96	14.02±0.32	12.71±0.51
		25	5.63±0.17	17.41±0.27	27.90±0.48	32.18±0.18
		50	16.66±0.67	19.21±0.86	31.22±0.63	58.22±0.21
		100	17.56±0.27	37.22±0.19	38.30±0.93	65.89±0.28
		200	65.6±0.51	43.08±0.05	44.11±0.06	74.01±0.89
Streptomycin		30	30.78±0.76	27.98±0.94	36.45±1.36	39.48±0.84

*All values are mean±SD of three determinations. SD: Standard deviation

Molecular docking

The molecular docking investigations of different mushroom compounds with three different protein targets were carried out. The binding modes of the ligands were sorted by their free binding energy according to the AutoDock 4.0 server. The free binding energy associated with each docked position is shown in Figure and the docking scores of the mushroom compounds with the targets are shown in Table 8.

In terms of low binding energy, chlorogenic acid [−7.1 Kcal/mol (2AXJ), −6.0 Kcal/mol (5OTF), −6.3 Kcal/mol (4KXR)] has the lowest value. This is closely connected to the number of non-covalent interactions; these drugs have with the surrounding residues in the active site of the protein target. Although all compounds act as potential compounds for protein targets, from the estimation of binding energy, chlorogenic acid from wild mushroom shows the highest binding affinity toward the protein targets.

Table 6: HPLC analysis results

S. No	Mushroom	Compounds	Retention time
1.	*Agaricus bisporus*	Cinnamic acid	4.164
		Ascorbic acid	5.0
		Mannitol	9.1
		P-coumaric acid	13.2
		Rutin	18.5
2.	*Ganoderma wiiroense*	Mannitol	3.58
		Gallic acid	4.563
		ganodermic acid	22.922
		Quercetin	24.885
		Ganodermic acid	29.422
3.	*Pleurotus ostreatus*	Mannitol	3.66
		Glutamic acid	4.45
		Lovastatin	8.34
		P-coumaric acid chlorogenic acid	13.03
			16.03
4.	*Pleurotus tuber-regium*	Chlorogenic acid	2.768
		Glutamic acid	4.331

Fig. 6 illustrates the docked position of the lowest energy conformers of two molecules, chlorogenic acid and ganodermic acid, as well as their related 2D interaction graphs. The docked postures conclusively illustrate the interactions between the drug molecules and the active region of the macromolecular structure.

Chlorogenic acid interacts with 4KXR protein with van der Waals force (TRP167), π-alkyl (VAL85), π-σ (LEU164), and conventional hydrogen bond (LEU164 and SER23). The bioactive compound binds with 2AXJ protein through conventional hydrogen bonds (GLN211, GLU156, THR172, TRP159). It forms van der Waals interactions with ILE234, GLN213, VAL212, LEU157, VAL170, VAL166, and GLU165. Further, carbon-hydrogen bond type of interaction has been found between chlorogenic acid and SER158, HIS167. In the case of 5OTF, the compound forms a conventional hydrogen bond with THR239, LYS202, and ARG84 and interacts through van der Waals force with PHE87, THR118, GLY85, ASP204, VAL237, ASP218, GLY238, SER221, ASP200 residues and forms π-σ bond with ALA86.

Ganodermic acid binds with 4KXR through conventional hydrogen bonds with GLY 108, and through van der Waals force with following residues: SER107, LYS11, GLN8, GLN 213, SER9, THR7, SER24, THR109, TYR215, PRO10, GLN213, THR232. In case of 2AXJ protein, the binding is through van der Waals force with ALA27, MET24, TYR153, PRO166, TRP167, TRP31, ILE89, THR165, VAL157, VAL85, LEU164, LEU161, ILE92. The compound interacts with 5OTF protein with conventional hydrogen bond (ARG412, THR405, ARP11), carbon-hydrogen bond (TRP19, HIS140), van der Waals force (GLU406, GLY413), alkyl bond (CYS133), and π-alkyl bond (ALA138).

In this study, blind docking was performed for ten compounds, namely chlorogenic acid, glutamic acid, lovastatin, *p*-coumaric acid, ganodermic acid, quercetin, vanillin, gallic acid, cinnamic acid, and ascorbic acid as ligands against three protein targets (PDB ID: 4KXR, 2AXJ, and 5OTF). From the estimation of binding energy of compounds extracted from the mushroom species, chlorogenic acid and ganodermic acid show more effective docking with all the three target proteins compared to other compounds.

DISCUSSION

For thousands of years, our ancestors have utilized mushrooms, rich in medicinal properties, as remedies for treating various diseases. In addition, they display good phytochemical activities [19]. The presence of polysaccharide (carbohydrate)-protein complex contents in the mushroom extract has great therapeutic applications on human health [5]. About 90% of the weight of most mushrooms is water. The remaining 10% is made up of 10–40% protein, 2–8% fat, 3–28% carbohydrate, 3–32% fiber, 8–10% ash, and some vitamins and minerals [20]. Proteins are essential for maintaining the structure and function of life for the growth and development. High level of proteins in mushrooms increases their commercial value and it is possible for isolating high amount of protein-based bioactive compounds from mushrooms [21]. The work done by [22] shows that in mushrooms, the amount of protein content is higher than carbohydrates. Mushrooms with low-fat and high fiber content tend to have higher nutraceutical properties.

The experiment done by Gogavekar *et al.* [23] estimated 29.3 g proteins, 62.97 g carbohydrates, 6.82 g total ash, 12.3 g crude fiber, and 0.91 g crude fat in *P. ostreatus*. Two commercial mushroom varieties, namely *A. bisporus* and *P. ostreatus,* were analyzed for their nutritional content. *P. ostreatus* shows higher amount of crude fiber and crude protein content. Both the varieties display no significant difference in their energy levels but contain low phytic acid and oxalate [24]. These results are similar to our present results of commercially edible mushrooms. The research on *Ganoderma* species shows the presence of non-volatile ingredients including 1.8% ash, 26–28% carbohydrate, 3–5% crude fat, 59% crude fiber, and 7–8% crude protein [20]. Similar results were found for *Ganoderma* species in the current work.

Antioxidants act as a first line of defense against free radical damage and are critical for maintaining the optimum health and well-being of an organism. They can deactivate the free radicals and prevent attack on the cells. The living system itself produces low amounts of antioxidants but they are not capable of destroying the free radicals. Hence, an external source is required for the body [1]. Macrofungi are enriched with bioactive compounds, which are used to prevent various diseases by diminishing oxidative damage. The antioxidant compounds extracted from wild mushrooms such as phenolics, tocopherols, ascorbic acid, and carotenoids help fight against the damage caused by free radicals [25]. Phenolics are potent antioxidants and free radical scavengers, and they have act against UV radiation and pathogens. Phenolic compounds have anti-inflammatory, antimicrobial, anti-thrombotic, anti-atherogenic, and cardioprotective properties. Quercetin, *p*-coumaric acids, caffeic acid, gallic acid, and catechin are some of the main phenolics present in mushrooms. The main characteristic of mushrooms is the presence of these phenolic compounds leading to anti-oxidant properties. Meroterpenoids and lucidenic/ganodermic acids were the major terpenoids isolated from mushrooms [26]. Polysaccharides, steroids, and terpenoids from *Ganoderma* have shown important biological activities [27]. For the treatment of various ailments including cancer, crude extracts of these species are used [28]. Inflammation is a complex biological response of vascular tissues to harmful stimuli caused by pathogens, damaged cells, or irritants. Polysaccharides, terpenes, fatty

Table 7: SwissADME analysis

S. No.	Compounds	Molecular Formula	Lipinski's rule of five	
			Properties	**Value**
1.	Chlorogenic acid	$C_{16}H_{18}O_9$	Molecular weight (<500 Da)	354.31 g/mol
			LogP (<4.15)	−1.05
			H-Bond donor (<5)	6
			H-Bond acceptor (<10)	9
			Violations	1 violation; NHorOH>5
2.	Glutamic acid	$C_5H_9NO_4$	Molecular weight (<500 Da)	147.13 g/mol
			LogP (<5)	2.35
			H-Bond donor (<5)	3
			H-Bond donor (<5)	5
			Violations	nil
3.	Lovastatin	$C_{24}H_{36}O_5$	Molecular weight (<500 Da)	404.54 g/mol
			LogP (<5)	3.57
			H-Bond donor (<5)	1
			H-Bond acceptor (<10)	5
			Violations	nil
4.	*p*-Coumaric acid	$C_9H_8O_3$	Molecular weight (<500 Da)	164.16 g/mol
			LogP (<5)	1.28
			H-Bond donor (<5)	2
			H-Bond acceptor (<10)	3
			Violations	nil
5.	Ganodermic acid	$C_{34}H_{50}O_6$	Molecular weight (<500 Da)	554.76 g/mol
			LogP (<5)	5.34
			H-Bond donor (<5)	1
			H-Bond acceptor (<10)	6
			Violations	2 violations: MW>500, MLOGP>4.15
6.	Quercetin	$C_{15}H_{10}O_7$	Molecular weight (<500 Da)	302.24 g/mol
			LogP (<5)	−0.56
			H-Bond donor (<5)	5
			H-Bond acceptor (<10)	7
			Violations	nil
7.	Vanillin	$C_8H_8O_3$	Molecular weight (<500 Da)	152.15 g/mol
			LogP (<5)	0.51
			H-Bond donor (<5)	1
			H-Bond acceptor (<10)	3
			Violations	nil
8.	Gallic acid	$C_7H_6O_5$	Molecular weight (<500 Da)	170.12 g/mol
			LogP (<5)	−0.16
			H-Bond donor (<5)	4
			H-Bond acceptor (<10)	5
			Violations	nil
9.	Cinnamic acid	$C_9H_8O_2$	Molecular weight (<500 Da)	148.16 g/mol
			LogP (<5)	1.90
			H-Bond donor (<5)	1
			H-Bond acceptor (<10)	2
			Violations	nil
10.	Ascorbic acid	$C_6H_8O_6$	Molecular weight (<500 Da)	176.12 g/mol
			LogP (<5)	−2.60
			H-Bond donor (<5)	4
			H-Bond acceptor (<10)	6
			Violations	nil

Table 8: The docking score of the mushroom compounds with three different protein targets

S. No.	Compounds	Binding Energy (Kcal/mol)		
		2AXJ	**5OTF**	**4KXR**
1.	Chlorogenic acid	−7.1	−6.0	−6.3
2.	Glutamic acid	−4.3	−4.1	−4.6
3.	Lovastatin	−5.8	−3.0	−6.2
4.	*p*-Coumaric acid	−6.2	−4.7	−5.5
5.	Ganodermic acid	−6.9	−5.4	−6.8
6.	Quercetin	−6.6	−5.2	−6.0
7.	Vanillin	−4.5	−4.6	−4.6
8.	Gallic acid	−4.9	−4.8	−5.0
9.	Cinnamic acid	−4.8	−4.2	−5.4
10.	Ascorbic acid	−4.5	−5.1	−4.6

acids, steroids, phenolic acids, and other metabolites have all been found to be anti-inflammatory substances in mushrooms. Numerous studies have shown that among them, polysaccharides, phenolics, and terpenoids appear to be the most significant contributors to the anti-inflammatory effect of mushrooms [29,30].

The use of computational techniques serves as significant and fundamental tool in every phase of drug discovery and development process [30]. Molecular docking and ADME studies are *in silico* techniques which are currently employed in drug discovery research [31]. The best ligand-protein interaction is closely connected to the number of non-covalent interactions that drugs have with the surrounding residues in the active sites of proteins and governs stability of the drug candidate [32-34]. Docking results show that chlorogenic acid and ganodermic acid have good binding energies.

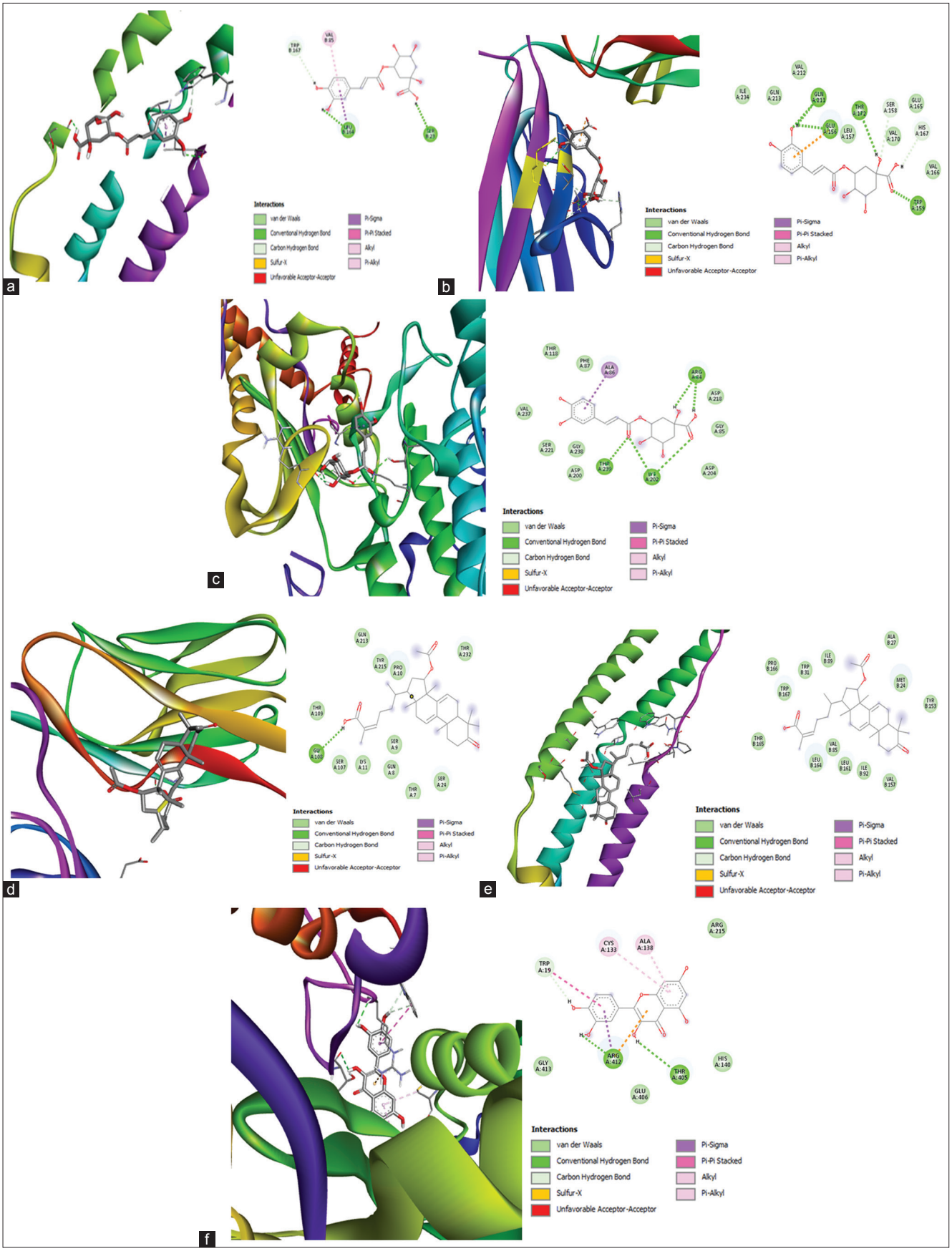

Fig. 6: Molecular docking of ganodermic acid against (a) 4KXR (antioxidant), (b) 2AXJ (antimicrobial), (c) 5OTF (anti-inflammatory) protein targets, (d) 4KXR (antioxidant), (e) 2AXJ (antimicrobial), and (f) 5OTF (anti-inflammatory) protein targets

CONCLUSION

In the present study, the chemical contents, food value, and medicinal properties of ethanolic and water extracts of various commercial and wild mushrooms were determined. The results concluded that carbohydrates, proteins, lipids, fiber, and phenolic substances are in various proportions in both commercial and wild mushrooms. These compounds have antioxidant, antibacterial, and anti-inflammatory properties. The current studies show that the compounds extracted from both commercial and wild mushrooms have an equivalent potential against the targeted diseases and their proteins. Further, *in vivo* studies are needed to prove the potential of mushrooms.

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AUTHOR'S CONTRIBUTIONS

Jeya Preethi S did the major experiments and drafted the manuscript, Sharmila P did the anti-inflammatory experiments, Sangeetha did the anti-microbial experiments, and Dr. P. Ponmurugan reviewed and edited the manuscript.

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Nil.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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