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# In vitro repress of breast cancer by bio-product of edible *Pleurotus ostreatus* loaded with chitosan nanoparticles

Aisha M. H. Al-Rajhi<sup>1</sup> and Tarek M. Abdel Ghany<sup>2\*</sup> 

## Abstract

Despite advances in early detection and therapy, cancer still is a significant health challenge with the highest priority for investigation. Breast cancer represents the most common cancerous disease among women in the world. The study's purpose is to estimate the cytotoxic activity of the edible mushroom *Pleurotus ostreatus* extract (PE), chitosan nanoparticles (ChNPs), and PE loaded with ChNPs (PELChNPs), as well as to identify the molecular docking of the cytotoxicity of methyl gallate (MG) as a main component of the PE against breast cancer (MCF-7) cell line. High-performance liquid chromatography (HPLC) analysis of PE exhibited the existence of various phenolic and flavonoid compounds such as MG, gallic acid, chlorogenic acid, hesperetin, naringenin, rutin, and cinnamic acid. The proliferation of the MCF-7 cell line was inhibited at 1, 3.9, and 62.50  $\mu\text{g}/\text{mL}$  of PELChNPs, PE, and ChNPs, respectively. PELChNPs were more effective against the MCF-7 cell line than PE, particularly at low concentrations. For instance, at 7.8  $\mu\text{g}/\text{mL}$  of PELChNPs and PE, the inhibitory % of MCF-7 proliferation was  $20.59 \pm 1.75\%$  and  $8.57 \pm 0.59\%$ , respectively. At 15.6  $\mu\text{g}/\text{mL}$  of PELChNPs and PE, the inhibitory % of MCF-7 proliferation was  $51.37 \pm 1.09\%$  and  $25.18 \pm 1.64\%$ , respectively. While there is slight difference in the inhibition % of MCF-7 cells ( $98.64 \pm 0.21$  and  $97.22 \pm 0.16\%$ ) at high concentration 500  $\mu\text{g}/\text{mL}$  of PELChNPs and PE, respectively.  $\text{IC}_{50}$  was  $15.25 \pm 0.54$   $\mu\text{g}/\text{mL}$ ,  $46.27 \pm 1.94$   $\mu\text{g}/\text{mL}$ , and  $337.38 \pm 13.68$   $\mu\text{g}/\text{mL}$  against MCF-7 cell line of PELChNPs, PE, and ChNPs, respectively. The value of  $\text{IC}_{50}$  documented the efficacy of PELChNPs compared with the  $\text{IC}_{50}$  ( $5.91 \pm 0.43$   $\mu\text{g}/\text{mL}$ ) of Vinblastine sulfate. Noticeable distortions were observed in the MCF-7 cell line mainly treated with PELChNPs, followed by PE alone. While ChNPs exhibited less effect on the morphology of the MCF-7 cell line. Antioxidant activity of ChNPs, PE, and PELChNPs was evaluated compared with Trolox, which reflected  $\text{IC}_{50} = 118.33 \pm 4.02$ ,  $85.63 \pm 3.96$ ,  $36.80 \pm 2.52$  and  $24.74 \pm 0.45$   $\mu\text{g}/\text{mL}$ . Methyl gallate binding interactions were assessed using molecular docking with the MOE-Dock tool against the target crystal structures of Breast cancer cell line 3HB5. The results shed light on how molecular modeling techniques can inhibit methyl gallate with possible uses in treating breast cancer.

**Keywords** Mushroom, Chitosan nanoparticles, Breast cancer, Molecular docking, Cytotoxicity

## Introduction

Cancer is one of the most severe diseases that threaten to kill people worldwide, although promising developments in treatment and medical diagnosis [1]. Among the various types of cancer, breast cancer is the most common in women worldwide [2]. Moreover, breast cancer is the second most significant common cancer and the number one killer among women. Breast cancer accounts for 12.5% of newly diagnosed cancers worldwide each year.

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In 2020, 684,996 fatalities and 2,261,419 new breast cancer cases were reported [2, 3]. There are numerous factors related to breast cancer, for example, diet, gender, alcohol consumption, physical activity, history of family, lifestyle, endocrine aspects, and both extrinsic and intrinsic aspects. Several other vital factors, such as previous benign tumors, may lead to breast cancer. However, it is still unclear which factors are most important in the development of breast cancer. Because mammography is unavailable for routine screening, breast cancer is usually detected too late, leading to poor and inadequate healing, pain control, and reassuring care for women. Breast cancer has vital implications for society and women's quality of life. Thus, leading to life-threatening conditions such as lowered productivity and premature death [3].

Chemotherapy remains one of the promising strategies for cancer treatment till now. Many chemotherapeutic agents from natural sources are currently used to treat cancer, including vinblastine, vincristine, bleomycin, camptothecin, and paclitaxel. So, extensive attention has been devoted to checking anticancer agents from natural, safe sources, comprising medicinal plants, fungi, and bacteria [4].

About 270 species of mushrooms have been detected to be potentially beneficial to the health of humans. However, few fungi have been investigated for bioactive constituents that could be useful in treating different diseases [5]. Across the past 30 years, scientific and therapeutic studies in China, Japan, South Korea, and most recently in the United States have demonstrated that mushroom-derived properties and unique constituents effectively prevent and treat cancer, among other chronic diseases. These species include *Cordyceps*, *Agaricus*, *Antrodia*, *Albatrorus*, *Carpathia*, *Kritocybe*, *Flammulina*, *Formus*, *Ganoderma*, *Hunria*, *Innonotus*, *Inocybe*, *Lactarius*, *Felinus*, *Russula*, *Pleurotus*, *Schizophyllum*, *Trametes*, *Zerocoms*, and *Suylas*. They display promising influence against cancer and may comprise substantial anticancer agents. Lectins (carbohydrate-binding proteins) are a great content in mushrooms that exhibit anticancer/antiproliferative activities with various mechanisms. Mushrooms are also rich in essential compounds such as phenolics and flavonoids which were recognized as antioxidants. Overall, the use of mushrooms appeared safe and free of side effects in oncological studies [6, 7]. Numerous mushrooms have been experimented in phase I or II clinical trials, primarily to treat breast cancer (18.6%), colon cancer (14%), and followed by prostate cancer (11.6%) [5]. *Pleurotus highking* extract was tested against the human breast cancer cell line (MCF-7) and exhibited potential anticancer activity [8]. Recently, Mishra et al. [9] concluded that *Pleurotus ostreatus* is a valuable source of vital components and a reservoir of

secondary metabolites imparting critical biological activities, including anticancer, antibacterial, and antioxidant in vitro and silico investigations. *P. ostreatus* has attracted the attention of researchers due to its potent antitumor properties with a promising  $IC_{50}$  (4.5  $\mu\text{g}/\text{mL}$ ) against breast cancer cell proliferation, besides apoptosis induction and angiogenesis inhibition, thereby halting the metastasis process [10].

Currently, nanotechnology is more effective in medicinal applications than traditional treatments, such as chemotherapy, in reducing side effects of chemotherapeutic agents. Therefore, the development of nanotechnology is in progress today in many investigations [11–15]. The biological activities of natural compounds were enhanced by incorporating nanomaterials, particularly polymers such as chitosan, gelatin, and starch [16–19]. According to some studies, chitosan nanoparticles (ChNPs) have proven efficacy against cancer proliferation alone or in combination with other compounds; besides, these polymers are widely applied as carriers of active therapeutic ingredients [20, 21]. Anticancer mechanisms associated with ChNPs selectively penetrate the cancer cells and reveal antiproliferative potential through immunopotential, anti-angiogenesis, apoptosis, antioxidant protection, and regulation of enzymes [22, 23]. Within the current decade, Silico tools like molecular docking are successfully utilized for giving knowledge into the binding besides interaction strengths of ligand inhibitors for drug discovery [16]. Silico tools includes a target-based approach based on the target and structure of ligand, thus saving time, effort, and cost as well [24]. The current study aimed to determine the impact of a combination between edible mushroom (*Pleurotus ostreatus*) extract and ChNPs against breast cancer cells.

## Materials and methods

### Chemical materials

The agriculture research center in Egypt provided a mushroom sample (*Pleurotus ostreatus*). Chitosan nanoparticles (ChNPs) were supplied from Primex, Siglufjördur, Iceland. The other chemicals including Tween 20 in the current investigation were supplied via Sigma Aldrich (USA). The characterized chitosan nanoparticles were provided from Professor Tarek M. Abdelghany [16].

### Extraction of *P. ostreatus* and loading with chitosan nanoparticles

Ten g of mature *P. ostreatus* were washed with distilled water, air-dried fruiting bodies, then dried in an oven at 40 °C were grounded and extracted with 100 mL of methanol under shaken conditions (150 rpm for 24 h) at 25°C. The extract was filtrated via Whatman No. 1 paper. Methanol was removed utilizing a rotary evaporator

(Model: Laborata 4001, Heidolph WB, Germany) at 40 °C, and the residual solution was lyophilized. The obtained crude extract was re-dissolved for preparing stock solution containing 5 mg/ mL in mixed solvent of methanol: DMSO (8:2). The extract was kept at 4 °C in the dark to stop oxidative damage until further analysis. ChNPs were prepared by the addition of chitosan (2%) in aqueous solution acetic acid (1%, v/v), then allied to stirred for 2 h. Subsequently tween 20 was added to obtain a concentration of 0.05% (v/v) that required to raise the wettability and adhesion properties of the solution. *P. ostreatus* extract (PE) was loaded with the solution of ChNPs via agitation and then ultrasonication for 25 and 45 min, respectively, to obtain 10% by weight. The construction of ChNPs was characterized [16].

#### HPLC analysis of mushroom contents of flavonoid and phenolic

##### HPLC conditions

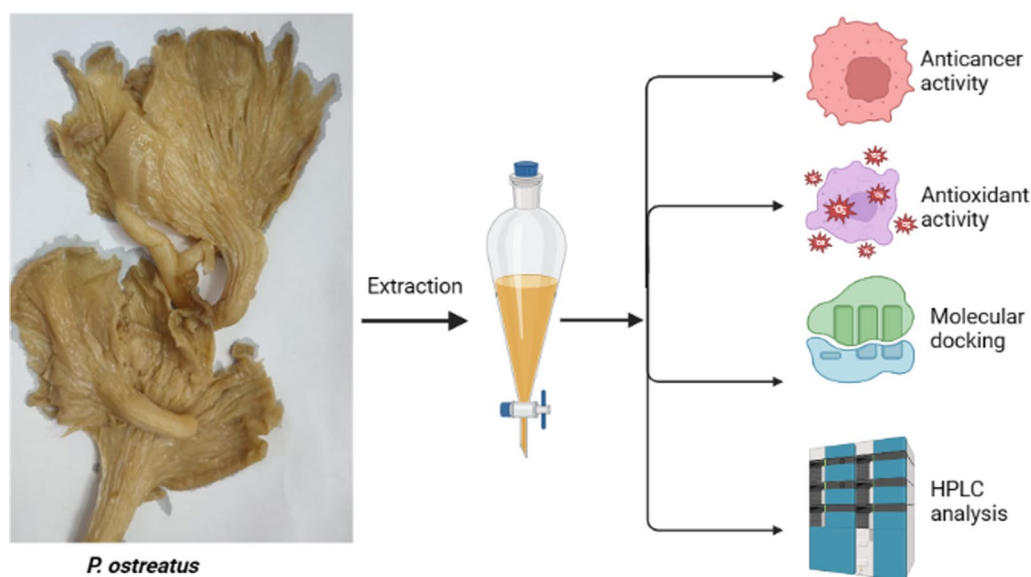
An agilent 1260 series was used for the HPLC analysis of *P. ostreatus* extract (5 µL were injected in HPLC). Eclipse C18 column was used to separate sample contents (4.6 mm x 250 mm i.d., 5 m). Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were the components of the mobile phase, which had a flow rate of 0.9 mL/min. The linear gradient was sequentially programmed into the mobile phase as follows: 0 min (82% A), 0–5 min (80% A), 5–8 min (60% A), 8 min (12%), 12 min (15%), 15 min (16%), and 16 min (20%). The column was kept at a constant temperature of 40 °C. The presence of phenolic compounds and flavonoids was determined using an

**Table 1** Flavonoid and phenolic compounds of *Pleurotus ostreatus* extract assessed by HPLC

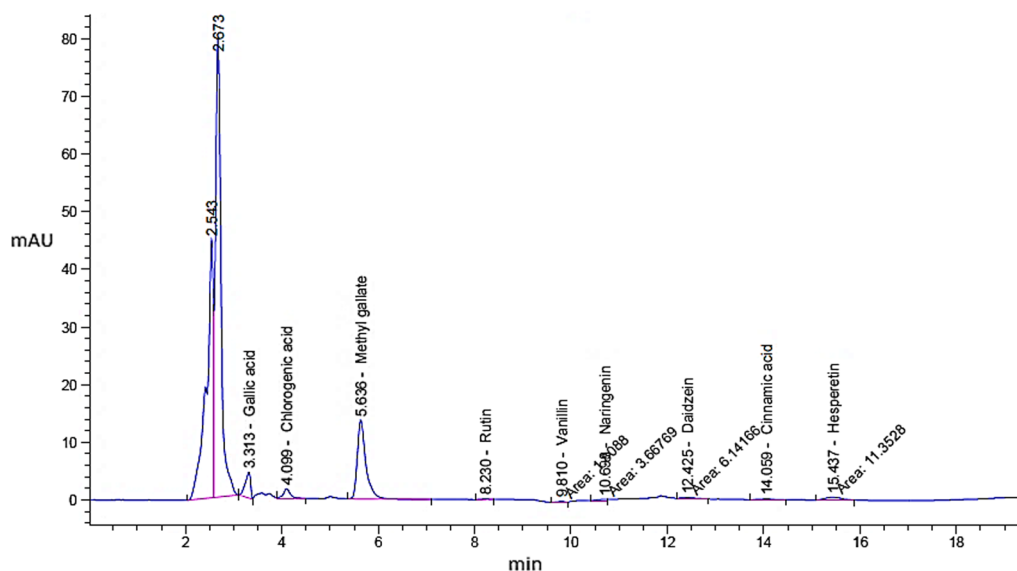
Compound	R.T.*	Area	Area%	Conc. (µg/g)	Molecular weight
Gallic acid	3.313	33.85	2.5149	28795.97	C <sub>6</sub> H <sub>2</sub> (OH) <sub>3</sub> CO <sub>2</sub> H.
Chlorogenic acid	4.099	17.90	1.3297	24470.58	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>
Catechin	4.615	0.00	0.00	0.00	–
Methyl gallate	5.636	169.47	12.5924	93932.64	C <sub>8</sub> H <sub>8</sub> O <sub>5</sub>
Coffeic acid	6.070	0.00	0.00	0.00	–
Syringic acid	6.610	0.00	0.00	0.00	–
Pyro catechol	6.800	0.00	0.00	0.00	–
Rutin	8.230	1.24	0.0921	1468.19	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>
Ellagic acid	8.899	0.00	0.00	0.00	–
Coumaric acid	9.185	0.00	0.00	0.00	–
Vanillin	9.810	1.81	0.1344	802.65	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>
Ferulic acid	10.268	0.00	0.00	0.00	–
Naringenin	10.698	3.67	0.2725	4489.95	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>
Daidzein	12.425	6.14	0.4563	3786.60	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>
Quercetin	12.769	0.00	0.00	0.00	–
Cinnamic acid	14.059	2.34	0.1738	439.95	C <sub>9</sub> H <sub>8</sub> O <sub>2</sub>
Apigenin	14.539	0.00	0.00	0.00	–
Kaempferol	15.039	0.00	0.00	0.00	–
Hesperetin	15.437	11.35	0.8435	6069.74	C <sub>16</sub> H <sub>14</sub> O <sub>6</sub>

R.T.\*, retention time

ultraviolet (UV) detector with a wavelength of 280 nm. Phenolic and flavonoid compounds were achieved qualitatively and quantitatively based on the injected authentic of phenolic and flavonoid compounds in HPLC. The



**Fig. 1** *P. ostreatus* sample and further bioprocess including extraction process, phenolic and flavonoid characterization, antioxidant activity, anticancer against MCF-7 cells, molecular docking interaction of the main detected compound in *P. ostreatus* extract with MCF-7 cells



**Fig. 2** Flavonoid and phenolic compounds chromatogram of *P. ostreatus* extract assessed by HPLC with different retention times and area

quantity of detected phenolic and flavonoid compounds was detected using the calibration curve through plotting the peak area in contrast to the concentration of the respective authentic compounds [25].

mL staining solution of MTT-PBS. The values of OD at 570 nm were measured via a Microplate reader model SunRise, TECAN, Inc., USA to calculate the viability % (V%) of cells via the following formula.

$$V (\%) = 1 - \frac{\text{The optical density of well treated with the tested extract}}{\text{The optical density of the untreated cells (Control)}} \times 100$$

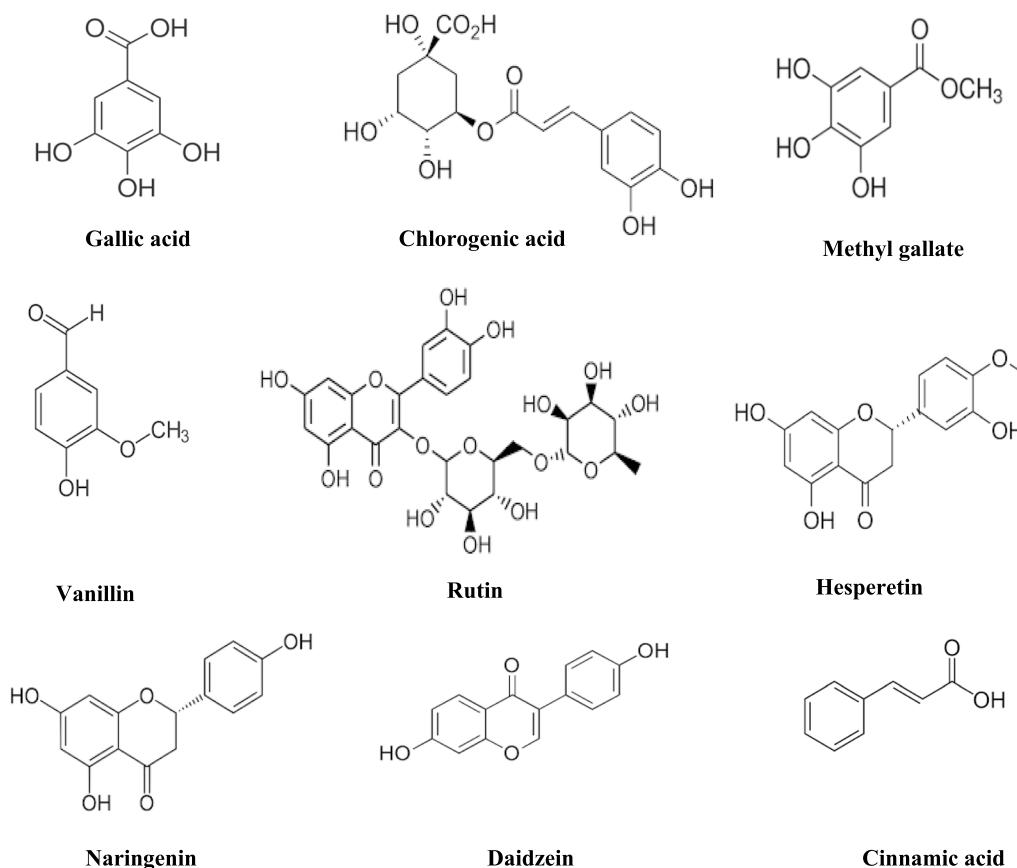
#### Cell line propagation, cytotoxicity assessment and microscopic studies

PE and PELChNPs were tested against the proliferation of human breast cancer cell line (MCF-7 cells) provided by the American Type Culture Collection (ATCC, Rockville, MD). Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum, L-glutamine (1%), and gentamycin (50 gm/L was used to propagate the tested cells. Under certain conditions (37 °C and a humid environment with 5% CO<sub>2</sub>), the tested cells were grown and maintained in DMEM medium. The tested cells were planted in 96-well plates. Each well containing 100 L of DMEM was seeded with 1 × 10<sup>4</sup> cells. The growth medium with various concentrations of the investigated extracts was added to wells after 24 h, followed by incubation. The viability and count of cells was detected via MTT [3 (4,5-dimethyl thiazol 2 yl) 2,5-diphenyl tetrazolium bromide] colorimetric approach. MTT solving solution (150 μL) were added per well after the incubating period (4 h) with the five mg/

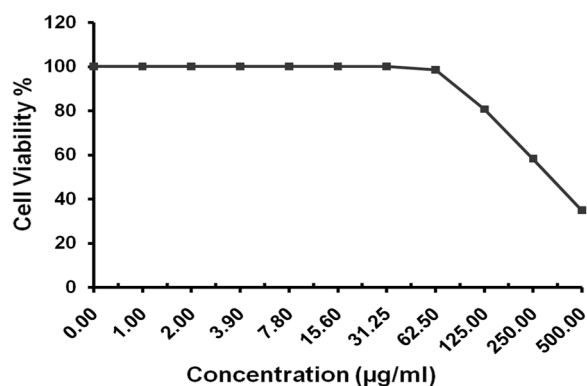
The relation between living cells and extract concentration was plotted to obtain the survival curve of MCF-7 after treatment. The 50% inhibitory concentration (IC<sub>50</sub>) is necessary to cause toxic effects in 50% of whole cells. IC<sub>50</sub> was assessed from the graphic plots of the quantity response curve for each concentration utilizing Graph-Pad Prism software (San Diego, CA., USA).

Microscopic characteristics of treated cells with tested substances were monitored using an inverted microscope (CKX41; Olympus, Tokyo, Japan) supplemented with a digital camera [26].

For this perform experiment, the prepared MCF-7 cell cultures with a 96-well plate were incubated for 12 h. The different concentrations of the tested substances were added to each well including the MCF-7 cell culture, then the plate was incubated for 3 days. At the end of incubation period, the cells were examined. Vinblastine sulfate at different concentrations was used as a positive control and applied against MCF-7 cells appropriate conditions as mentioned in case assay of PE or PELChNPs.



**Fig. 3** Chemical formula of the detected phenolic and flavonoid compounds in *P. ostreatus* extract



**Fig. 4** Anticancer activity of ChNPs against MCF-7 cells indicated by cell viability %

doses (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 g/mL) were combined with one milliliter of a 0.1 mM solution of DPPH in methanol. The mixture was shaken for 30 min at 22 °C, after which the absorbance (Abs) of the reaction mixture was determined at 517 nm using a spectrophotometer (UV-VIS milton roy). Radical absorbance without the tested PE and PELChNPs was utilized as control. A log dose inhibition curve documented the concentration of tested substances required to obstruct 50% of the DPPH free radical (IC<sub>50</sub>) [16]. All results of antioxidant activity were compared with ascorbic acid as a standard agent. DPPH scavenging (%) was calculated via the next equation:

$$\text{DPPH scavenging (\%)} = \frac{\text{Abs control} - \text{Abs in the existence of tested sample}}{\text{Abs control}} \times 100$$

#### Antioxidant activity

PE and PELChNPs were tested for their antioxidant capacity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test. Three milliliters of the dissolved tested substances in ethanol at various

#### Docking studies

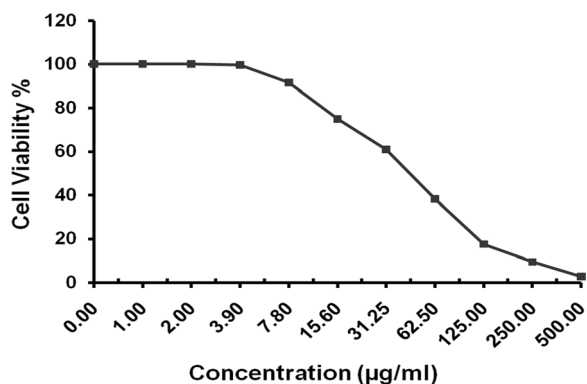
Molecular docking analyses of Methyl gallate via Molecular Orbital Environment (MOE) software were developed to explore the binding modes between the

**Table 2** Anticancer activity of ChNPs, PE, PELChNPs and Vinblastine sulfate against MCF-7 cells

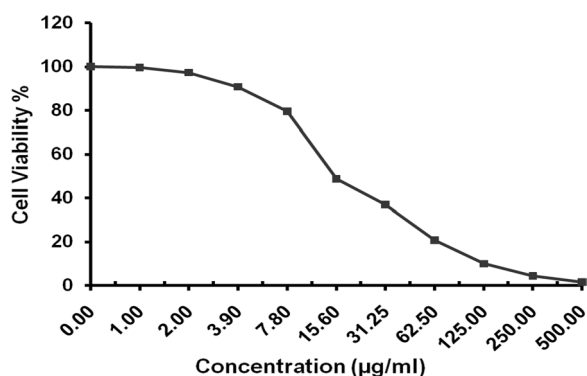
Conc. (µg/mL)	ChNPs		PE		PELChNPs		Vinblastine sulfate	
	Viability %	Inhibitory %	Viability %	Inhibitory %	Viability %	Inhibitory %	Viability %	Inhibitory %
500	34.76	65.24±2.48	2.78	97.22±0.16	1.36	98.64±0.21	5.49	94.51±0.23
250	58.19	41.81±2.07	9.47	90.53±0.39	4.29	95.71±0.07	7.82	92.18±0.16
125	80.65	19.35±1.39	17.52	82.48±0.74	9.85	90.15±0.13	15.18	84.82±0.49
62.5	98.42	1.58±0.64	38.17	61.83±1.29	20.64	79.36±0.22	23.87	76.13±1.59
31.25	100	0.0	60.95	39.05±2.71	36.79	63.21±0.83	31.95	68.05±0.72
15.6	100	0.0	74.82	25.18±1.64	48.63	51.37±1.09	40.56	59.44±1.18
7.8	100	0.0	91.43	8.57±0.59	79.41	20.59±1.75	47.21	52.79±2.35
3.9	100	0.0	99.56	0.44±0.12	90.64	9.36±0.52	52.94	47.06±1.78
2	100	0.0	100	0.0	97.23	2.77±0.31	58.76	41.24±0.24
1	100	0.0	100	0.0	99.52	0.48±0.16	67.16	32.84±0.32
0	100	0.0	100	0.0	100	0.0	100	0.0
IC <sub>50</sub>	337.38 ± 13.68 µg/mL		46.27 ± 1.94 µg/mL		15.25 ± 0.54 µg/mL		5.91 ± 0.43 µg/mL	

ligand and the target (Breast cancer cell line 3HB5). The compounds' structure was drawn using Chem-Draw Ultra 12.02 and saved as MDL files (".sdf") for

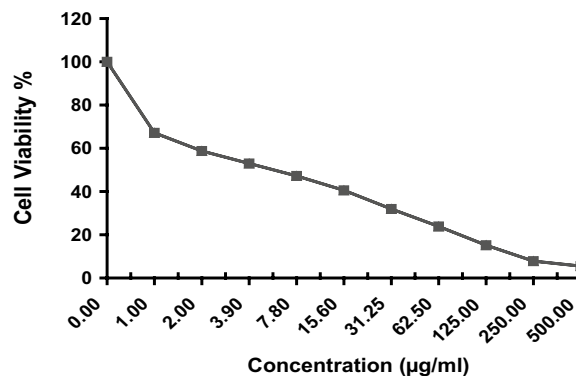
MOE. The crystal structures of Breast cancer cell line 3HB5 were obtained from the protein data bank (<http://www.rcsb.org/pdb>, accessed on 20 June 2021). Hydrogen atoms were supplied after the water molecules around the protein were removed. The parameters and charges were assigned using the MMFF94x force field. After alpha-site spheres were made using the site finder module of MOE, our methyl gallate was docked in the active site using the DOCK module of MOE. The MOE program's dock scoring was calculated using the London dG scoring formula, placement: triangle matcher, retain 10, and refinement: force field. The leading conformations of the docked ligands were determined by considering the root mean square deviation (RMSD) values, binding energies, and binding modes with the chosen residues [26].



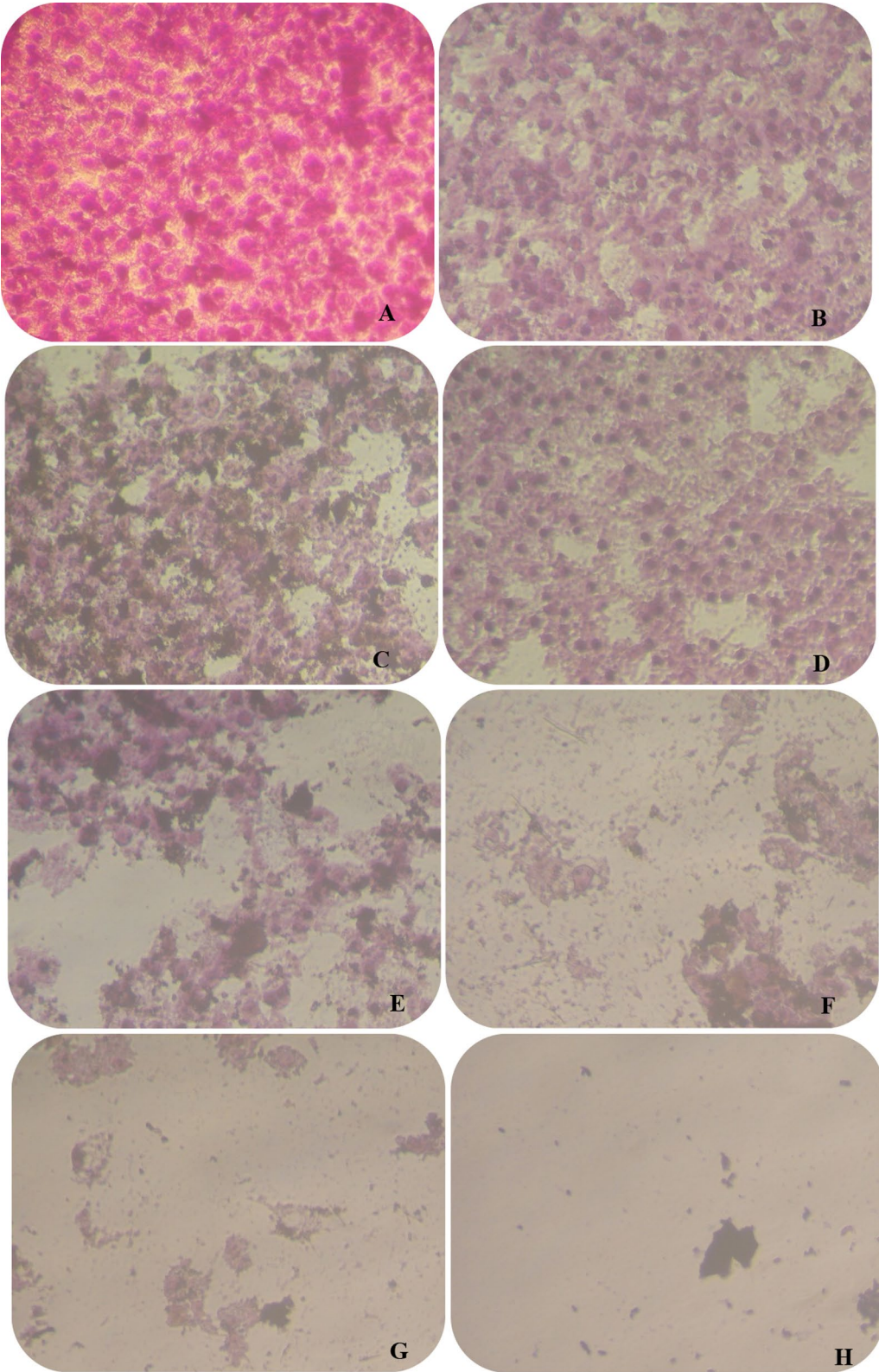
**Fig. 5** Anticancer activity of PE against MCF-7 cells indicated by cell viability %



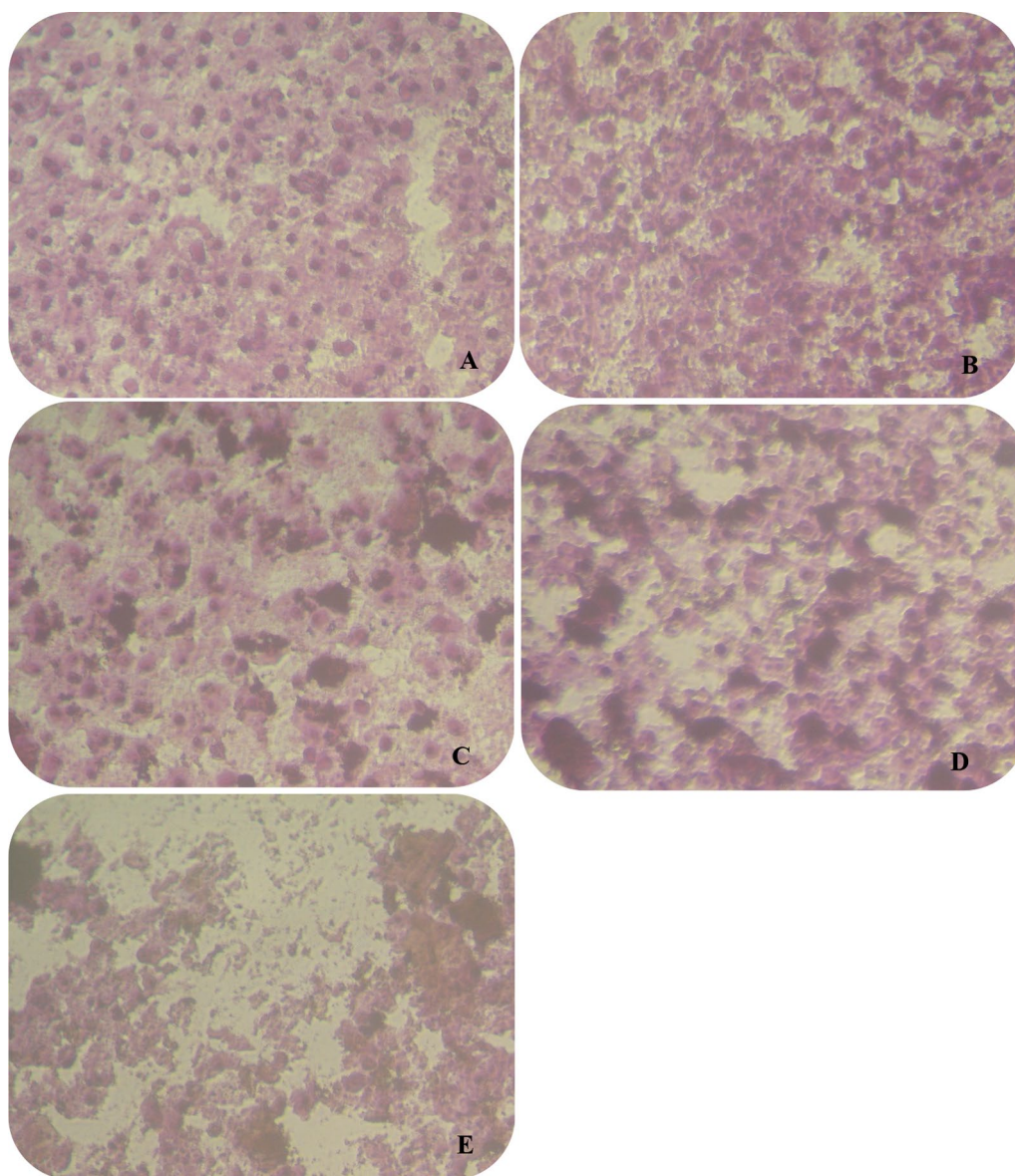
**Fig. 6** Anticancer activity of PELChNPs against MCF-7 cells indicated by cell viability %



**Fig. 7** Anticancer activity of Vinblastine sulfate against MCF-7 cells indicated by cell viability %



**Fig. 8** Morphological changes of MCF-7 treated with different concentrations of PE, **A** (Control): 0, **B**: 7.8, **C**:15.6, **D**: 31.25, **E**:62.5, **F**:125, **G**:250, and **H**: 500 µg/mL



**Fig. 9** Morphological changes of MCF-7 treated with different concentrations of ChNPs, **A**: 31.25, **B**:62.5, **C**:125, **D**:250, and **E** 500 µg/mL

#### Statistical analysis

Results are expressed as mean  $\pm$  standard deviation (SD) based on triplicate experiments.

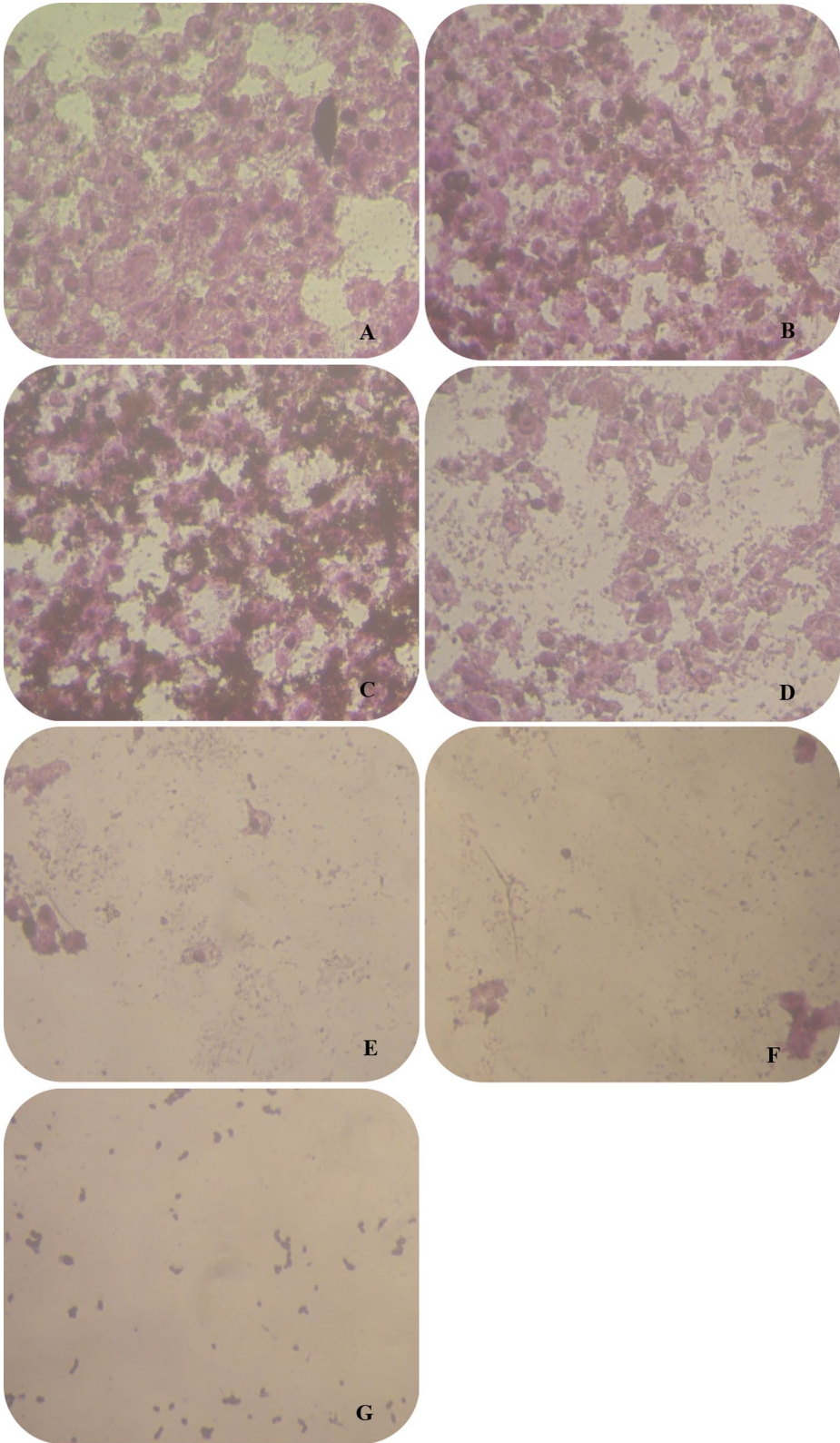
#### Result and discussion

##### Phenolic and flavonoid compound analysis in *P. ostreatus* extract

The collected mushroom was subjected to extraction, followed by HPLC analysis to recognize flavonoid and phenolic compounds content (Fig. 1). Methyl gallate (MG) represents the main constituent of mushroom 28795.97 µg/g, followed by gallic acid (28795.97 µg/g)

and chlorogenic acid (24470.58 µg/g). Other biologically active compounds were detected but with moderate concentrations, including hesperetin (6069.74 µg/g), naringenin (4489.95 µg/g), and rutin (1468.19 µg/g). Cinnamic acid was also recognized in the extract but in low quantity (439.95 µg/g) (Table 1 and Fig. 2). Compared with identified standards in HPLC, compounds such as caffeic acid, syringic acid, pyro catechol, ellagic acid, coumaric acid, ferulic acid, quercetin, apigenin, and kaempferol were not recognized in the extract of mushroom. The chemical formulas of all detected compounds are shown in Fig. 3. MG exhibited antioxidant activity besides





**Fig. 10** Morphological changes of MCF-7 treated with different concentrations of PELChNPs, **A:** 7.8, **B:** 15.6, **C:** 31.25, **D:** 62.5, **E:** 125, **F:** 250, and **G:** 500  $\mu\text{g/mL}$

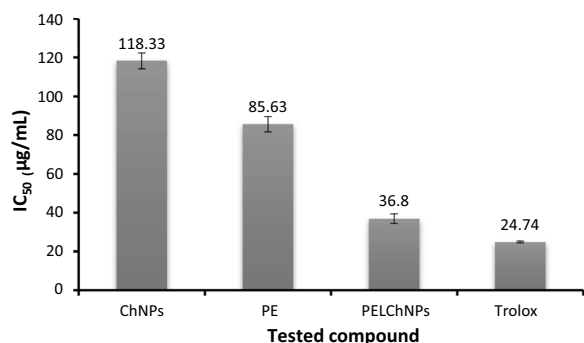
several other biological properties, including antispasmodic, antiatherogenic, anti-inflammatory, and antibacterial activities [27]. In addition, it has shown antitumor activity. It may be an effective therapeutic adjunct for patients with hepatocellular carcinoma [28]. According to the literature review of Podkowa et al. [29], gallic acid and Chlorogenic acid were identified in numerous species of mushrooms, including *Pleurotus ostreatus*, *Lactarius deliciosus*, *Morchella esculenta*, *Russula aurora*. Naringenin and rutin were also found in various edible mushrooms and exhibited killer effects against tumor cells [30].

#### Anticancer activities and morphological studies on MCF-7 cancer cell line

ChNPs, up to 31.25  $\mu\text{g/mL}$ , exhibited no anticancer activity against the MCF-7 cell line. Insignificant anticancer activity was observed at higher concentrations (Table 2; Fig. 4). PE and PELChNPs increase in concentration increased their antitumor activity. PE and PELChNPs concentrations of 3.9 and 1  $\mu\text{g/mL}$  were the minimum effective concentrations, respectively (Table 2; Figs. 5 and 6). Significant differences among the cytotoxicity of PE and PELChNPs at relatively low concentrations, where the inhibition of MCF-7 proliferation was  $8.57 \pm 0.59$  and  $20.59 \pm 1.75\%$  at 7.8  $\mu\text{g/mL}$ ,  $25.18 \pm 1.64$  and  $51.37 \pm 1.09\%$  at 15.6  $\mu\text{g/mL}$ ,  $39.05 \pm 2.71$  and  $63.21 \pm 0.83\%$  at 31.25  $\mu\text{g/mL}$ , respectively. Concentrations higher than 125  $\mu\text{g/mL}$  showed insignificant differences in the inhibition of MCF-7 proliferation not exceeding 10%. Generally, ChNPs enhanced the antitumor activity of PE with  $\text{IC}_{50}$   $15.25 \pm 0.54$   $\mu\text{g/mL}$  compared to  $\text{IC}_{50}$  of PE alone ( $46.27 \pm 1.94$   $\mu\text{g/mL}$ ) and ChNPs ( $337.38 \pm 13.68$   $\mu\text{g/mL}$ ). All these results were compared with the standard anticancer agent Vinblastine sulfate, which exhibited  $\text{IC}_{50}$   $5.91 \pm 0.43$   $\mu\text{g/mL}$  (Table 2; Fig. 7). The anticancer activity may be due to the existence of active compounds in PE, such as methyl gallate, gallic acid naringenin, and rutin.

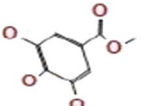
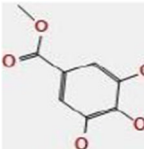
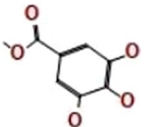
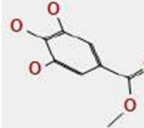
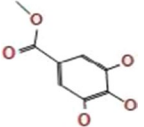
The enhancement of the anticancer activity of PELChNPs could be explained based on the unique properties of nanomaterials. Polysaccharides-based nano-formulation has been booming in biological activities [16, 18]. As [31] mentioned, ChNPs enhance the bioavailability and regulate the release of loaded therapeutic compounds, besides raising cellular uptake, and targeting tumor cells. Our findings were promising compared to other mushroom extracts; for instance, *Ganoderma lucidum* extract  $\text{IC}_{50}$  was  $209.6 \pm 0.24$   $\mu\text{g/mL}$  against MCF-7 [32]. In another study, using the extract of *Flammulina velutipes* against MCF-7 and MDA-MB-231 showed  $\text{IC}_{50}$  ranged from 17.7  $\mu\text{g/mL}$  to 38.36  $\mu\text{g/mL}$  and ranged from 114.5  $\mu\text{g/mL}$  to 184.2  $\mu\text{g/mL}$ , respectively [33]. Polysaccharides from *Agaricus bisporus* succeeded in repressing the proliferation of MCF-7 cells but had little activity against other cancer cells, including prostate, gastric cancer, colon, and murine Sarcoma 180 cells [34]. In vivo study [35], ChNPs loaded by extract of *Pleurotus eryngii* compared with extract alone could synergistically have potent antioxidant and antiapoptotic activity against doxorubicin-induced testicular injury in rats [35].

After 24 h of treatment with different concentrations of PE, ChNPs, and PELChNPs, the morphology of the MCF-7 cell lines was visualized in (Figs. 8, 9 and 10, respectively). Marked deformations and alterations were observed on the cell surface, which could be attributed to the impact of tested compounds, particularly with PELChNPs followed by PE alone. While ChNPs showed less effect on cell morphology. The increment of PELChNPs and PE concentration was accompanied to the increment of cell morphology and apoptotic alteration. The untreated cells exhibited remarkable confluence and had adherent progress and a polygonal form; besides, it demonstrated dense cell populations. Mechanisms of chitosan's antitumor activity are associated with membrane-disrupting and apoptosis-inducing actions [36]. Jedinak and Sliva [37] demonstrate that PE explicitly represses the growth of breast cancer and colon cells without any influence on normal cells and induces morphological changes causing the elongation of MCF-7 cells. Mishra et al. [9] observed numerous changes in the morphological features of MCF-7 treated with PE, including apoptotic cells with cytoplasmic membrane blebbing, cells shrinkage, and nuclear fragmentation, while intact stretched morphological structures were observed in the untreated MCF-7 cells. Our results showed that the treated cells' structural changes varied in a concentration-dependent manner.



**Fig. 11**  $\text{IC}_{50}$  values of antioxidant activity of ChNPs, PE, PELChNPs and Trolox as a positive control

**Table 3** Docking scores and energies of methyl gallate with MCF-7 cells 3HB5

Mol	rseq	mseq	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
	1	1	-5.01197	0.62351	2.645754	-66.1692	-10.8736	-26.0427	-5.01197
	1	1	-4.99384	1.59043	1.470515	-70.3849	-10.8288	-24.7089	-4.99384
	1	1	-4.90764	1.466602	7.633593	-68.9083	-12.8659	-21.0736	-4.90764
	1	1	-4.90188	2.290448	6.923578	-62.4627	-10.9264	-28.2436	-4.90188
	1	1	-4.88986	1.417585	2.700965	-82.8003	-12.373	-25.8585	-4.88986

### Antioxidant activities

Antioxidant activity of ChNPs, PE, and PELChNPs was evaluated compared with Trolox, which reflected  $IC_{50}$  of  $118.33 \pm 4.02$ ,  $85.63 \pm 3.96$ ,  $36.80 \pm 2.52$  and  $24.74 \pm 0.45$   $\mu\text{g/mL}$ . The antioxidant activity of PE and ChNPs was detected. The antioxidant activity of PELChNPs was superior to PE, which suggests that ChNPs enhance PE antioxidant activity (Fig. 11). The antioxidant properties of PE may occur due to the presence of flavonoid and phenolic compounds. In a previous report, strong antioxidant capacity was associated with mushroom fungi such as *Pleurotus columbinus* with  $IC_{50}$  of  $35.13$   $\mu\text{g/mL}$  and *Pleurotus sajor-caju* with  $IC_{50}$  of  $40.91$   $\mu\text{g/mL}$ , but it was less with using *Agaricus bisporus* with  $IC_{50}$  of  $83.93$   $\mu\text{g/mL}$  [38]. Recently, a DPPH assay was used to assess the antioxidant potential of the extracts of edible termite mushrooms including

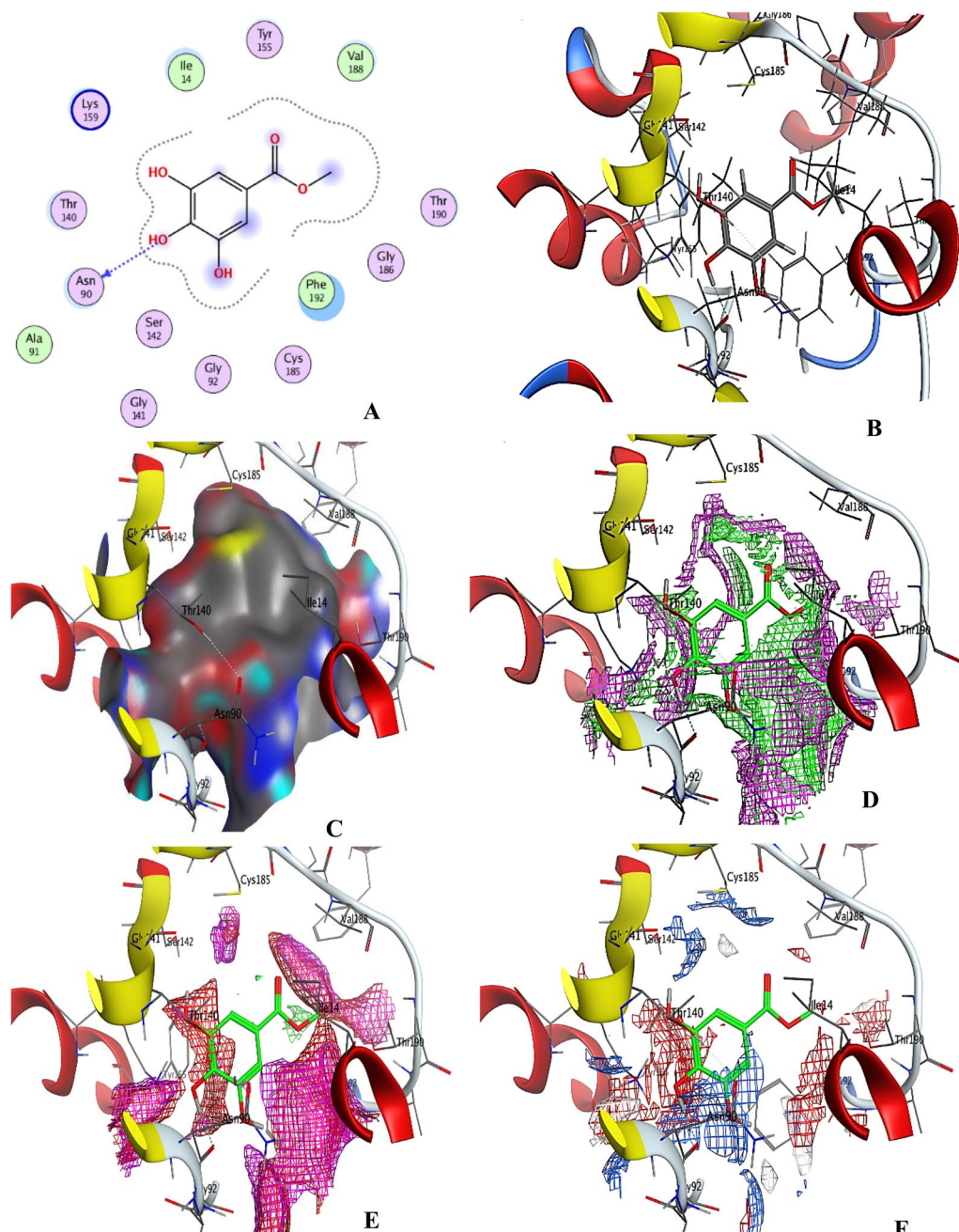
*Termitomyces eurhizus*, *T. albuminosus*, and *T. robustus*,  $IC_{50}$  ranging from  $710.00$  to  $714.05$   $\mu\text{g/mL}$  was detected and correlated to the bioactive secondary metabolites which can be utilized as safe antioxidants [39]. The antioxidant activity of *Agaricus bisporus* extract was compared to *A. bisporus* extract loaded on ChNPs to evaluate the role of ChNPs [40] demonstrated that ChNPs improved the antioxidant capacity of the extract. An earlier study explained the enhanced antioxidant activity of natural compounds loaded by ChNPs due to the stability and continuous release of active compounds from ChNPs [41]. Extracts with low  $IC_{50}$  (less than  $10$   $\text{mg/mL}$ ) have an antioxidant capacity and give hydrogen atoms to scavenge free radicals [42].

### Molecular modeling: docking study of Methyl gallate with MCF-7 cancer cell line

To evaluate the MOE-Dock program's accuracy, the co-crystallized ligand was removed from the active site and re-docked within the inhibitor binding cavity. RMSD value was determined to be  $0.62351\text{\AA}$ , demonstrating that our docking method is appropriate for the inhibitors under study and that the MOE-Dock method is trustworthy for docking these inhibitors. Methyl

**Table 4** Interaction of Methyl gallate with MCF-7 cells 3HB5

Mol	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
Methyl gallate	O 9	O ASN 90 (X)	H-donor	3.28	-0.7



**Fig. 12** Molecular docking process of Methylgallate with 3HB5 showing, **A** The interaction between Methylgallate and activesites of 3HB5 protein, **B** Theidentified most likely bindingconformation of Methyl gallate and the corresponding intermolecularinteractions, **C** Molecularsurface of Methylgallate - 3HB5 complex, **D** The contact preference of Methyl gallate with 3HB5, **E** Interaction potential of Methylgallate with 3HB5, and **F** TheElectrostatic map of Methylgallate with 3HB5

gallate was bound deeply with 3HB5 enzyme making interaction by ASN 90 residue and the O 9 of ligand. The docking score was found to be  $-5.01197\text{Kcal/mol}$ , and the results obtained are calculated in Tables 3 and 4. Recently molecular docking analysis by Hamed et al. [43] showed that methyl gallate bound with the

epidermal growth factor receptor tyrosine kinase (EGFRwt) (PDB ID: 1M17) through hydrogen bonding with MET-769, THR-766, GLN-767 amino acid residues. In addition, methyl gallate is bound with ASP-831 in the EGFR kinase domain activation loop. The binding pose score was  $-4.5287\text{ kcal/mol}$  with a root mean

square deviation (RMSD) value of 1.69 Å. Figure 12 shows 2D and 3D docking modes via MOE visualizing tool. Recently Mishra et al. [9] concluded that *P. ostreatus*, via In silico studies, is a rich source of biologically active compounds that play a vital role in repressing the MCF-7 cancer cell line. Docking scores study was reported in numerous studies reflecting the efficacy of active compounds against cancer cells as well as pathogenic microorganisms [12, 26, 44, 45].

#### Acknowledgements

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number WE-44-002.

#### Author contributions

AMHAR conceptualization the study and writing—manuscript and editing, ATM; performed study methods, writing the paper; AMHAR writing—original draft preparation. All authors have read and agreed to the published version of the manuscript. Both authors read and approved the final manuscript.

#### Funding

Funded from Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number WE-44-002.

#### Availability of data and materials

Not applicable.

#### Declarations

#### Competing interests

All authors declare no competing interests.

Received: 4 January 2023 Accepted: 8 May 2023

Published: 3 June 2023

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