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Antioxidant and Cytotoxic Activities of A Novel Isomeric Molecule (PF5) Obtained from Methanolic Extract of *Pleurotus Florida* Mushroom

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ABSTRACT

The *Pleurotus florida* is recognized as a medicinal and edible mushroom and the present study intends to reveal the active isomeric molecules from this mushroom. The *P. florida* was cultivated using different nutrient supplements: groundnut husk, maize powder, horse gram powder and coconut oil-cake powder. Horse gram supplement showed the higher mushroom yield and henceit was used for the cultivation of *P. florida*. Methanolic extract of *P. florida* was found to be efficient in antioxidant activity among ethanol, aqueous, ethyl acetate and hexane extracts. The bioactive fraction 3-methoxy-4-hydroxy cinnamic acid (PF5) was isolated and purified from the methanolic extract of *P. florida* by column chromatography, thin layer chromatography (TLC) and gas chromatography-mass spectrum (GC-MS) and further it was characterized by Nuclear magnetic resonance (NMR). The PF5 was tested for its DPPH and reducing power assays, and the IC_{50} values were found to be 21.7 μg/mL and 105 μg/mL, respectively. We found that the cytotoxic effect of 3-methoxy-4-hydroxy cinnamic acid was tested against the lung cancer cell line using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), sulphorodamine B (SRB) and trypan blue assays which exhibited a higher cytotoxic effect (CTC_{50}, 645 μg/mL). These results suggested that 3-methoxy-4-hydroxy cinnamic acid from *P. florida* could be explored as a novel and potent natural antioxidant and cancer preventive agent, alternative to existing synthetic molecules.

1. Introduction

In recent years, there has been a heightened awareness of antitumor molecules in the fast-growing field of biotechnology. For millennia, mushrooms have been valued as fit for human consumption and traditional ancient therapy for mankind. The *Pleurotus* mushroom that belongs to the family Pleurotaceae, is an economically important mushroom widespread in temperate, subtropical and tropical zones in worldwide (Kong, 2004). The *Pleurotus* sp. contains various essential nutrients, including dietary fiber, protein, amino acids, vitamins and minerals (Singh et al., 2011). The *Pleurotus* sp. is promising as medicinal mushrooms, exhibiting antibacterial, antiviral, hematological, hypocholesterolic, antitumor and immunomodulation activities (Venturella and Gargano, 2017). In addition to its unique taste and high nutritional and biological value, these kinds of mushroom contain biologically active compounds like polysaccharides (Dey et al., 2013).

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In modern decades, active compounds isolated from natural sources like algae, lichens higher plants and mushrooms have attracted a great deal of attention in the biomedical field because of their broad spectrum of therapeutic properties, relatively low toxicity and do not cause significant side effects (Zhao et al., 2012). In some of the studies, the researchers used the plant and seed extracts as an antioxidant and antitumor agent (Choi et al., 2006; Khodabande et al., 2017). Pleuran, lectin and PS-I and II (pure polysaccharide) are some of the active components in *P. florida* mushroom (Bergendiova et al., 2011; Rana et al., 2012). Many polysaccharides have exhibited strong antioxidant and immunostimulating properties which can be explored as novel potential antioxidants and immunostimulators (Bhunia et al., 2012; Maity et al., 2013). The *P. florida* has the significant characteristics of gas production, organic matter digestibility, metabolizable energy, net energy lactation and short-chain fatty acid production (Nasehi et al., 2017). Methanolic extract of *P. florida* fruiting bodies showed profound antitumor activity against the Ehrlich's ascites carcinoma (EAC) cell line induced solid tumor model in mice (Janardhanan et al., 2000). The *Pleurotus* sp. showed a potent antioxidant activity (Finimundy et al., 2013; Menaga et al., 2013) and narrow antibacterial activity (Menaga et al., 2012). On the other hand, the photo-irradiated *P. florida* mushroom was used for the synthesis of biofunctionalized silver nanoparticles (AgNPs) which showed admirable antioxidant effects (Bhat et al., 2013; Owaid, 2019). Kong et al. (2016) studied antioxidant properties and toxicology evaluation of the sclerotium of tiger milk mushroom *Lignosus tigris* cultivar E. They observed the presence of high nutritional value without any toxic substance and antioxidant properties from the edible mushroom. Recently, an in vitro approach was studied to evaluate the anti-adipogenic effect of *Myrica nagi* Thunb (Prashar and Patel, 2020).

The *P. florida* showed hepatoprotective activity against paracetamol-induced liver damage in Wister albino rats (Sumy et al., 1970). Hypercholesterolemia-induced oxidative alteration is also recovered by the feeding of *P. florida* in Wister rats (Khan et al., 2011). The antiplatelet-aggregating activity along with the anti-inflammatory activities, suggests its potential therapeutic use against vascular disorders (Jose et al., 2004). Ganeshpurkar et al. (2011) reported in vitro anticataract activity of *P. florida* on the isolated goat eye lens. A novel substance β-glucan from *Pleurotus* sp. was isolated and used for cytotoxic effect against PA1 cells (Maity et al., 2019). In another study, Selvi et al. (2011) demonstrated the anti-tumor potential of the ethanolic extract of *P. florida* mushroom against T24 bladder cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and DNA fragmentation assay. In the present investigation, the potential of bioactive compounds isolated from the methanolic extract of *P. florida* has been evaluated on its antioxidant and cytotoxic effect against A549 lung cancer cell line.

2. Materials and Methods

2.1. Mushroom cultivation and extraction

The *P. florida* mushroom spawn was collected from The Tamil Nadu Agricultural University (TNAU), Coimbatore and cultivated on paddy straw substrate by using groundnut husk, maize powder, horse gram powder and coconut oil-cake powder as a nutrient supplement. Since the growth of *P. florida* mushrooms enhanced by the horse gram supplement, in the present study, horse gram nutrient supplemented mushroom was used (Menaga et al., 2012). The *P. florida* mushrooms were cultivated, air dried, powdered and extracted with five different solvents: methanol, ethanol, aqueous, ethyl acetate and hexane. The crude extract was used as the compound for the further study.

2.2. Antioxidant activity of P. florida mushroom extracts by DPPH radical scavenging assay

The antioxidant activity of the different extracts of *P. florida* mushroom was determined in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) carried out by using the method from Molyneux (2004). About 1 mL of 100 μmol/L DPPH solution in methanol and equal volume of 20–100 μg/mL concentrations of the extracts in methanol were added and incubated in the dark for 30 min and 1 mL of methanol served as control. The change in colour was observed in terms of absorbance using Ultraviolet-visible (UV-Vis) spectrophotometer (Cyberlab UV 100, USA) at 517 nm. The different concentrations of ascorbic acid were used as reference compounds. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated as Equation (1) according to the report by Chen et al. (2019).

$$Percentage of\ DPPH assay(\%) = (Absorbance control - - Absorbance sample)/Control OD \times 100\% \quad (1)$$

2.3. Antioxidant activity of P. florida mushroom extracts by reducing power assay

The reducing power was determined according to the method of Berker et al. (2010). About 2.5 mL *P. florida* extracts (100–500 μg/mL) was mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer and 2.5 mL of 1% potassium ferricyanide and the mixture was incubated at 50 °C for 20 min. After the addition of 2.5 mL of 10% trichloroacetic acid, the reaction mixture was centrifuged at 3000 r/min for 10 min. About 5 mL of the upper layer was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. The IC_{50} values (μg extract per mL) are the effective concentration at which the 0.5 absorbance for reducing power and ascorbic acid was used as a standard.

2.4. Purification of active compounds through column chromatography

The antioxidant activity showed that the methanolic extract of *P. florida* mushroom was effective in evoking an antioxidant response. Hence, the methanolic extract was subjected to column chromatography. The crude methanolic extract of *P. florida* mushroom was dissolved in petroleum ether at a ratio of 10:1 (V/w) of petroleum ether-to-crude extract. The solution was applied to a dimension of 30 cm × 2.0 cm silica gel column, which was preconditioned with petroleum ether. It was eluted with the solvent systems starting with petroleum ether followed by increasing polarity up to methanol, until all the fractions were eluted. For each eluent mixture about 100 mL was used and each fraction was collected in a beaker and air dried.

2.5. Purification of active compounds through thin layer chromatography

Thin layer chromatography (TLC) was performed with silica gel precoated on aluminium plates (60, F254 Merck) with the dimension of 20 cm × 20 cm. Ten micro liter of each fraction collected from column chromatography was loaded to the marked points about 10 mm from the bottom of silica plate. Glass jars were saturated overnight by the different solvent systems such as *n*-hexane, dichloromethane, diethyl ether, ethyl acetate and methanol with the proportions of *n*-hexane:ethyl acetate:methanol (7:2:1 and 7.5:2:0.5), *n*-hexane:diethyl ether (8:2) and *n*-hexane:dichloromethane:methanol (6:3.8:0.2). The spotted aluminium sheets were placed in the glass jar containing solvents and it was run completely. After few min TLC spots were visualized under iodine fume and UV light with short (254 nm) and long (360 nm) wave and in addition, adequate TLC reagents like vanillin sulphuric acid reagent and folin-ciocalteu reagentwere used to detect the phenolic compounds. Individual retention factor (R_f) values for each spot were determined (Stahl, 1969). Finally, each fraction was tested for antioxidant activity as before to know the active fraction.

$$R_f = Distance travelled by the substance / Distance travelled by the solvent front \quad (2)$$

2.6. The GC-MS analysis

Gas chromatography-mass spectrum (GC-MS) was adopted for the identification and determination of the molecular weights and structure of the purified bioactive compounds. The 3-methoxy-4-hydroxy cinnamic acid (PF5) of *P. florida* was subjected to GC-MS analysis. It was performed on GC Clarus 500 Perkin Elmer systems under the following conditions: injector temperature 250 °C, using a capillary column Elite 5 (100% dimethyl poly siloxcane), mass range 40–500 m/z, oven temperature from 110 °C to 280 °C at 5 °C/min, with an initial hold of 2 min and it was detected by using a turbo mass detector.

2.7. Identification of active compounds

Interpretation of mass-spectrum by GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62 000 patterns. The spectrum of unknown components was compared with known component spectra stored in the NIST library. The name, molecular formula, molecular weight, retention time and structure of the components of the test materials were ascertained.

2.8. Nuclear magnetic resonance (NMR)

The powdered isolated compound of methanolic extract of *P. florida* was also subjected to ^{1}H-NMR. The NMR experiment was partly performed with a Bruker Avance 200 in chloroform ($CHCl_3$), internal standard tetramethylsilane (TMS) or alternatively on a JEOL Eclipse 400 NMR spectrometer (JEOL Ltd., USA). ^{1}H chemical shifts (δ, mg/L) are relative to residual solvent signals. The abbreviations, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad, are used throughout; coupling constants (*J*) are reported in Hz.

2.9. Cytotoxic activity of 3-methoxy-4-hydroxy cinnamic acid on A549 cell line

Lung cancer cell line-A549 was procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% of inactivated Fetal Bovine Serum (FBS), 100 IU/mL of penicillin, 100 μg/mL of streptomycin and 5 μg/mL of amphotericin B in a humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were dissociated with Trypsin Phosphate Versene Glucose (TPVG) solution [0.2% trypsin, 0.02% ethylenediaminetetraacetic acid (EDTA) and 0.05% glucose in phosphate buffered saline (PBS)]. The stock cultures were grown in 25 cm^2 culture flasks (Tarsons India Pvt. Ltd., Kolkata, India).

The viability of the cells was determined by trypan blue dye exclusion technique. The monolayer cell culture was trypsinized and the cell count was adjusted to 1×10^5 cells/mL using DMEM containing 10% FBS. To each of 40 mm petridish, 1 mL of the diluted cell suspension approximately 100 000 cells was added. After 24 h, when a partial monolayer was formed, the supernatant was removed, washed the monolayer once with medium and 1 mL of different concentrations (62.5, 125, 250, 500 and 1000 μg/mL) of 3-methoxy-4-hydroxy cinnamic acid was added on to the partial monolayer in culture dishes. The dishes were kept under incubation at 37 °C for 3 d in 5% CO_2 atmosphere. Further, the microscopic examination was done and observations were noted often at every 24 h time interval. After 72 h, the drug solution in the wells was removed and cells were trypsinized. The cells were suspended in PBS

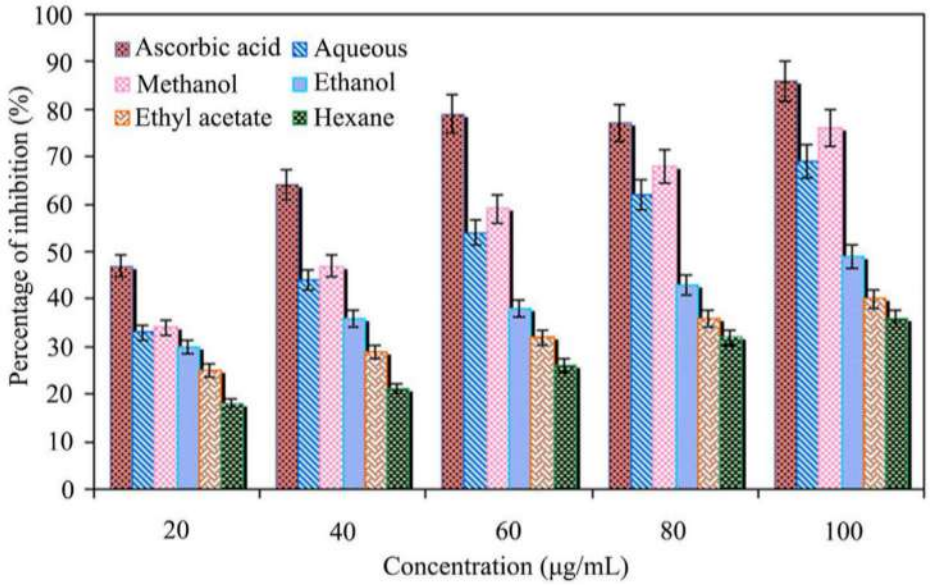


Fig. 1. Effects of five different extracts of *Pleurotus florida* on DPPH•scavenging activity.

and centrifuged to separate cell pellet, re-suspended in 1 mL of fresh medium and performed dye exclusion test, i.e., equal quantity of the drug treated cells and tryphan blue (0.4%) were mixed and left for 1 min. It was then loaded in a haemocytometer and viable and non-viable cell count was recorded within 2 min. The percentage growth inhibition was calculated according to Equation (3) and cytotoxic effect (CTC_{50}) value was generated from the dose-response curves for each cell line (Unnikrishnan and Kuttan, 1988).

$$Percentagegrowthinhibition = 100 - (Totalcells - -Deadcells)/Totalcells \times 100\% \quad (3)$$

The viability of the cells was also determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and sulphorodamine B (SRB) assays. The monolayer cell culture was trypsinized and the cell count was adjusted to 1×10^5 cells/mL using the medium containing 10% FBS and was used for the determination of cell viability by MTT and SRB assays as described by Denizot and Lang (1986) and Skehan et al. (1990). The absorbance was taken using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was deliberated using the following Equation (4). The concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) value was generated from the dose-response curves for each cell line.

$$Percentagegrowthinhibition = 100 - Meanopticaldensity(OD)ofindividualtestgroup/MeanODofcontrolgroup \times 100\% \quad (4)$$

2.10. Statistical analysis

The DPPH•scavenging activity and reducing power assay were studied with triplicate and the results were expressed with error bars. Both the mean and standard deviation were determined where appropriate, using the statistical package in Microsoft Excel version 2019. The biochemical parameters studied were subjected to statistical analysis using SPSS Version 16. One-way ANOVA followed by post-hoc analysis using Duncan's least significance difference (DLSD) was adapted to all the other parameters under study to test the level of statistical significance.

3. Results

3.1. Antioxidant activity of P. florida *mushroom extracts*

The different crude extracts of *P. florida* were screened for antioxidant activity by DPPH•scavenging and reducing power assay. The methanolic extract from *P. florida* showed the highest DPPH•scavenging activity (76%) followed by aqueous (69%), ethanol (49%), ethyl acetate (40%) and hexane (36%) at 100 µg/mL extract (Fig. 1). Reducing power of the five extracts of fruit bodies of *P. florida* was 0.51–0.97 at 500 µg/mL. The reducing power of the methanolic extract was found to be 0.97, which was relatively more prominent than that of standard ascorbic acid, which was 0.78 at 500 µg/mL (Fig. 2).

3.2. Isolation of compound PF5

The column fractions 50 to 55 (PF5) were bulked together as they showed a similar TLC distinct spot with same R_f value (0.56 cm) which was found in the solvent system of *n*-hexane:diethyl ether:methanol (60:30.8:0.2). Further, it was then subjected to preparative TLC, which was found to be pure at TLC screening. By means of visualizing agent fraction PF5 reveals the presence of phenolic

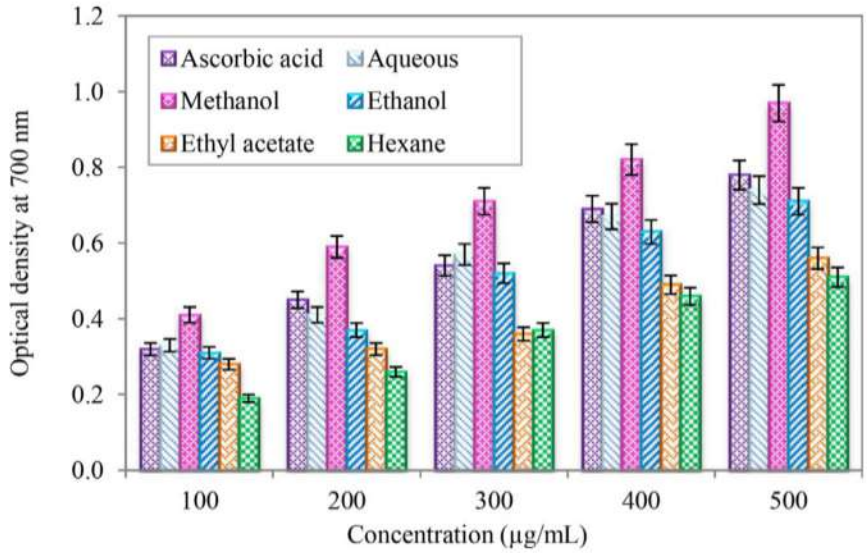
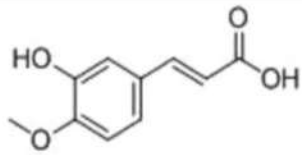


Fig. 2. Effects of five different extracts of *P. florida* on reducing power assay.

Table 1
Compound identified from methanolic extract of *Pleurotus florida* using gas chromatography-mass spectrum (GC-MS).

Fraction	Probable compound	Retention time (min)	Molecular weight	Molecular formula	Compound nature	Probable compound structure
PF-5	3-hydroxy 4-methoxy cinnamic acid	19.09	194	$C_{10}H_{10}O_4$	Acid phenol ester trans alkene	

compound which was further confirmed with GC-MS. By the method of the TLC, the rapid quantitative and qualitative analysis from crude extracts offers a practical and simple procedure for many applications.

3.3. The GC-MS analysis

Interpretation of mass spectrum by GC-MS was performed by means of the database of NIST having over 62 000 patterns. Based on the peak area, molecular weight and molecular formula of the phytochemical compound (PF5) was identified as 3-methoxy-4-hydroxy cinnamic acid coming under isomeric molecule (Table 1 and Fig. 3).

3.4. Nuclear magnetic resonance (NMR)

The structure of the isolated product (PF5) has been confirmed with the help of ^{1}H NMR, which is recorded with the Brucker instrument at 400 MHz using chloroform as a deuterated solvent. The δ value at 3.9 mg/L singlet corresponds to the methoxy protons, singlet at 5.4 mg/L for the –OH proton, two doublets at 6.2 and 7.6 mg/L for the olefinic protons, and multiplets at 6.9–7.1 mg/L corresponds to the three aromatic protons and finally the singlet for the acidic proton appears at 10.4 mg/L. Hence, from the ^{1}H NMR interpretation, we concluded that the proposed structure of PF5 was 3-methoxy-4-hydroxy cinnamic acid. Singlet for one proton at 6.9 mg/L and doublet of doublet for two proton appears at 7.0–7.1 mg/L indicate that one proton is present in the carbon adjacent to the olefinic and hydroxy moiety, i.e., ortho to olefinic substitution. Two protons may be present on the carbon adjacent to the olefinic and other one adjacent to the methoxy moiety. The m/z value of GC-MS was obtained at 40–500 m/z and recorded in the electron impact ion mode. From the NMR and m/z value from GC-MS, we concluded that the compound PF5 was 3-methoxy-4-hydroxy cinnamic acid (ferulic acid) (Fig. 4).

3.5. Antioxidant activity of 3-methoxy-4-hydroxy cinnamic acid

The 3-methoxy-4-hydroxy cinnamic acid from the methanolic extract of *P. florida* mushroom was investigated for the effect on the in vitro generation of free radicals. The results of the study showed that the maximum extent inhibition of free radical generation was occurred. The PF5 demonstrated H-donor activity. The DPPH radical scavenging activity was detected and compared with vitamin

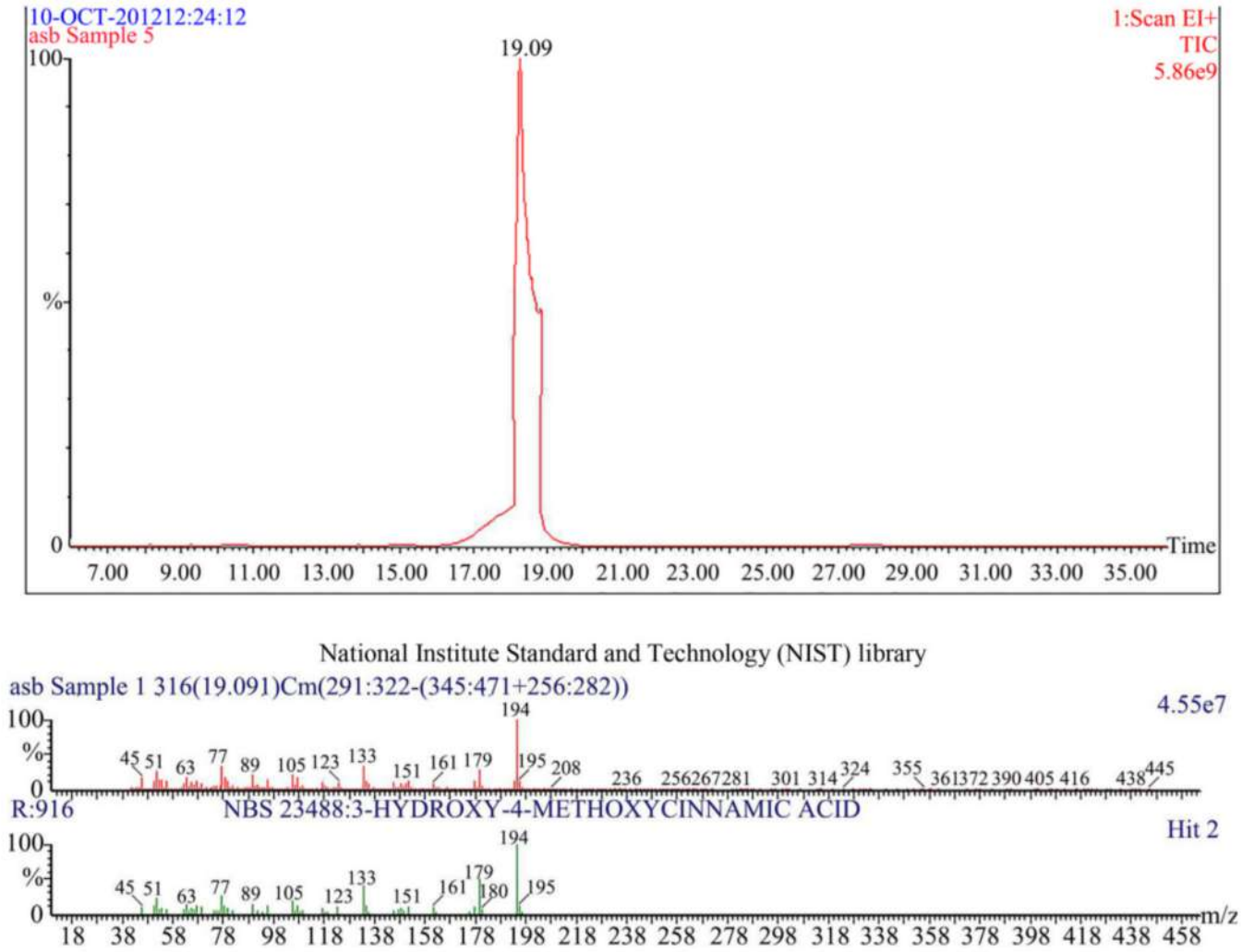


Fig. 3. The GC-MS analysis of PF5 from methanolic extract of *P. florida*.

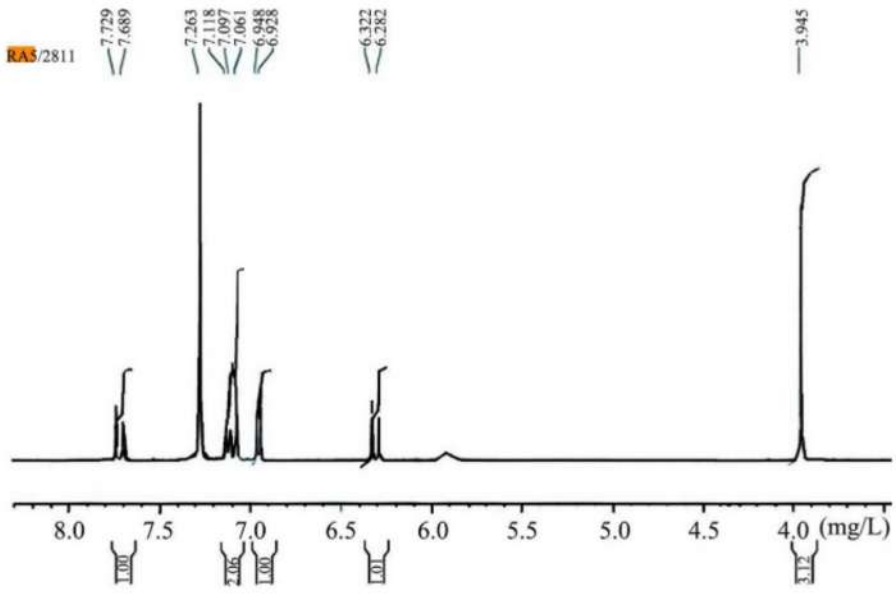


Fig. 4. The ^{1}H NMR spectrum of 3-methoxy 4-hydroxy cinnamic acid.

Table 2
The IC_{50} values of methanolic fractions from *P. florida* for antioxidant properties.

Sample No.	Purified compound	IC_{50} value (μg/mL)	
		DPPH radical	Reducing power assay
1	PF5	21.7	105
2	Vitamin C	50.0	225

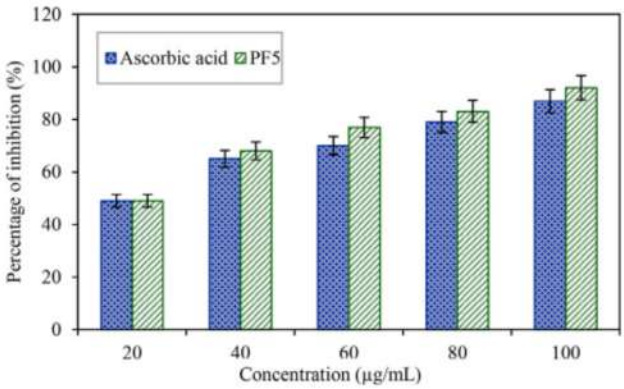


Fig. 5. Effect of methanolic fraction (PF5) of *P. florida* on DPPH radical scavenging activity.

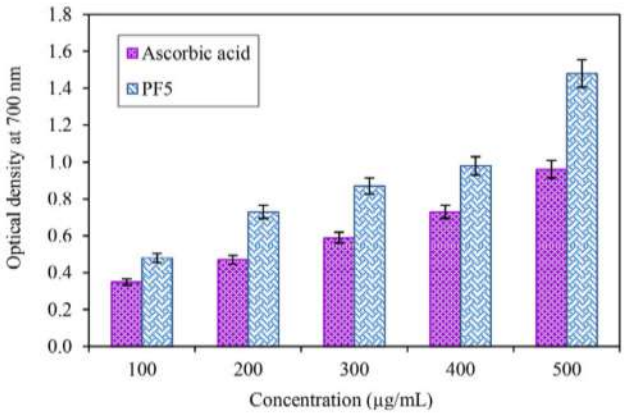


Fig. 6. Effect of methanolic fraction (PF5) of *P. florida* on reducing power assay.

C. The IC_{50} value was recorded 21.7 and 50.0 μg/mL, respectively (Table 2). The results revealed that PF5 possessed excellent antioxidant capacity than vitamin C (Fig. 5).

The high reducing power was indicative of the hydrogen donating ability of the active fraction present in the extract. The reducing power of the PF5 was shown in Fig. 6. The IC_{50} value for the PF5 was determined as 105 μg/mL, which was relatively more pronounced than that of standard ascorbic acid (225 μg/mL) (Table 2). The reducing power of the PF5 was excellent when the concentration of the extract was increased.

3.6. Cytotoxic activity of 3-methoxy-4-hydroxy cinnamic acid on A549 cell line

In the present study, SRB, MTT and Trypan blue assay were used to test the cytotoxicity of 3-methoxy-4-hydroxy cinnamic acid isolated from the methanolic extract of *P. florida* mushroom. The results of the cytotoxicity tests conducted against human lung adenocarcinoma epithelial cell line (A549) showing a certain degree of selectivity against the different methods used and the observations are given in Figs. 7–9.

The cytotoxicity of the 3-methoxy-4-hydroxy cinnamic acid against the A549 cancer cell line showed a potential cytotoxic activity in MTT assay (CTC_{50} value, (645.67 ± 5.77) μg/mL) comparing with SRB (CTC_{50} value, (676.00 ± 15.28) μg/mL) and Trypan blue (CTC_{50} value, (749.67 ± 7.33) μg/mL) assay against the tested A549 cancer cell line. The cytotoxic activity was also found to be dose dependent, and higher the concentration higher the activity. The values obtained in the present study are presented in Table 3 and Table 4. One-way ANOVA, followed by Duncan's post hoc test, evaluated the statistical differences. The mean differences were significant at all the tested levels ($P > 0.05^{*}$, $P > 0.01^{**}$, $P > 0.001^{***}$) (Fig. 10).

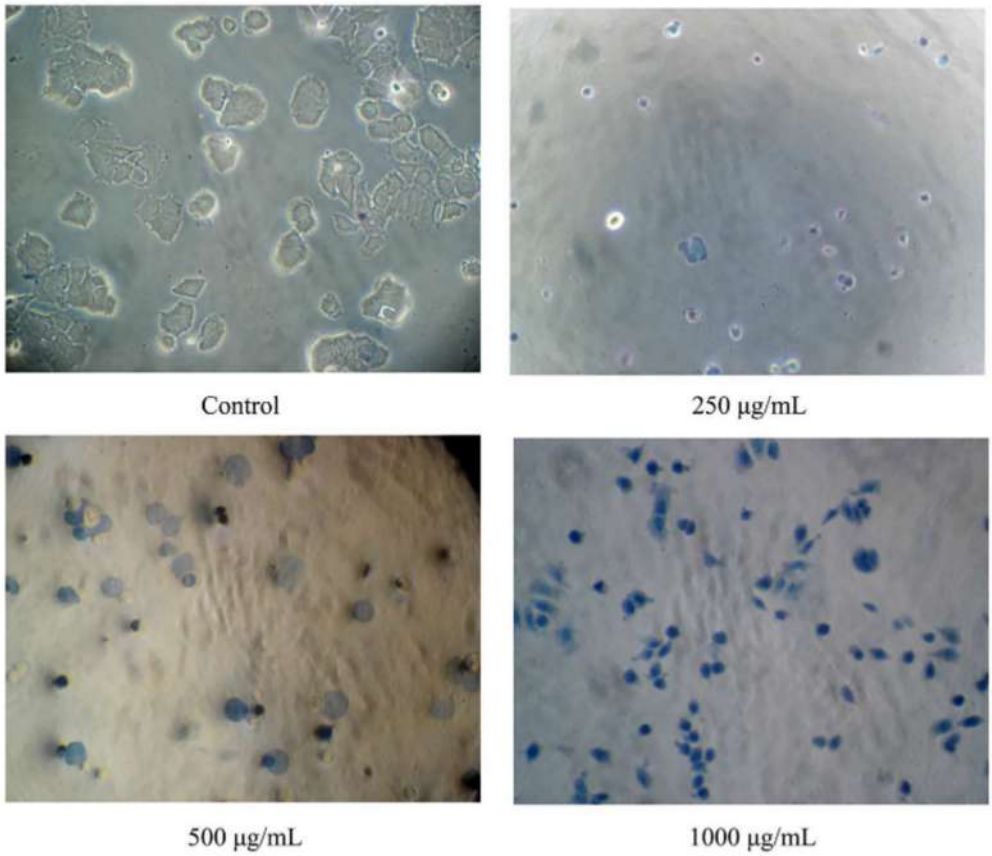


Fig. 7. Microscopic photographs of the tested cell line in trypan blue assay at 100 × magnification.

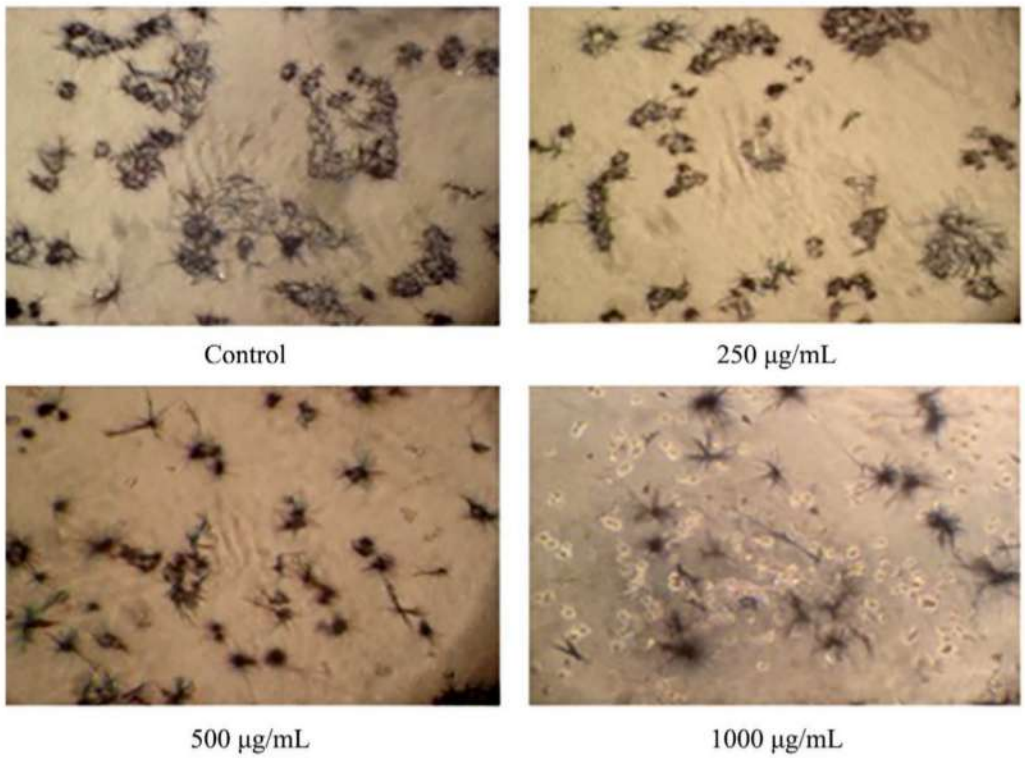


Fig. 8. Microscopic photographs of the tested cell line in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay at 100 × magnification.

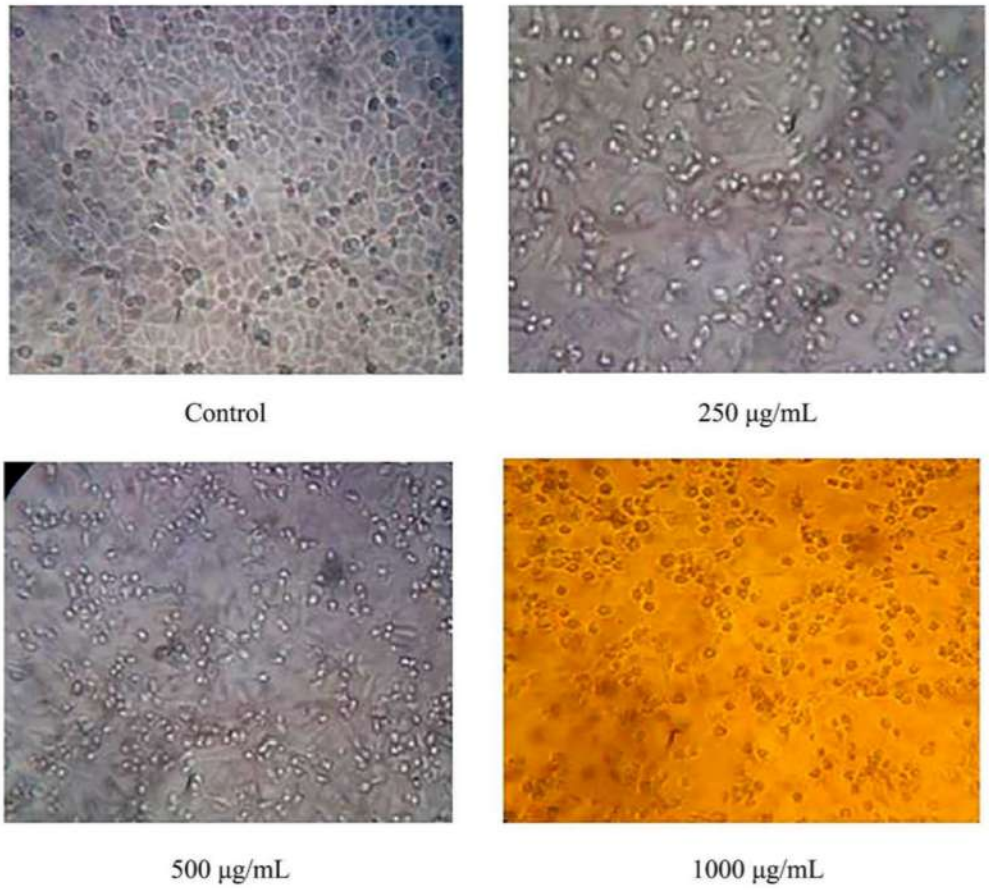


Fig. 9. Microscopic photographs of the tested cell line in sulphorodamine B (SRB) assay at 100 × magnification.

Table 3
Cytotoxicity of 3-hydroxy-4-methoxy cinnamic acid of *P. florida* mushroom against A549 cell line.

Sample No.	Test concentrations (µg/mL)	Cytotoxicity (%)		
		Trypan blue	MTT	SRB
1	1000	68.00±0.58	73.59±0.33	70.52±0.83
2	500	39.56±0.12	40.80±0.19	42.57±0.33
3	250	26.67±0.41	25.87±0.08	26.04±0.64
4	125	22.22±0.91	21.26±0.26	21.81±1.16
5	62.5	20.00±0.36	15.26±0.15	18.01±0.07

Notes: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; SRB, sulphorodamine B.

Table 4
The CTC_{50} value of three different assays of 3-hydroxy-4-methoxy cinnamic acid against A549 cell line.

Sample No.	Name of the assay	CTC_{50} (µg/mL)
1	Trypan blue	749.67±7.33
2	MTT	645.67±5.77
3	SRB	676.00±15.28

Note: CTC50, cytotoxic effect.

4. Discussion

The *P. florida* is an edible mushroom having excellent flavor and taste and is increasingly becoming popular as protein-rich delicious vegetable. The *P. florida* cultivation is popular due to low-cost technology and easy availability of various substrates for its cultivation. The use of mushrooms has been recommended in Traditional Medicine meant for treatment of different disease including cancers. Previous findings indicated that the cultivation of *P. florida* in the paddy straw substrate with the supplementation of horse

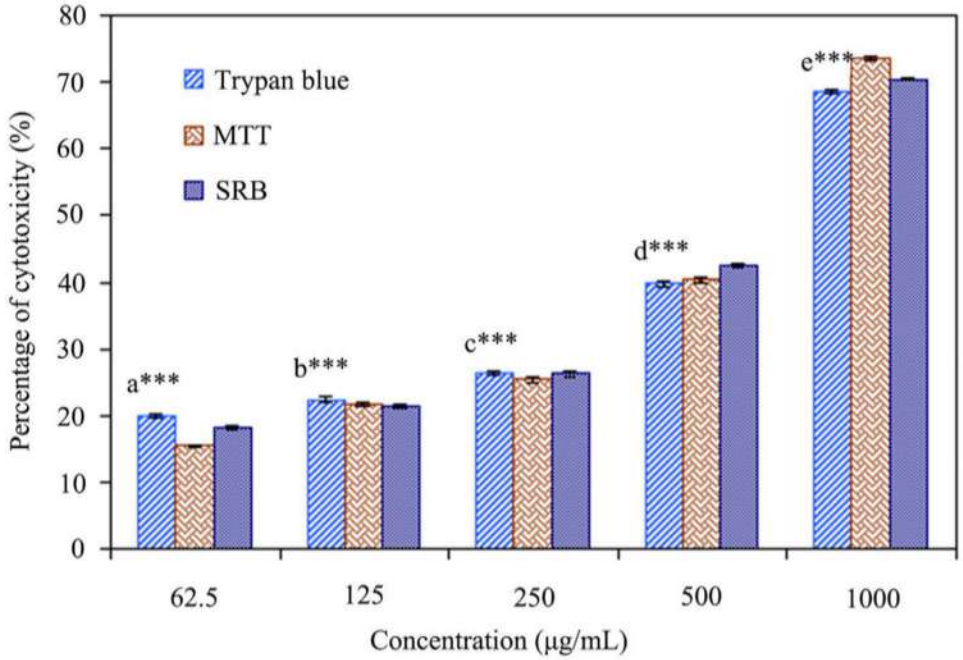


Fig. 10. Columns represent cytotoxicity of 3-methoxy 4-hydroxy cinnamic acid on A549 cell line in different concentration of drug. Experiments were repeated three times and values are expressed as mean ± Scanning Electron Microscope ($n = 3$). a–e*** denote significant variations in each concentration.

gram enriched the significant yield of mushroom when compared with other supplements tested. Consequently, the horse gram supplementation on *P. florida* accumulated high total protein. Generally, it is known that the horse gram is a good source of protein (17.9%–25.3%), carbohydrates (51.9%–60.9%), essential amino acids, energy and a low content of lipid (0.58%–2.06%) and is also an excellent source of iron and molybdenum (Sreerama et al., 2010a).

In this study, we investigated different extracts of *P. florida* for the radical scavenging assays. We found that the methanolic extract of *P. florida* showed the highest antioxidant activity, which was further investigated for the effects on the in vitro generation of free radicals. In our study, methanol extraction showed the best solvent for extraction, isolation of bioactive secondary metabolites from dried *P. florida* mushroom followed by ethanol and aqueous extracts. However, hexane and ethyl acetate extracts of *P. florida* showed low activity and comprised low phenolic compound content. It may be due to the high lipophilic nature of bioactive metabolites. This methanolic extraction provided the effective isolation of bioactive principles from *P. florida*.

The methanolic extract of *P. florida* was subjected to column chromatography, TLC and GC-MS for purification of active compounds. The purified fraction PF5 was further characterized by NMR and identified as 3-methoxy-4-hydroxy cinnamic acid. Ferulic acid is a strong membrane antioxidant in humans and is known to protect against cancer, cold, flu, influenza, diabetes, cholesterol, skin aging, degenerative diseases and muscle wasting (Fazary and Ju, 2007).

A white rot fungus isolated from decaying wood has the ability to metabolize ferulic acid, transforming it into 4-vinyl guaiacol, which was further metabolized to give acetovanillone (Mabinya et al., 2006), a product with potential applications in the pharmaceutical industry (van den Worm et al., 2001). Xie et al. (2010) suggested that when edible mushroom mycelium grown in wheat bran, could have the capacity to release ferulic acid by secreting cellulase and ferulic acid esterase enzyme. This may be the reason for the presence of high amount of ferulic acid in *Pleurotus* mushroom. Doğan and Akbaş (2013) identified phenolic compounds like catechin, ferulic acid, *p*-coumaric acid and cinnamic acid from Caesar's mushroom using gas chromatography. In this study, the *P. florida* mushroom was cultivated using paddy straw as substrate with horse gram as a nutrient supplement. The composition of phenolic compounds in paddy straw and horse gram reported in the literature and their analysis revealed that ferulic acid was the dominant phenolic acid, which possesses significant antioxidant activity (Sreerama et al., 2010b; Sreerama et al., 2012).

High radical scavenging activity against DPPH radical has been reported in water soluble heteroglycan isolated from aqueous extract of edible hydrid mushroom *P. florida* and *Calocybe indica* var. APK2 (Maity et al., 2011). Finimundy et al. (2013) obtained similar results while evaluating the aqueous extracts of *Lentinus edodes* and *P. sajor-caju.* In the present study, 3-methoxy-4-hydroxy cinnamic acid exhibited a better (92%) DPPH radical scavenging activity from methanolic extract of *P. florida* which was a good agreement with the above reports.

The reducing power of bioactive molecules is coupled with antioxidant activity. Thus, it is necessary to find out the reducing power of phenolic constituents to reveal the relationship between their antioxidant effect and their reducing power (Siddhuraju et al., 2002). In the present study, the fraction PF5 from the methanolic extract showed maximum reducing power assay which was supported by the findings of Liu et al. (2013) who reported that the methanolic extract of *Agaricus bisporus* showed excellent reducing power activity (IC_{50} value, 0.37 mg/mL). This observation indicates the strong antioxidant activity of *P. florida* mushroom.

In this study, the 3-methoxy-4-hydroxy cinnamic acid from the methanolic extract of *P. florida* was tested for the cytotoxicity against A549 cells, where we found a remarkable decrease in the viability of cancer cells. Reactive oxygen species play an important role in pathological processes like aging, cancer, coronary heart disease, alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataract formation and inflammation (Song et al., 2013). But, excessive production of reactive oxygen species (ROS) may cause oxidative damage to proteins, DNA, genomic instability and function of macromolecules. Accumulation of excessive ROS favours the acquisition of mutations and ultimately results in the cellular functional alterations in cancer cells (Vera-Ramirez et al., 2011). On the other hand, several studies have reported that substances from *P. florida* harbour the antioxidant properties, which can prevent the generation of cancer cells. Methanolic extract of *P. florida* showed significant effects in anti-inflammatory and platelet aggregation inhibiting activities (Jose et al., 2004).

Ferulic acid derivative was reported against breast, colon, stomach and tongue cancers (Fazary and Ju, 2007). It was noted that ferulic acid has a variety of pharmacological properties including hepatoprotective, antimalarial, antioxidant and antityrosinase activities (Prabhakar and Doble, 2011). In this study, we found that the effective cytotoxic activity of 3-methoxy-4-hydroxy cinnamic acid conducted against A549 lung cancer cells. The results obtained from the present study showed that the 3-methoxy-4-hydroxy cinnamic acid is cytotoxic in nature and may possess antitumor activity. It may be used as a supportive supplement to decrease the toxicity of anticancer drugs.

5. Conclusions

The results obtained in the present study demonstrated that the methanolic extract of *P. florida* was found to be potential among the all other extracts. The PF5 from the methanolic extract of *P. florida* was purified and characterized as one of the isomeric molecule 3-methoxy-4-hydroxy cinnamic acid. Antioxidation test in vitro showed that the PF5 possessed significant antioxidant activities in a dose-dependent manner. The IC_{50} value of DPPH was 21.7 μg/mL and reducing power was 105 μg/mL. Moreover, PF5 showed potent inhibitory effects (CTC_{50}, 645 μg/mL) on growth of A549 lung cancer cells. These results can be as a support to this views that 3-methoxy-4-hydroxy cinnamic acid from the methanolic extract of *P. florida* is a promising source of potential antioxidant and anticancer principles and may be efficient as preventive agents in the pathogenesis of some diseases. This study was a positive demonstration of the utility of mushroom for their food and medicinal values and this may be a good natural antioxidant alternative to existing synthetic antioxidant and anticancer drug in the food and medicinal industries.

Declaration of Competing Interest

There are no conflicts to declare.

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