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Evaluation of ethanolic and hexane extracts of *Trechispora pallescens* as antioxidant activity and anticancer potentiality against A549 cancer cell line

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ABSTRACT

Trechispora pallescens (Bres.) K.H. Larse comb. nov. was collected and identified by molecularly (ITS) in West Bengal, India by us in our laboratory. In this article the research aimed to elucidate the antioxidant contents and properties of ethanol (TEE) and hexane (THE) extracts of *Trechispora pallescens*, along with assessing their potential anti-cancerous effects on lung cancer A549 cells line. The result showed that the extraction yields were 0.57 ± 0.03% for TEE and 0.72 ± 0.05% for THE. Phenolic content was significantly higher in TEE (44.33 ± 1.10 mg GAE/g) compared to THE (29.15 ± 0.79 mg GAE/g), whereas flavonoid content was slightly higher in TEE (25.9 ± 0.42 mg QE/g) than in THE (21.7 ± 0.14 mg QE/g). TEE also exhibited higher tannin content (11.78 ± 0.47 mg TAE/g) than THE (8.94 ± 0.39 mg TAE/g). Total antioxidant contents which determine the antioxidant capacity were found to be 98.56 ± 1.41 mg AAE/g for TEE and 67.11 ± 1.57 mg AAE/g for THE. Both extracts displayed concentration-dependent DPPH radical scavenging activity, with TEE exhibiting an EC_{50} of 8.25 ± 0.34 mg/mL and THE showed an EC_{50} of 9.82 ± 0.72 mg/mL, relative to ascorbic acid (EC_{50} = 0.0473 ± 0.0002 mg/mL). Cellular and nuclear morphological changes were noted on A549 cell line by treatment with both TEE and THE. In MTT assays, TEE and THE showed dose-dependent inhibition of growth of A549 cells, with TEE achieving IC_{50} values of 1402.16 ± 128.00 µg/mL (24 h) and 1173.03 ± 45.22 µg/mL (48 h), THE achieving IC_{50} values of 1887.45 ± 155.00 µg/mL (24 h) and 1732.11 ± 40.23 µg/mL (48 h). Percentage of apoptosis were dose dependent manners by both extracts (TEE and THE). Leakages of LDH from A549 cells were occurred by both extracts. It was a novel work because before this work none did work on antioxidant content, activity and anticancer potentiality of this mushroom. These findings certified the both extracts of *T. pallescens* as valuable sources of antioxidant and anticancer agents, suggesting their relevance for pharmaceutical and functional food applications.

Figures : 04 References : 60 Table : 01
KEY WORDS : A549 cell line, Anticancer activity, Antioxidant content, DPPH, Ethanolic extract, FRAP, Hexane extract, Lung cancer, *Trechispora pallescens*

Introduction

Free radicals are generated in our body by unhealthy lifestyles and rapidly developing environmental pollutants and disrupt the body's system[16]. Well-designed antioxidant defense mechanisms are present in our body system that is responsible for the prevention of oxidative stress[49]. In this respect, the enzymatic defenses like glutathione peroxidase, catalase and superoxide dismutase[2] are found to protect against free radicals. But when excessive free radicals are accumulated in our body, they overpower the body's defense mechanisms and induce oxidative stress[17]. Due to this, scientists have focused recently on searching for new non-enzymatic, exogenous antioxidant sources, as they play an important role in boosting the body's antioxidant capacity against reactive oxygen species (ROS) and consequently preventing oxidative stress[13,45,46].

Cancer stands as a leading global cause of death, claiming approximately 10 million lives in 2020, according to the World Health Organization (WHO)[28]. The focus on anti-cancer activity is heightened due to the rising number of cancer patients. In 2020 alone, 19.3 million people were diagnosed with cancer, resulting in nearly

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TABLE - 1 : Yield percentage, TPC, TFC, TTC and TAC of TEE and THE

Extract	Yield of extract (%)	TPC (mg GAE/g dry weight of extract)	TFC (mg QE/g dry weight of extract)	TTC (mg TAE/g dry weight of extract)	TAC (mg AAE/g dry weight of extract)
TEE	0.57 ± 0.03	44.33 ± 1.10	25.9 ± 0.42	11.78 ± 0.47	98.56 ± 1.41
THE	0.72 ± 0.05	29.15 ± 0.79	21.7 ± 0.14	8.94 ± 0.39	67.11 ± 1.57

10 million deaths. If current trends persist, it is projected that there will be 28.4 million cases of cancer by 2040[53]. Due to the side effects associated with conventional pharmaceutical agents, there is an increasing interest in exploring natural products as alternative therapies. Natural products are gaining attention for their potential to effectively control cancer and provide safe, long-term prevention. Recent approaches have focused on utilizing food and edible medicinal herbs for these purposes[18,33,42]. It has been found that approximately 74% of new anticancer compounds are either natural products or natural product derived[12,14,30,54].

Mushrooms have been used as source of nutrient and remedy for a number of diseases. They contain high amount of carbohydrates, proteins, dietary fiber, and low level of fat[39], and bioactive phenolic compounds, carotenoids, and unsaturated fatty acids have been reported in mushrooms[32]. So, like plant-derived supplements, medicinal mushrooms have gained considerable global attention in recent years[11]. Globally, there are approximately 140,000 mushroom species, with only 10% identified so far. Of these, about 2,000 species are edible, and 650 species are recognized for their therapeutic and pharmacological properties[1].

In this study, the objectives were to assess the antioxidant content, evaluate the antioxidant activity and anticancer potentiality of the hexane extract (THE) and ethanol extract (TEE) of *Trechispora pallescens* mushroom.

Materials and Methods

Collection and identification of mushrooms

Mushrooms were collected in September 2021 from Masinan, Pursurah, Hooghly, West Bengal, India. Morphological characteristics (color, shape, size) and microscopical observations of the mushrooms were recorded and identified by the identification keys[20,35]. After that, the genomic DNA was extracted and amplified the ITS region of rDNA (ITS1-5.8s-ITS2) with fungus specific primers - ITS1 (forward primer) & ITS4 (reverse primer) by using PCR technology by the method previously described[43]. Then the amplicon was sent to GCC Biotech Lab., Kolkata, India for sequencing the region. After getting the FASTA file from them, the FASTA file was submitted to NCBI for homology searching and then it was published in NCBI with Accession no. At the herbarium of Ramakrishna Mission Vivekananda Centenary College (Autonomous), Rahara, Kolkata, India, a voucher specimen was deposited.

Solvent extraction of mushrooms

To remove dirt, the mushrooms were washed with distilled water and air-dried at room temperature (27 ± 2 °C) for 3 days. The dried mushrooms were then cut into small pieces and finely powdered using a mixer grinder. Subsequently, the mushroom powder was subjected to solvent extraction first with hexane (99% purity) and then with ethanol (99% purity) in a conical flask with sealed mouth conditions, in a stepwise manner for 72 hours at room temperature (27 ± 2 °C) using the dipping method with continuous stirring. All extracts were then filtered first through Whatman No. 4 filter paper, followed by Whatman No. 1 filter paper. The filtrates were then concentrated under reduced pressure using a rotary vacuum evaporator at 40 °C (SUPERFIT, India). Next, the concentrated filtrates were dried using a lyophilizer (BIOBASE, China), and the dry weights were measured. The final yields of all extracts were calculated and labeled as THE (*Trechispora* hexane extract) and TEE (*Trechispora* ethanol extract) and were stored under airtight conditions in a refrigerator at 4 °C until use.

Determination of the antioxidant contents of TEE and THE

The total phenolic contents (TPC) of the TEE and THE were determined by Folin–Ciocalteau method[3] using gallic acid (GA) as a standard. From the calibration curve of GA, TPCs of different extracts (TEE and THE) of the mushroom were determined and expressed as mg gallic acid equivalent (GAE) per g of extract.

Total flavonoid content (TFC) was determined according to the Dowd method[5] using quercetin as a standard. The TFCs of the extracts were measured using a calibration curve of quercetin. The data (TFC) were expressed as mg quercetin equivalent (QE) per g of dried extracts.

The total tannin content (TTC) was assessed by the method[47]. Tannic acid was used as a standard. TTCs of the extracts were estimated from the calibration curve of different concentration of tannic acid standard (concentration ranging from 5-25 μg/mL). TTCs were expressed as mg tannic acid equivalent (TAE) per g of dry weight of extracts.

Total antioxidant content (TAC) was evaluated by phospho-molybdenum assay according to the method[48] using ascorbic acid as a standard. The TACs of TEE and THE were calculated from a calibration curve of ascorbic acid standard and were expressed as mg ascorbic acid equivalent (AAE) per g of dry weight of the extracts. All the experiments were conducted in triplicate and represented as mean ± standard deviation.

Determination of antioxidant activity of TEE and THE

DPPH free radical scavenging activity assay

The ability of the extracts of the mushroom to scavenge free radical was estimated by DPPH (2,22 - diphenyl-1-picrylhydrazyl) assay to evaluate antioxidant activity of the extracts[8] .

The radical scavenging activity was determined by the percentage of DPPH• radical scavenged and it was estimated according to following equation:

$$\%\ \text{of DPPH• scavenging activity} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

Where $Abs_{control}$ = Absorbance of the control, Abs_{sample} = Absorbance of the test sample.

The EC_{50}, which indicates the concentration needed for 50% scavenging of the DPPH• radical, was derived from a graph where scavenging percentages were plotted against different concentrations of the extract. Ascorbic acid was used as a standard.

Ferric reducing antioxidant potential (FRAP) assay

According to the method described by Benzie and Strain[29]. The ferric reducing powers of the mushroom extracts were determined[5]. This assay evaluates the antioxidant effect of a substance by assessing its reducing ability. From the calibration curve of different concentrations of ferrous sulphate, the FRAP values were calculated and the values were expressed as mM Fe^{2+}/mg of sample.

Cell culture

The human lung cancer cell line (A549) was purchased from the National Centre for Cell Science (NCSS), Pune, India, and cultured in fresh media (DMEM)[19].

Effect of TEE and THE extracts on cellular and nuclear morphology of A549

To investigate the cell morphology, 2 × 10^5 cells /well of the cell line A549 and HEK 293T (normal cell line) were seeded into 6-well plates separately, and at 80-90% confluence the cells were treated with the concentrations of TEE and THE (1000 μg/mL) where DMEM was used as vehicle control and after 48 h, we observed under compound and phase contrast microscope and images of the cells were captured using an Olympus phase contrast microscope (at a magnification of ×10; Olympus Corporation, Tokyo, Japan). For nuclear morphology examination, treated cells were stained with fluorochrome strain (DAPI) and observed under fluorescence microscope[21].

Antiproliferative assay by MTT

The effects of THE and TEE (concentrations ranging from 100 to 800 μg/mL) on cell proliferation in the A549 cell line at 24 and 48 hours were assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) assay[41]. The HEK 293T cell line (Human embryonic kidney cell line) was subjected to MTT assay at the highest tested concentration of different sample (1000 μg/mL) to check the cytotoxicity effect of these extracts toward a normal human cell line.

The percentage of cell cytotoxicity was obtained using the following formula:

$$\text{Cell cytotoxicity (\%)} = 100 - \left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}})}{\text{Abs}_{\text{control}}} \times 100\right]$$

Where $Abs_{control}$ = Absorbance of the control, Abs_{test} = Absorbance of the test sample.

The IC_{50}, which is the dose required to achieve 50% cell death, was determined by plotting inhibition percentages against various extract concentrations on a graph. The experiment was conducted three times for each extract, and the data points presented as the mean ± standard deviation.

Apoptosis analysis

The nuclear morphology of A549 line treated by TEE and THE was examined after they were stained with DAPI to determine whether or not apoptosis had occurred[22]. Using an inverted fluorescent microscope, each experiment involved the observation and counting of apoptotic cells. In each experiment, at least five optical fields containing a total of 200 cells were counted after 48 h.

The percentage of apoptotic cells was calculated as follows:

$$\text{Percentage of apoptotic cells (\%)} = \frac{\text{Number of apoptotic cells}}{\text{Total cells counted}} \times 100$$

Lactate dehydrogenase assay

Cancer cells were seeded and grown separately in 96-well plates (4×10^3 cells/well) in medium containing 10% FBS for 24 h at 37°C and 5% CO_2. After washing the cells with PBS, 100 µL of each concentration of TEE and THE extracts were added separately to the wells, and 100 µL of medium was added to the control well, and the cells were incubated for 48 h. The cells were incubated and the lactate dehydrogenase (LDH) kit was used to perform the assay (Thermo Fisher Sci. Ltd, USA). A hundred microliter of (10:1000) sample was mixed with working reagent and incubated for 1 minute at 37°C before being observed at 340 nm and calculated according to the manufacturer's instructions[22].

Results and Discussion

Identification of mushrooms

The mushrooms were morpho-anatomically and molecularly identified as *Trechispora pallescens* (strain SKG24). The NCBI GenBank Accession number was PP163384.1.

Yield and antioxidant content of the extracts

The extraction yields for TEE and THE were 0.57 ± 0.03 and 0.72 ± 0.05% respectively (Table-1). The total phenolic contents (TPCs), total flavonoid contents (TFCs), total tannin contents (TTCs) and total antioxidant contents (TACs) of the above extracts were given in Table-1. Phenolic compounds are a diverse group of secondary metabolites produced by the majority of plant life activities[4] and play an important role for protecting against oxidation. They show antioxidant properties and found to scavenge free radicals, donate hydrogen, and quench singlet oxygen[15]. High amount of phenolic compounds are also found in mushrooms[9] and the frequent phenols in mushrooms are generally phenolic acids, flavonoids, tannins, and other polyphenols[60]. The TPCs of TEE and THE were 44.33 ± 1.10 and 29.15 ± 0.79 mg GAE/g dry weight of the extracts respectively. An investigator[7] reported total phenolic content (TPC) of five edible mushrooms of three different extracts (Water, 50% (v/v) ethanol and diethyl ether). The highest amount of TPC was found in *Lentinus edodes* and among the three extracts of it, the amount of TPC was found maximum in water extract (36.19 ± 0.59 mg GAE/g dw). Whereas, the lowest amount of TPC was found in diethyl ether extract of *Auricularia auricular* (2.17 ± 0.40 mg GAE/g dw). But except *Volvariella volvacea,* the order of the TPC was (for *Lentinus edodes, Pleurotus eous*, *Pleurotus sajor-caju* and *Auricularia auricular*) like water >50% (v/v) ethanol>diethyl ether. In case of *Volvariella volvacea,* the order was 50% (v/v) ethanol> water >diethyl ether. In our study, the order of the TPC was TEE>THE, and our result was higher than the above result.

Flavonoids are the most prevalent and widely distributed group of phenolic compounds[58]. The total flavonoid contents of TEE and THE were 25.9 ± 0.42 and 21.7 ± 0.14 mg QE/g dry weight of extracts respectively. Workers[40]showed high total flavonoid content of *P. ostreatus* ranging from 50.79–77.54 mg QE/g dw.

Total tannin contents found in TEE and THE were 11.78 ± 0.47 and 8.94 ± 0.39 mg TAE/g dry weight of extracts respectively. It was found that the tannin content of the *Pleurotus ostreatus* mushroom (cultivated on walnut sawdust) was 1.011 ± 0.088 CE mg/g[59].

Consuming foods rich in phytochemicals that possess potent antioxidant activity is commonly known to reduce the risk of chronic diseases associated with oxidative stress[35]. The total antioxidant content of mushroom extract is determined by its capability to reduce Mo (VI) to Mo(V) and is assessed by the subsequent formation of a green phosphate/Mo (V) complex at an acidic pH[52]. In our study, the total antioxidant contents (TAC) of TEE, and THE were 98.56

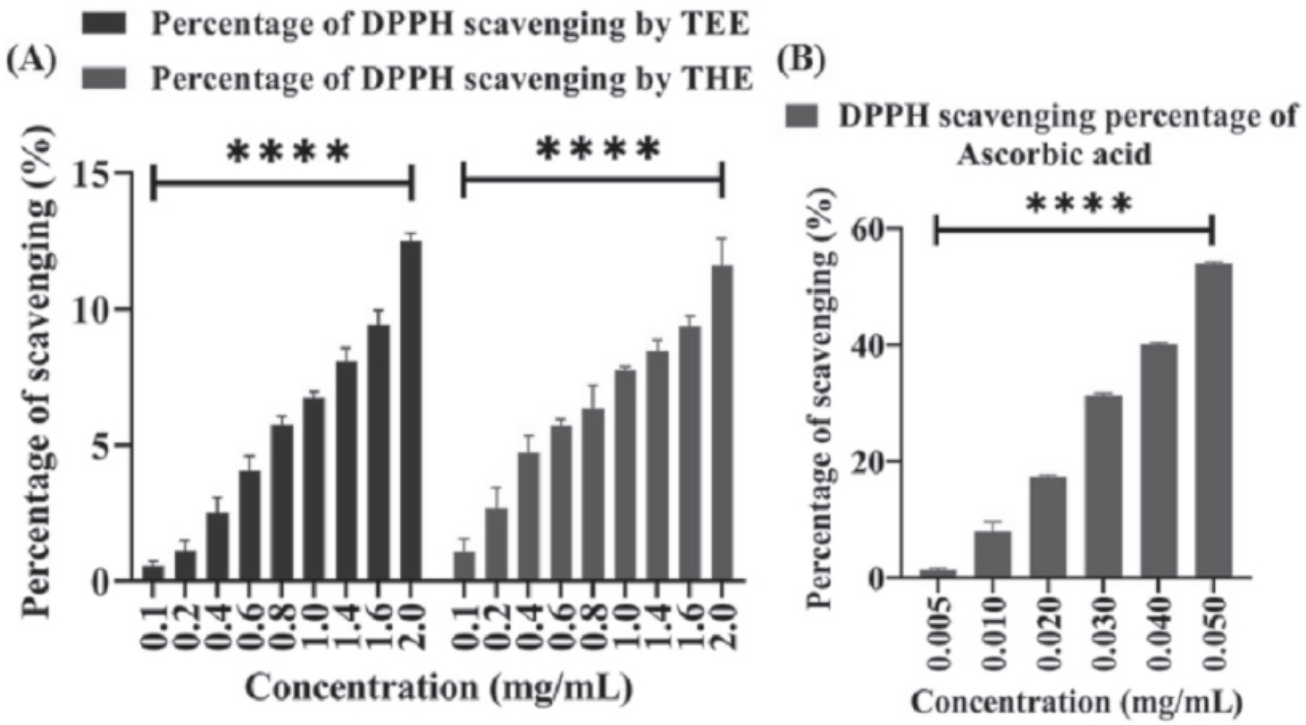

Fig. 1: DPPH scavenging percentages of (A) TEE and THE respectively (B) Ascorbic acid

±1.41 and 67.11 ± 1.57 mg AAE/g dry weight of extracts respectively. So, the result showed that, TEE and THE were rich in antioxidant contents.

Antioxidant activity

DPPH scavenging activity

DPPH assay is widely used in research and industry as it takes short time to complete the analysis[44]. Antioxidant's ability to scavenge DPPH free radical is linked to their capability to donate hydrogen, which is potentially influenced by their phenolic contents[55] and flavonoid contents[50]. The result showed that TEE and THE exhibited DPPH radical scavenging activity and the scavenging percentages were increased with increased concentration (Fig. 1). They showed the scavenging activity in a concentration range of 0.1-2 mg/mL, but the scavenging percentages were different for three different extracts. The EC_{50} values were calculated of two extracts and the EC_{50} values of TEE and THE were 8.25 ± 0.34 and 9.82 ± 0.72 mg/mL respectively. Commercial synthetic antioxidant, ascorbic acid, was used as a standard and the EC_{50} value of ascorbic acid for scavenging DPPH free radical was 0.0473 ± 0.0002 mg/mL. The Pearson correlation coefficient test showed a statistically significant relationship ($p<0.05$) between the concentrations of TEE, THE and the DPPH free radical scavenging percentages. The EC_{50} values of methanolic extract of *L. edodes* and *Agaricus blazei* were 26.32 and 6.77 mg/mL respectively for DPPH scavenging[10].

Ferric reducing antioxidant potential (FRAP) assay

FRAP is a simple and reliable way of assessing the antioxidant activity of extracts[27,51]. The ferric reducing antioxidant potentials of TEE, and THE were 0.402 ± 0.004, and 0.341 ± 0.004 mM Fe^{2+}/mg of the extracts respectively. It was found that FRAPs of acetone and ethyl acetate extracts (1 mg/mL concentration) of Enoki caps (*Flammulina velutipes)* were 0.339 ± 0.001 and 0.291 ± 0.001[56]. At 2 mg/ mL concentration, the ferric reducing powers of the ethyl acetate extract and methanol extract of *Pleurotus eous* were 0.635 and 0.250 respectively[52].

Anticancer activity

Cancer cell morphology was examined under broad field microscope and nuclear morphology by DAPI staining under fluorescence microscope

After 48 h of exposure with TEE and THE separately at a concentration of 1000 µg/mL, A549 cells were examined under bright field microscope. Untreated cancer cells (DMEM vehicle control) had a normal spindle shape and reached 90% confluence after 48 h of culture (Fig. 2A). Cells treated with TEE at the above concentration lost their spindle shape, had lower cell confluence, and there was a lot of debris (Fig. 2B). In case of THE treatment at the above concentration, cell confluency was also reduced and cells lost their original shape (Fig. 2C). But the effectivity of TEE was better

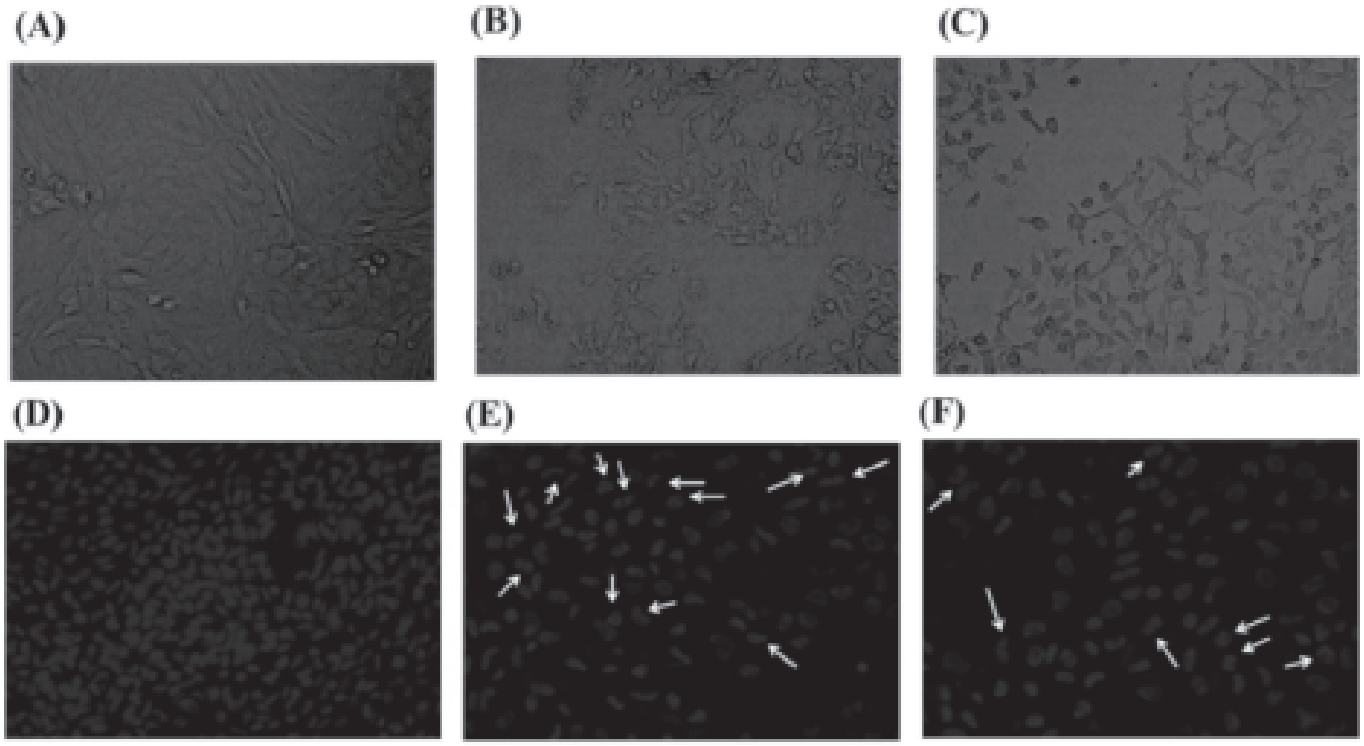

Fig. 2: Changes of cellular and nuclear morphology of A549 cells (A) Negative control showed the normal cells of A549 cells. (B, C) Cell confluences reduced after the treatment with TEE and THE at a concentration of 1000 µg/mL for 48 h. (D) Negative control showed normal nuclei of A549 cells (E, F) Clear apoptotic nuclei (fragmented nuclei, chromatin condensed) observed after treated with 1000 µg/mL of TEE and THE respectively at 48 h (at 10×magnification; Olympus Corporation, Tokyo, Japan)

than THE. In case of normal human cell line HEK 293T, treatment of TEE and THE exhibited no morphological changes but slight confluency change in respect to control (Fig. not shown here). Nuclear morphological study by DAPI staining showed under inverted fluorescence microscope, the control or untreated A549 took light blue uniformly (Fig. 2D) while treated (1000 µg/mL of TEE and THE separately) cells exhibited bright blue nuclei due to chromatin condensation or irregular in shape or fragmented nuclei (Fig. 2 E, F). It indicated that this TEE and THE were effective to induce apoptosis in this cancer cell line. In case of normal human cell line (HEK 293T), at the highest concentration of 1000 µg/mL, showed very few apoptotic nuclei. It has been widely accepted that any chemical that has ability to change morphology of cancer cells must have anticancer potentiality. Workers[21,23] demonstrated changes of cancer cell morphological and confluency levels in both CaSki and HeLa and noted changes of cell (CaSki) morphology under the exposure of methanolic and ethanolic extract of *Agaricus bisporus* from normal elongated to round. The nucleus condensation, fragmentation, and DNA cleavage are indicative of apoptosis[34]. DAPI staining exhibited chromatin condensation and breaking in both HeLa and CaSki under exposure of EAE of *C. indica*[21]. Induction of apoptosis is suggested to be one of the major action mechanisms of chemotherapeutic anticancer drugs on malignant cells[38].

Cytotoxicity or Antiproliferative assay by MTT

A well-established preclinical assay is MTT assay and it is used to assess the anticancer efficacy of drugs[36]. In our study, MTT assay was used to evaluate the cytotoxic/anti-proliferative effect of TEE and THE against lung cancer A549 cell line. The inhibition percentages of cell growth ranged from 8.64 ± 0.96 to 31.26 ± 0.85%; and 5.70 ± 1.91 to 24.30 ± 0.72% at 24 h for TEE and THE respectively, in the concentration range of 100-800 µg/mL (Fig. 3A). Whereas at 48 h, the inhibition percentages of TEE and THE ranged from 11.92 ± 1.16 to 36.45 ± 0.84%, and 6.07 ± 1.94 to 26.94 ± 1.58% respectively, with a similar concentration range (Fig. 6B). IC_{50} (Inhibitory concentration 50) determines the effectiveness of a drug's action during examination[37]. The IC_{50} values were evaluated for two extracts and the IC_{50} values were 1402.16 ± 128.00, and 1887.45 ± 155.00 µg/mL for TEE and THE respectively at 24 h. At 48 h, the calculated IC_{50} values were 1173.03 ± 45.22

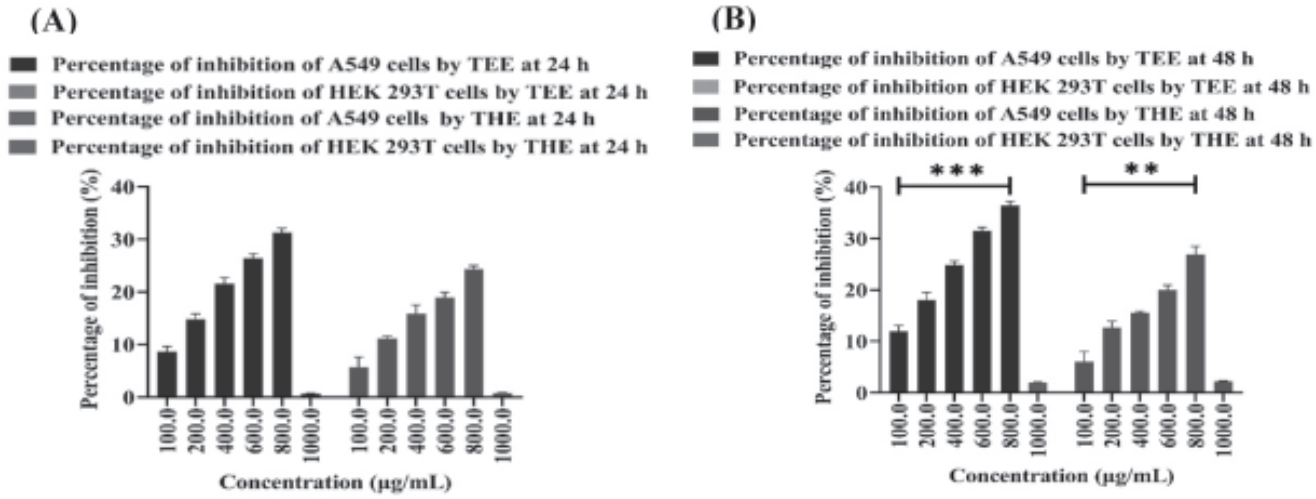

Fig. 3: Antiproliferative activity of TEE and THE against A549 cell line at (A) 24 h and (B) 48 h

and 1732.11 ± 40.23 µg/mL respectively for TEE and THE. The result demonstrated that, no significant changes were observed for TEE and THE at 48 h of treatment compared to 24 h of treatment. Similarly, the anticancer effect of ethanol extract of *Hexogonia glabra* was found on HeLa, SiHa, and CaSki and the IC_{50} values were 60.45 ± 6.21, 99.89 ± 7.45, 140.3 ± 15.32 µg/mL respectively[24].

Apoptosis inducing activity of TEE and THE extract

The percentage of apoptosis in A549 cells was determined at 500, 750, 1250 and 1750 µg/mL of TEE and THE. The percentage of cells that undergo apoptosis was found to be 10.01 ± 1.12, 20.15 ± 2.21, 40.02 ± 2.82, 60.04 ± 3.32%, for TEE ($p<0.05$) at above concentrations respectively. Cell apoptosis was found to be 5.06 ± 1.01, 15.05 ± 1.82, 25.01 ± 2.32 and 55.11 ± 3.54% respectively at the above concentrations of THE ($p<0.05$) (Fig. 4A). Similarly earlier workers[22] demonstrated evaluation of apoptosis by DAPI staining. The nucleus condensation, fragmentation, and DNA cleavage are indicative of apoptosis[34]. Induction of apoptosis is suggested to be one of the major action mechanisms of chemotherapeutic anticancer drugs on malignant cells[38]. Workers[21] demonstrated the changes of percentages of the apoptotic cells of CaSki and HeLa cell lines by different concentrations of EAE of *C. indica*. It was reported that the extracts (WE and ME) of *C. indica* were anti-proliferative against sarcoma and breast cancer cell lines[25]. The methanolic extract of *P. ostreatus* was recorded to anti cancerous on MCF-7, MDA-MB-231 (breast cancer), and HT-29 and HCT-116 (colon cancer) cell lines[31] and they noted changes of the morphology of HT-29 and MCF-7 cells by chromatin condensation and fragmentation of DNA which indicated the appearance of apoptosis[34]. According to Wang et al.[57] ethanolic extract of *Pleurotus ferulae* induced apoptosis *via* caspase 3 activation and by reduction of the MMP (mitochondrial membrane potential).

LDH assay of TEE and THE extracts on A549 cell line

The graphical representation in figure 4B demonstrated that LDH leakages occurred in dose-dependent manners from A549 cancer lines. TEE showed that the leakages of LDH were 7.04 ± 1.01, 15.01 ± 1.84, 29.11 ± 2.05 and 43.01 ± 2.92 (Unit/mL) at the concentrations of 500, 750, 1250 and 1750 µg/mL respectively. Similarly, THE exhibited that the leakages of LDH were 5.01 ± 0.92, 9.11 ± 1.23, 22.01 ± 2.12 and 31.05 ± 2.67 (Unit/mL) at the concentrations of 500, 750, 1250 and 1750 µg/mL respectively. This cell line was more sensitive to TEE at all concentrations than THE.

Some workers[22] recorded the effect of ethanolic extract of *Calocybe indica* on the leakage of LDH from pancreatic cancer cell lines (PANC-1 and MIAPaCa2 cells) after 24 h of treatment. Methanolic extract of *A. bisporus* had positive effect on LDH leakage on MCF7, HeLa and MDA-MB-231cancer cell lines[26].

Conclusion

TEE exhibited higher extraction yields, phenolic, flavonoid, and tannin contents compared to THE, and highlighting its superior antioxidant capacity. Both extracts of *T. pallescens* showed concentration-dependent DPPH radical scavenging activity, with TEE showing lower EC_{50} values than THE. Furthermore, TEE and THE exhibited dose-dependent anti-proliferation of A549 cell, and induction of cell apoptosis and phenomenon of LDH leakage from A549 cell suggesting their potential as anticancer agents. These results

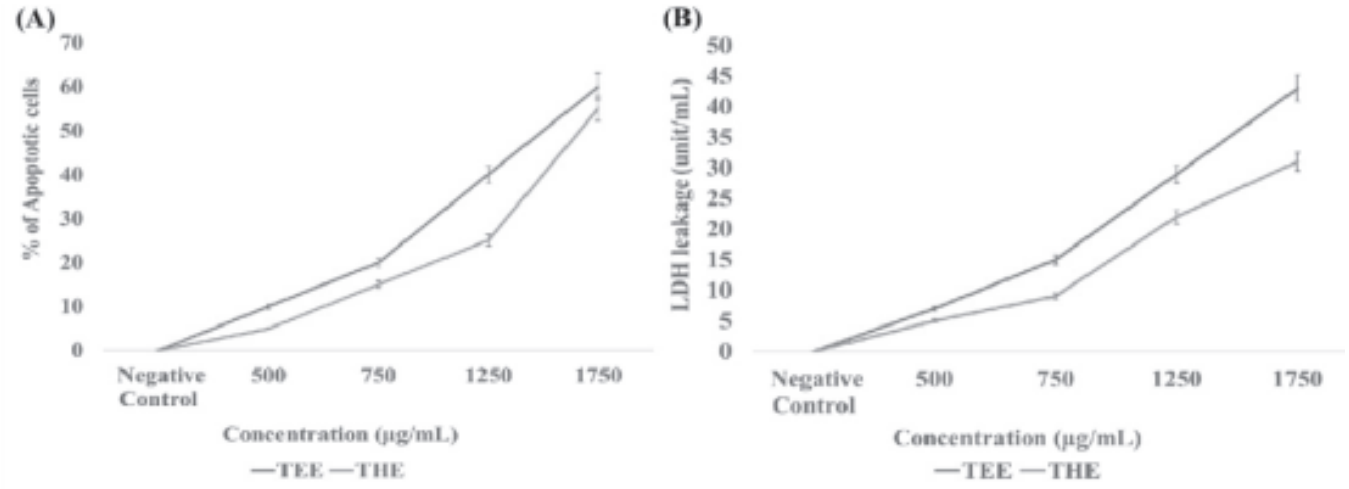

Fig. 4: Effect of TEE and THE of *T. pallescens* on apoptosis and LDH leakage on A549, (A) Percentage of apoptotic cells, (B) LDH leakage

highlighted the potential of *T. pallescens* extracts in pharmaceuticals and functional foods.

Data Availability Statement : All data generated or analyzed during this study are included in this article or can be obtained from the corresponding author upon reasonable request.

Ethics declarations

Competing interest All authors declare that there is no financial and non-financial conflict of interest for the publication of this article.

Human and animal rights : No animal and human trial has been conducted in this research work.

Consent for Publication : All authors gave consent for publication of this article

Informed Consent : Not applicable.

Institutional Review Board : Not applicable

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