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In vitro evaluation of growth reticence and anticancer potential of 5α,8α-epidioxy-24ξ-methylcholesta-6,22-dien-3β-ol and ergosta-5,7,22-trien-3β-ol bioactive isolated from an edible mushroom *Lentinus tuberregium* (fr.)

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Highlights

- Employing two bioactive compounds of *Lentinus tuberregium* (Fr.) for anticancer studies.
- Bioactives maximal growth inhibition in the order as A673>HCT116>MCF7.
- The dose-dependent and compound-specific apoptotic induction reported.

- The compounds evince a significant growth arrest in the A673 cell cycle.

Abstract

The focus point of this current work is to evaluate the anticancer and growth inhibitory efficacy of compounds 5α,8α-epidioxy-24ξ-methylcholesta-6,22-dien-3β-ol (LT1), and Ergosta-5,7,22-trien-3β-ol (LT2) of Lentinus *tuberregium* (Fr.) on three cell lines such as A673 (Rhabdomyosarcoma), MCF7 (breast cancer), and HCT116 (colorectal carcinoma) by MTT assay. LT1 and LT2 exerted maximal growth inhibition in the order as A673>HCT116>MCF7. Comparatively, LT1 was more potent in causing cell growth inhibition than LT2 in the A673 cancer cell line. Based on the MTT assay, A673 cells alone proceeded further as a model to evaluate the anticancer potential of LT1 and LT2 at three different semilogarithmic concentrations (3, 10, 30 μM). The cells exposed with compounds at 24 and 48 h were analyzed by flow cytometry. Exposure of LT1 at 3 and 10 μM concentrations for 24 h caused a G2-M arrest. At 10 μM concentration, cells also accumulated in the G0-G1 phase, indicating a G1 block. These effects were only transient as prolonged exposure (48 h) of LT1 treatment brought back the cell population to normalcy. Both the compounds only at 30 μM concentration have the potential to induce a hypodiploid peak (sub G0), indicating an induction of apoptosis which was explicit by nuclear condensation and fragmentation of nuclei in cells. The dose-dependent and compound-specific apoptotic induction was further confirmed by caspase activity higher in LT1 than LT2. The results highlight the significant growth inhibitory activity and anticancer potential of LT1 and LT2 which are recommended for further in-depth analysis.

Introduction

Cancer remains one of the world's distressing diseases and it's characterized by an unusual growth of cells and tissues which causes a high rate of disease-related–mortality. The most profound treatment technique is the use of chemotherapeutic drugs along with radiotherapy (Pugazhendhi et al., 2018; Shanmuganathan et al., 2019; Wu et al., 2020). The chemotherapeutic drugs must be target-specific to a cancer cell and should not affect the normal cells. But most of the available commercial chemotherapeutic drugs show various adverse effects on normal cells. Hence, there is growing demand to explore safe anticancer drugs, especially from biological sources to counteract these negative impacts of the existing drugs (Ahmed et al., 2020; Ray et al., 2020).

Since the serendipitous discovery of the fungal metabolite penicillin as an antibiotic drug, several fungal species owing to the presence of a plethora of bioactive compounds are being investigated as a major source of new pharmaceuticals and drug lead compounds for various diseases (Morris et al., 2016; Rathore et al., 2017). In this regard, mushrooms with a long history of medicinal use include *Grifola frondosa* (maitake), *Lentinula edodes* (shiitake), *Pleurotus florida*, *Phellinus rimosus, Cordyceps sinensis* and *Ganoderma lucidum* (Lingzhi or Reishi), which exhibit a wide range of biological properties ranging from antioxidant, anti-inflammatory, anticancer, antidiabetic, antimicrobial, and antilipemic to immunomodulatory (Friedman, 2016; Patel et al., 2021; Román et al., 2020). Polysaccharides, lipids, proteins, alkaloids, phenolics, ergosterol, folate, selenium, enzymes, and organic acids are some of the bioactive substances present in mushrooms (Park, 2022). Cordycepin, anthroquinonol, lectin, hispolon, polysaccharide, krestin, lentinan, Maitake D Fraction and sulfated polysaccharide are some of the anticancer bioactives of mushrooms (Ayeka, 2018). Only a small number of mushrooms are now approved for usage in clinical settings (Joseph et al., 2018). Studies have shown that mushroom-derived compounds either alone or in combination with chemotherapeutic drugs have great potential to destroy tumor cells without affecting normal cells (Ina et al., 2013). These anti-cancer compounds act at different stages of cancer, by modulating cell proliferation, apoptosis, cell cycle progression, inflammation, angiogenesis, and immune evasion and contributes to cancer prevention (Mishra et al., 2021).

Chihara et al. were the first to decipher the anticancer effect of a water-soluble polysaccharide which was named lentinan from the fruit bodies of shiitake (Chihara et al., 1969). *In vivo* studies revealed that lentinan potentiated the chemotherapeutic effect of trastuzumab and significantly suppressed tumor growth. The role of lentinan in immuno-chemotherapy when given in combination with fluoropyrimidine, which prolonged the survival of patients with advanced gastric cancer, as compared to chemotherapy alone (Ina et al., 2013). *Lentinus tuberregium* is a wild edible mushroom, well known for its medicinal values in Malaysia and Africa. It is a valuable medicine against diarrhea (Burkill, 1966; Jones et al., 2007) especially effective in children (Lee, 2006) and it has been used in much herbal preparation for coughs, indigestion, and dysentery (Lee, 2006). Most of the anticancer studies on *Lentinus* spp. Were confined to the crude extract of *L. edodes* and its isolated polysaccharide fraction of the compound, Lentinan. However, *L. tuberregium,* species of *Lentinus* has not been worked out intensively for the anticancer effect.

There is a lot of evidence to support the anti-tumor assertions made of lentinan (Antonelli et al., 2020; Hong et al., 2011; Zhang et al., 2018), and its acceptance into clinical medicine should perhaps highlight its efficacy (Suo et al., 2021). So, it is anticipated that *Lentinus* spp.

Can provide potential bioactive lead compounds to combat cancer diseases. Apart from lentinan polysaccharide, *Lentinus* spp. Is the source of several well-studied preparations with proven pharmacological activities (Azeem et al., 2020). It was therefore hypothesized that an edible and medicinal mushroom *L. tuberregium* has compounds that may have anticancer activity. To accomplish this hypothesis, the present study aimed to evaluate the cytotoxicity, cell cycle progression, and apoptotic induction by two compounds (assigned as LT1 and LT2) isolated from the fruit body of *L. tuberregium* using selected cell line models.

Section snippets

Cell lines and reagents

Human cancer cell lines such as HCT-116 (colorectal carcinoma), A673 (Rhabdomyosarcoma), and MCF (breast cancer) were procured from the National for Cell Science (NCSS), Pune. Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and phosphate-buffered saline (10 *X*) were obtained from Gibco, Invitrogen Life Technologies. Propidium iodide (PI) ethylenediaminetetraacetic acid, trypsin, sodium bicarbonate, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl

assay for cell growth inhibition

The results of MTT assay for the compounds LT1 and LT2 at 5 logarithmic concentrations (100, 10, 1, 0.1 and 0.01 μM and DMSO as vehicle control), in three cancer cell lines *viz.*, HCT116 (colorectal carcinoma), A673 (rhabdomyosarcoma), and MCF7 (breast cancer), for anti-proliferation activity are depicted in Fig. 1 and Table 1. Similar cell lines are used for the evaluation of anticancer activity of extracts of marine sponge by promoting tubulin polymerization as evidenced mitotic arrest and

Conclusion

L. tuberregium is an edible mushroom which not been explored for its anticancer efficacy in cell line models such as A673, HCT-116, and MCF. The bioactive compounds such as 5α,8α-epidioxy-24ξ-methylcholesta-6,22-dien-3β-ol (LT1) and Ergosta-5,7,22-trien-3β-ol (LT2) isolated from *L. tuberregium* exhibited potent cytotoxicity in A673cell lines than HCT-116 and MCF. The overall findings of the current research revealed that both LT1 and LT2 retarded the growth and proliferation of cells by causing

Credit author statement

J. Manjunathan: Conceptualization, Methodology, Formal analysis, Data curation, Software, Investigation, Writing-original draft, Visualization. **S. Shyamalagowri**: Review & Editing, Supervision. **M. Kamaraj**: Review & Editing. **S.P. Thyagarajan**: Review & Editing. **V. Kaviyarasan**: Review & Editing, **Kathirvel Brindhadevi**: Review & Editing, Supervision.

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Declaration of competing interest

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