Uncovering key pathways of *Lentinula edodes* against cancer via network pharmacology

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Introduction

Cancer, a widely orchestrated by inflammatory responses, can impair the patient's immune system that can be utilized to be sensible and destroy tumor cells or their survival environment Cancer is characterized by excessive inflammation, further, is driven by genetic mutations to control survival and migration to other organs Cancer -related signaling pathways associated with these alterations can manage cancer cell proliferation, apoptosis, and metastasis, can be distorted to accelerate inflammation. The inflammatory responses are the main factors, suggesting that an inflammatory microenvironment can enhance mutation rates with increasing the growth of mutated cells. At present, the usage of aspirin or other Non-steroidal anti-inflammatory drugs (NSAIDs) alleviates the rate of breast, lung, bladder, colorectal, and esophageal cancers. As alternative agents, herbal medicines are relatively safe, comfortable sources although some have unexpected side effects such as constipation, diarrhea, headache, dizziness, fatigue, and anorexia. The herbal medicines with natural-oriented secondary metabolites have contributed to anticancer medication development: paclitaxel from the bark of *Taxus brevifolia* Nutt. (Taxaceae), homoharringtonine from *Cephalotaxus harringtonia* var. drupacea (Sieb and Zucc.) (Cephalotaxaceae), and elliptinium from Fijian medicinal plant *Bleekeria vitensis* A. C. Sm. The medicinal mushrooms are one of significant natural medicines which have been used as antimicrobial, antibacterial, anti-inflammatory, immunomodulatory, antidiabetic and anticancer agents (Zhang et al. 2016a). A study demonstrated that aqueous extracts of *Lentinula edodes* (LE) exerted inhibitory effect on the growth of the human cancer cell lines laryngeal carcinoma (Hep-2) and cervical adenocarcinoma (HeLa). In addition, another study showed that α-glucans with very hydrophilic properties might be a potential anticancer agent with a plausibility. On contrary, mushroom-derived glucans with low bioavailability owing to poor absorption rate in intestinal barrier are not optimal for oral administration, furthermore, most data have been produced using cell cultures or model organisms. A human-based study proved that the number of 10 healthy male volunteers tookβ-glucans of 1000 mg once daily for a week, however, β-glucans in their serum samples were not detected at all time points for 20 days. Moreover, β-glucans have drug interactions to be able to induce unexpected side effects with most Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) : Ibuprofen, Ketoprofen, Indomethacin, Naproxen, Ketorolac, Nabumetone, Sulindac, Aspirin, Meclofenamate, Oxaprozin, Flurbiprofen, Piroxicam, Fenoprofen, Etodolac. Commonly, ligand's cell permeability is diminished typically, when topological surface area (TPSA) is beyond 140 Å^2. Subsequently, we infer thatβ-glucan as a representative compound of mycelium is very hydrophilic compound and cannot be designated as a potential ligand due to unseemly physicochemical properties (TPSA: 268.68 Å^2 or Lipinski's rule violation). Thus, our study was to uncover lipophilic compounds from LE, filtering out hydrophilic compounds like glucans or its derivatives. In particular, promising drug-like compounds (DLCs) and pharmacological mechanism(s) of LE against cancer have not been substantiated completely. Furthermore, mushroom extracts have been valued by human as s significant culinary and ethnopharmacy source due to a wide spectrum efficacy, including anti-cancer.

Accordingly, we implemented protein-protein interaction (PPI), pathway- target- compound (PTC) analysis of LE ethanolic extract through network pharmacology. Network pharmacology is s holistic concept to elucidate complex biological mechanisms on compound- target interaction. It is a significant tool to identify uppermost compound or target or pathway on system biology-based methodology (Oh et al. 2021b). This analysis is an efficient method to recognize the mechanism(s) of action on herbal extracts and to unravel efficacy of collective compounds. Presently, network pharmacology has been utilized to reveal new targets and ligands' relationships, which gives hint to evaluate therapeutic capability of natural products.

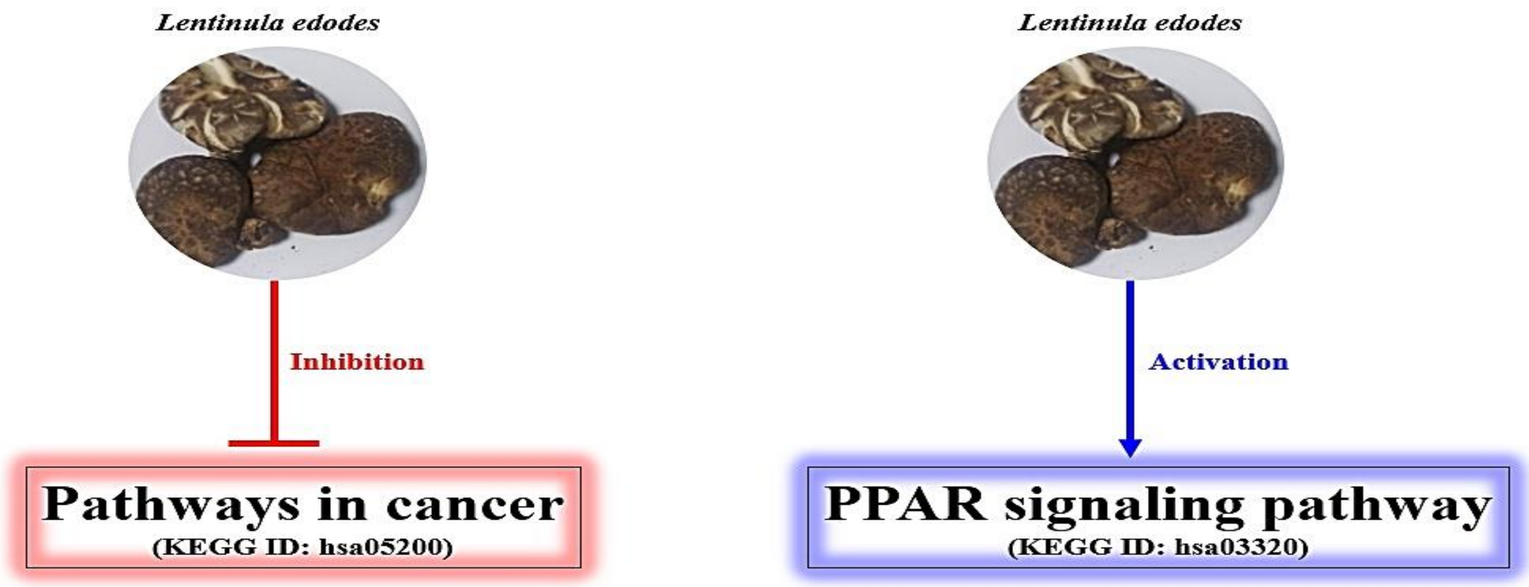
Abstract

Lentinula edodes (LE) is known as a good food source with potent anticancer efficacy, but its active compounds and mechanisms against cancer has not been revealed. This study was to investigate the bioactive compounds and mechanisms of LE against cancer via network pharmacology. Compounds from LE detected by Gas Chromatography Mass Spectrometry (GC-MS) and screened drug-like compounds by SwissADME. Targets were identified by two public bioinformatics, and overlapping targets were selected by Venn diagram. Then, protein-protein interaction (PPI) and pathways-targets-compounds (PTC) networks were constructed by RStudio. Lastly, we identified key compounds and targets via molecular docking test (MDT) on AutoDockVina. A total of 34 compounds from LE were selected as drug-like compounds (DLCs). The 34 compounds were associated with 108 targets and a key target (COX2) was identified through PPI networks. Most significantly, out of 9 pathways, inactivation of Pathways in cancer and activation of Peroxisome Proliferator Activated Receptor (PPAR) signaling pathway were uppermost pathways of LE. On MDT, we identified a key compound (Indole, 2-methyl-3-phenyl) on COX2 related to inactivation of Pathways in cancer, additionally, the number of 6 ergostane steroids associated with both pathways might be dual efficacy to alleviate inflammation against cancer. Overall, 13 targets, 11 compounds, 2 key pathways of LE were identified as the primary elements to treat cancer. Therefore, this study provides therapeutic evidence to evaluate the promising clinical efficacy of LE on cancer, suggesting that LE is a good food pharmacy with synergistic effects against cancer.

Results

Graphical abstract

Figure.1 Key pathways of *Lentinula edodes*



Materials and methods

Compounds identification from LE and confirmation on drug-like property

The chemical compounds from LE were detected via GC-MS analysis. The compounds identified by GC-MS validated "Drug-likeness" physicochemical property via Lipinski's rule on SwissADME (http://www.swissadme.ch/) (accessed on 28 June 2021). The validated compounds converted into Simplified Molecular Input Line Entry System (SMILES) format through PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 29 June 2021) to perform MDT.

Targets related to compounds from LE

Targets associated with compounds from LE were retrieved via both Similarity Ensemble Approach (SEA) (http://sea.bkslab.org/) (accessed on 01 July 2021) and SwissTargetPrediction (STP) (http://www.swisstargetprediction.ch/) (accessed on 02 July 2021). The overlapping targets between SEA and STP identified by Venn diagram tool; InteractiVenn (http://www.interactivenn.net/) (accessed on 02 July 2021) .

The construction of PPI networks

The overlapping targets from two databases (SEA, STP) were utilized to analyze PPI networks on STRING database (https://string-db.org/) (accessed on 03 July 2021) (D et al. 2019). The RPackage software was used to signify the degree of values on PPI networks.

Key pathways of LE and functional enrichment analysis

The STRING database (https://string-db.org/) (accessed on 03 July 2021), a widely utilized genomic-based annotation tool, was used for analysis of key pathways and gene ontology (GO) analysis of the overlapping targets from LE. The collective data were obtained on human genes and false discovery rate (FDR)≤0.05 as the cut-off value.

The construction of PTC networks

The PTC networks were used to construct a size map, according to the degree of values. In this size map, yellow rectangles (nodes) stood for pathways; red triangles (nodes) stood for targets; and orange circles (nodes) stood for compounds; its sizes represented degree value. The size of red triangles represented the number of connectivity with pathways; the size of orange circles represented the number of connectivity with targets. The merged networks were built by utilizing RPackage.

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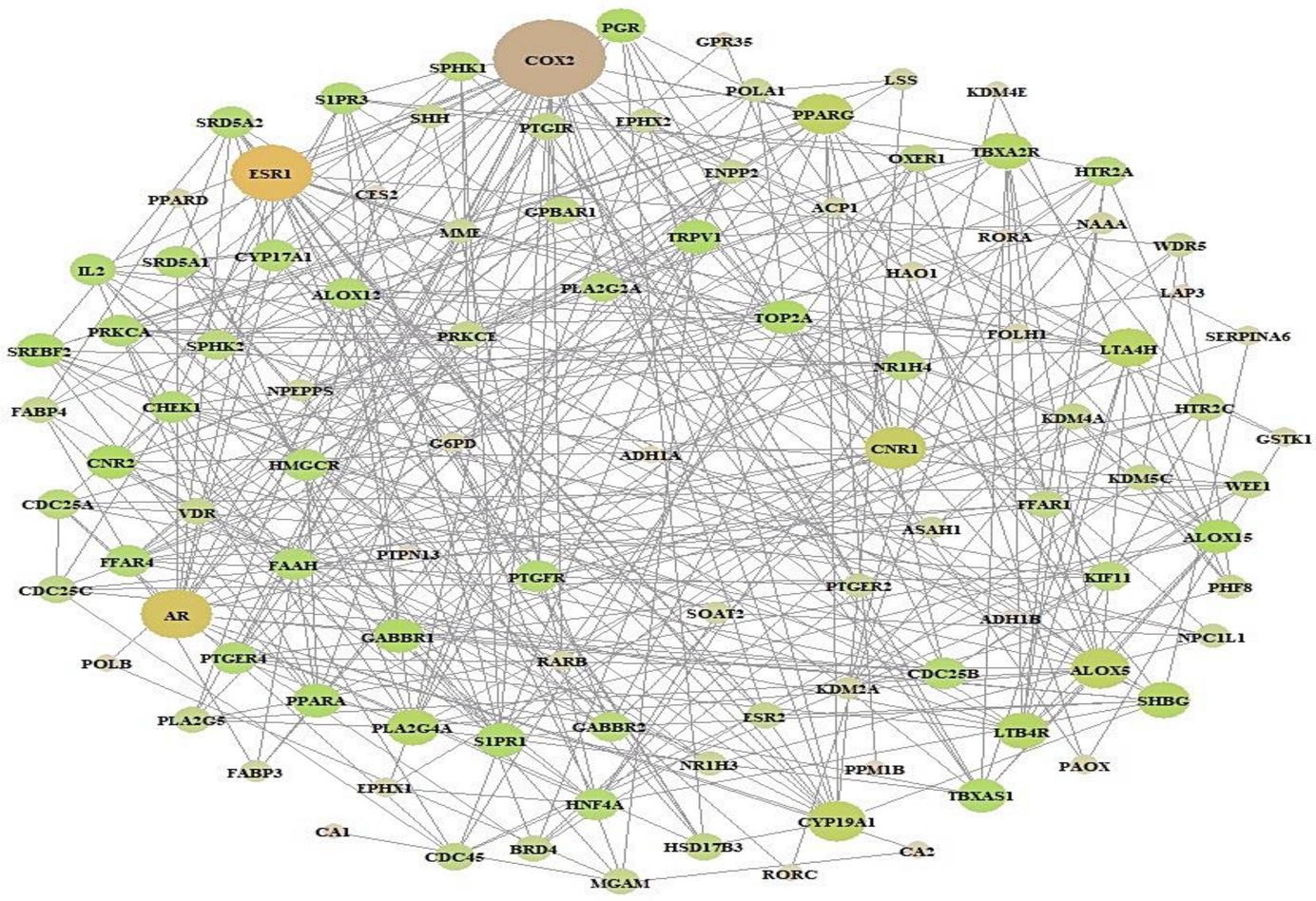
Figure.2 PPI networks of *Lentinula edodes*



Conclusion

The study provides 13 key targets, 11 key compounds, 2 key pathways of LE which might be underlying synergistic effects against cancer. Out of 13 key targets, COX2 related to Pathways in cancer is a key target against cancer. Out of 11 compounds, Indole, 2-methyl-3-phenyl related to COX2 is a key compound to inhibit inflammation against cancer. The inactivation of Pathways in cancer, and activation in PPAR signaling pathway are of significant pathways of LE against cancer. Also, our findings provide that ergostane steroids have dual efficacy on the two pathways. Thus, this work suggests that LE might be promising combined efficacy against cancer.

References

1. Zhang J-J, Li Y, Zhou T, et al (2016a) Bioactivities and Health Benefits of Mushrooms Mainly from China. Molecules 21:. https://doi.org/10.3390/MOLECULES21070938
2. Oh K, Adnan M, Cho D (2021b) Uncovering Mechanisms of Zanthoxylum piperitum Fruits for the Alleviation of Rheumatoid Arthritis Based on Network Pharmacology. Biology 2021, Vol 10, Page 703 10:703. https://doi.org/10.3390/BIOLOGY10080703