Research article

Predicting the bioactive compounds of *Lentinula edodes* and elucidating its interaction with genes associated to obesity through network pharmacology and *in-vitro* cell-based assay

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ABSTRACT

Mushrooms are traditionally used for various medicinal purposes in traditional oriental medicine. The Japanese and Chinese are familiar with the medicinal macro fungus *Lentinula edodes* (*Shiitake* mushroom). This study aims to evaluate the role of chemical compounds from *L. edodes* using network pharmacology and *in-vitro* studies for management of Obesity. Bioactive compounds in extracts of *L. edodes* were identified by GC-MS analysis. Compounds were later screened for their drug-like property by Lipinski's rule. In addition, public databases (SEA, STP, Omim and DisGenet) were searched to identify genes associated with selected molecules and obesity, as well as genes that overlap obesity target genes with genes related to *L. edodes*. Additionally, analysis was performed using Enrichr KG to predict the disease targets of *L. edodes*. Finally, network was constructed between the overlapping genes and bioactive molecules using Rstudio. Further *in-vitro* studies were carried out using 3T3-L1 cell line. The genes related to the selected compounds and obesity were identified and overlapped. The disease targets of *L. edodes* was predicted by enrichment analysis and was found to be linked to obesity. Furthermore, the hub gene was found to be fatty acid amide hydrolase, and the key bioactive compound was hexadecanoic acid methyl ester. The *in-vitro* cell culture studies confirmed the inhibition of adipogenesis in mushroom extract-treated 3T3-L1 cells and the augmentation of adiponectin. The study suggests that the hub gene fatty acid amide hydrolase might alleviate obesity by inhibiting arachidonoyl ethanolamide signaling, which would enhance the action of fatty acid amide hydrolase and limit appetite in *L. edodes* extract.

1. Introduction

Mushrooms have attracted a growing amount of interest for their potential pharmacological effects in recent years, and it has been suggested that some mushrooms are like miniature pharmaceutical factories that produce compounds with miraculous biological properties. Moreover, mushrooms are traditionally used as a nutritious food due to their medicinal and nutritional properties.

According to the edibility factor, they are categorized into three groups: edible mushrooms, inedible mushrooms, and poisonous mushrooms [1]. Edible mushrooms are an elixir for treating major metabolic disorders such as diabetes and obesity. The low-fat content, high polysaccharides, and dietary fiber in mushrooms possess anti-inflammatory and immunomodulatory properties, posing positive health risks to the body [2,3]. The secondary metabolites (polysaccharides, lipids, proteins, carbohydrates, and terpenoids) present in the mushroom play a crucial role in the regulation of glucose homeostasis, inflammatory factors for improving lipid metabolism and insulin resistance, and in assisting the actions of glucose transporter IV [4,5]. According to Wang et al., 2020 [6], *L. edodes* can be used to treat a number of illnesses, including diabetes, cancer, the flu, heart disease, high blood pressure, and weakness. It can do this by boosting the immune system, fighting free radicals, bacteria, and high cholesterol. Moreover, it has been reported to reduce gut dysbiosis, which results in reducing insulin resistance [7].

Additionally, network pharmacology is a well-organized, systemic, and useful bioinformatic method used to study how bioactive substances, proteins, genes, and diseases of interest interact with each other [8,9]. It is possible to decode compound mechanisms by looking at network pharmacology from a multifunctional point of view, emphasizing how multiple factors interact instead of just one compound for a given target [10]. Therefore, network pharmacology is a useful way to find possible key compounds (from natural sources) that can stop the development of several diseases and, more often than not, to figure out how they work together with other bioactive compounds [11]. Li and Zhang, 2013 [12] reported that network-based drug discovery has dramatically improved as a result of rapid advances in bioinformatics and system biology. A similar study using network pharmacology was carried out by Oh et al., 2021 [13] to evaluate the bioactive ingredients and mechanism of *Phellinus linteus* mushroom on type 2 diabetes mellitus.

In the present study, network pharmacology was applied to determine whether *L. edodes* mushroom was effective and to predict its activity against obesity. Later in the study, different *L. edodes* extracts were tested to see if they could slow down the process of adipogenesis using differentiated 3T3-L1 cells.

2. Materials and methods

2.1. Collection of fruiting body and chemicals

Dried *L. edodes* was procured from local market in Mumbai, India. The mushrooms were ground and passed through sieve no. 45 to obtain a fine powder. The powder was stored in an airtight container. LC-MS-grade methanol and acetonitrile were procured from Thomas Bakers Pvt. Ltd., Mumbai, India and S.D. Fine Chemicals Ltd, Mumbai, India. The 3T3-L1 adipocytes were procured from NCCS, Pune, India (Request Number: 868/2021-22). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), antibiotic (penicillin-streptomycin solution), Oil Red O, formalin, dexamethasone, insulin, 3-isobutyl-1-methylxanthine (IBMX), and isopropanol were provided by HiMedia, India. Other chemicals used were of analytical grade and obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

2.2. Extraction of L. edodes fruiting body

For extraction, 10 g of mushroom fruiting body powder was extracted using reflux method for 3 h with 100 mL of aqueous, ethanol, a mix of ethanol and aqueous in the ratio of 50:50, 25:75, 75:25 and methanol as solvents. The alcoholic extracts were further evaporated by rotary evaporator and aqueous extracts by lyophilization to obtain yield and stored for further use.

Lipid extraction was carried out using "Folch" wash method described by Marekov et al., 2012 [14] and Zhou et al., 2015 [15]. 1 g of mushroom extract was dissolved in 10 mL of methanol. To the above solution, 20 mL of chloroform was added and stirred for 30 min. The organic layer was evaporated. The extract was re-dissolved in 12 mL of mixture of chloroform-methanol (2:1) and was subjected to folch wash (3 mL of 0.88% KCl in aqueous). After removing aqueous layer, the organic layer was rewashed with 3 mL methanol-saline (1:1). The resulting solution was centrifuged, and aqueous layer was discarded. The organic layer was collected and dried. The lipid extract was dissolved in 2 mL of chloroform-methanol (2:1) and was subjected to analysis for GC-MS.

2.3. GC-MS analysis

The GC-MS analysis was carried out to identify the chemical compounds from *L. edodes* mushroom. Shimadzu GCMS- QP2010 Ultra was used to carry out the analysis. The GC was equipped with a 30 m × 0.25 mm x 0.25 μm capillary column. Initially, a temperature of 100 °C was maintained for a period of 3 min and was further raised to 300 °C at a rate of 10 °C/min. The temperature was then maintained for 17 min at the end of this period. The samples were injected in split mode. The injection port temperature and the helium flow rate were 260 °C and 1.21 mL/min. The MS scan range was set at 40 m/z – 650 m/z. The NIST Library MS database was used to compare the fragmentation pattern of the mass spectra [14].

2.4. Construction of database and drug-likeness filtering of myco-chemicals obtained from L. edodes

Based on the GC-MS analysis of myco-chemicals obtained from *L. edodes*, Lipinski's rule was applied through SwissADME (http://www.swissadme.ch/) [16] to identify their drug-like property. After the Lipinski's rule was able to assess the screen evaluation as "acceptable," the molecules were sorted out as potential bioactive ingredients. To identify SMILES (Simplified Molecular Input Line Entry System) of molecules, the Pub-Chem program was utilized (https://pubchem.ncbi.nlm.nih.gov/).

2.5. Searching of target genes associated to bioactive ingredients and obesity

SMILES was used to select target genes related to the active molecules through both Similarity Ensemble Approach (SEA) (http://sea.bkslab.org/) [17] and Swiss Target Prediction (STP) (http://www.swisstargetprediction.ch/) [18] by applying "Homo Sapiens" mode. Genes associated with obesity were extracted using DisGeNET (https://www.disgenet.org/) and Online Mendelian Inheritance in Man (https://omim.org). Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) was used to categorize and visualize the genes overlapping with selected molecules and obesity target genes [13].

2.6. Disease investigation of overlapping genes

Enrichr - KG [19] was used for disease investigation of overlapping genes using the DisGenet database. Based on DisGenet disease results, possible disease targets for *L. edodes* were derived.

2.7. Network visualization between bioactive molecules and overlapping genes

Using STP, SEA, and a network visualization tool, Rstudio (ver. 2023.03.0 + 386), we analysed the connections between compounds and overlapping genes. Configuring "degree values" of molecules and genes that were predicted by exploring network topology led to identifying the key active ingredients of *L. edodes* against obesity. On the network scale, compound or gene degree values represent the number of edges each compound or gene has [20]. In general, compounds or genes with a higher degree value are more valuable for the therapeutic effects of *L. edodes* against obesity [13].

2.8. Cell culture and differentiation

The 3T3-L1 preadipocytes were cultured in DMEM supplemented with 10% FBS and 1% antibiotic at 37 °C. The preadipocytes were maintained in the same medium for two days until growth arrest (day 0). Two days later, after full confluency, the cells were shifted to differentiation medium for another two days (day 2) containing 0.5 mM IBMX, 1 μM dexamethasone, and 10 μg/mL insulin. After two days, the cells were re-fed with complete medium containing 10 μg/mL insulin (differentiation progression medium). After two days, the differentiation progression medium was withdrawn (day 4) and the cells were maintained in the post differentiation medium until day 8 [20,21]. At this stage, the cells were treated with a concentration of 1 mg/mL for both the extract and the standard, and 250 μL of this solution was introduced to cells within a medium volume of 1.25 mL, resulting in a total volume of 1.5 mL in the well.

2.9. Oil Red O staining of cells

The differentiated and treated adipocyte cells were assessed for lipid accumulation by Oil Red O staining. Eight days post differentiation, cells were washed twice with PBS and fixed with 10% formalin at room temperature for 30 min. The fixed cells were again washed three times with distilled water and were stained using Oil Red O (60% Oil Red O solution) for 30 min. The cells were again washed three times with distilled water and microscopic images were taken for visualizing the lipid droplets in the cells. The cellular lipid content was measured by dispensing isopropanol into the cells. After 30 min, the supernatant was collected and measured at 490 nm using a microplate reader [22,23].

2.10. Measurement of adiponectin

The cellular adiponectin content was measured using Mouse ADP/Acrp30 (Adiponectin) ELISA kit (Wuhan Fine Biotech Co. Ltd., China). Briefly, the differentiated cells were washed three times with ice cold PBS and then scraped and centrifuged. The cell pellet was lysed and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and used as whole-cell lysate and adiponectin content was determined using the ELISA kit as per the instruction given by the manufacturer.

2.11. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.0. All the data were presented as the mean ± standard error of the mean (SEM) of triplicates. The p values < 0.05 were considered statistically significant.

3. Results and discussion

3.1. Potential active ingredients of L. edodes

The GC-MS analysis of different extracts of *L. edodes* (Supplementary Figure S1a–f) revealed 46 chemical compounds in aqueous extracts, 67 chemical compounds in ethanolic extract, 29, 37 and 48 chemical compounds in various hydroalcoholic extracts (50:50, 25:75, 75:25), and 46 chemical compounds in methanolic extract. Lipinski's rule further confirmed the drug-like characteristics of these molecules. Based on SEA and STP databases, 31, 55, 17, 23, 39, and 33 active ingredients (i.e., compounds that interacted with genes such as FAAH, CNR1, PPARG, PPARD, ALOX5) were selected respectively for aqueous, ethanolic, hydroalcoholic (50:50, 25:75

and 75:25) and methanolic extracts (Supplementary Table S1).

3.2. Target genes related to the active molecules and obesity

Based on SMILES, genes from SEA and STP associated with compounds were extracted. Based on the Venn diagram, the two databases contain 63 genes from aqueous extract, 137 genes from ethanolic extract, 61, 87, and 82 genes from hydroalcoholic extracts (50:50, 25:75, 75:25), and 71 genes from methanolic extract that overlap (Fig. 1). The two public databases, OMIM and DisGeNet, provided 400 genes associated to obesity (Supplementary Table S2). Based on the Venn diagram, the number of genes that shared overlapping regions among 400 obesity-related genes and the overlapping genes was 12, 15, 9, 20, 14 and 12 genes in the respective extracts (Fig. 2). The overlapping genes associated with the disease are enlisted in Supplementary Table S3.

3.3. Possible disease targets of L. edodes

All the extracts of the mushroom target diseases such as diabetes mellitus, obesity, metabolic syndrome X, non-alcoholic fatty liver disease, steatohepatitis, bulimia nervosa, and dyslipidemias (Supplementary Figure S2a–f). Agunloye & Oboh, 2022 [24] showed that *Pleurotus ostreatus* and *Letinus subnudus* can help people with diabetes by lowering blood sugar and lowering the activity of enzymes that break down carbohydrates and enzymes that are linked to high blood pressure. Moreover, both the mushrooms can protect the body from ROS produced by hyperglycaemic conditions in diabetes, suggesting that consuming *Pleurotus ostreatus* and *Letinus subnudus* may help prevent diabetes from progressing. Similarly, Yu et al., 2016 [25] found that *Lentinula edodes* mushroom reduced lipidemia-related factors in rats regardless of their sex, and that *L. edodes*-induced reductions in lipophilic antioxidant capacity may reflect an improved metabolic profile as a result of a lower prooxidative state.

Our findings indicate that all the possible disease targets associated with the genes of *L. edodes* extracts are indirectly and directly corelated with the metabolic syndrome, targeting insulin secretion, breakdown of glucose, glucose uptake, gluconeogenesis lipogenesis, antioxidant targeting and other factors related to obesity. However, it can be deduced that *L. edodes* may regulate lipid metabolism and catabolism, fatty acid metabolism and biosynthesis and the inflammatory response to reduce obesity.

3.4. Bioactive molecules of L. edodes and hub genes associated with obesity

Finally, the overlapping genes interacted with respective molecules and their interactions were visualized with the network containing various nodes and edges (Fig. 3). We assessed the genes associated with obesity for *L. edodes* extract based on "degree value". The level of compounds in all the extracts (Supplementary Table S4) showed that hexadecanoic acid methyl ester was highly concentrated except for the hydroalcoholic (50:50) and methanolic extracts and connected to a maximum of 11 genes. In addition, FAAH interacted with most compounds (41 in total) in all of the *L. edodes* extracts. This meant that FAAH was thought to be the key gene in *L. edodes* that fights obesity (Table 1).

A study discovered that hexadecanoic acid could change most of the proteins and the P13K-Akt pathway, which in turn improved metabolism [26]. FAAH is an enzyme associated with membranes and responsible for degrading endocannabinoids. The role of FAAH and endocannabinoid system in lipid metabolism, oxidative stress, and inflammation have been studied by Bátkai et al., 2004 [27,28].
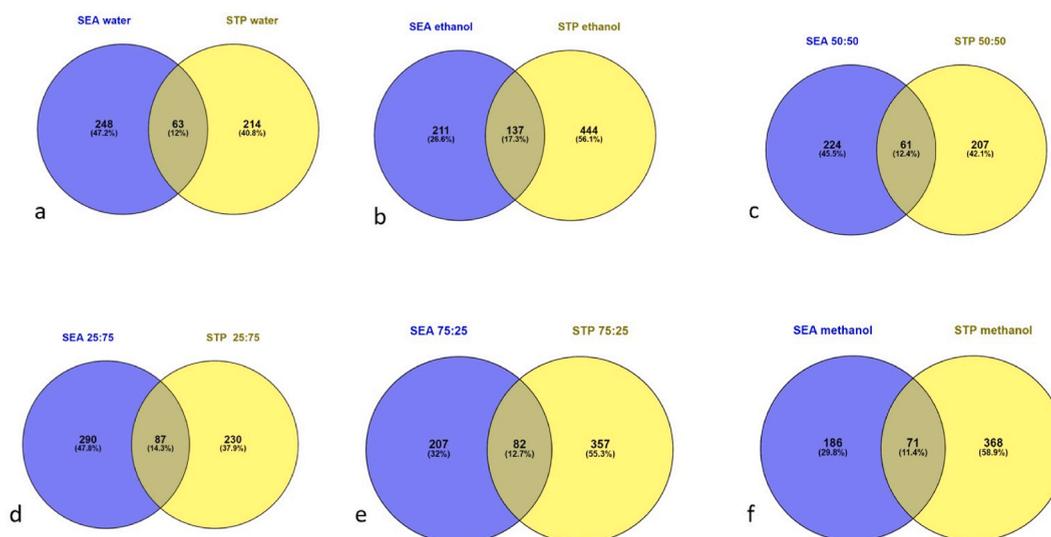
Fig. 1. Overlapping genes between compound-related genes of, a: 100% aqueous extract; b: 100 % ethanolic extract; c: 50:50 hydroalcoholic extract; d: 25:75 hydroalcoholic extract; e: 75:25 hydroalcoholic extract; f: methanolic extract, from SEA and STP database.
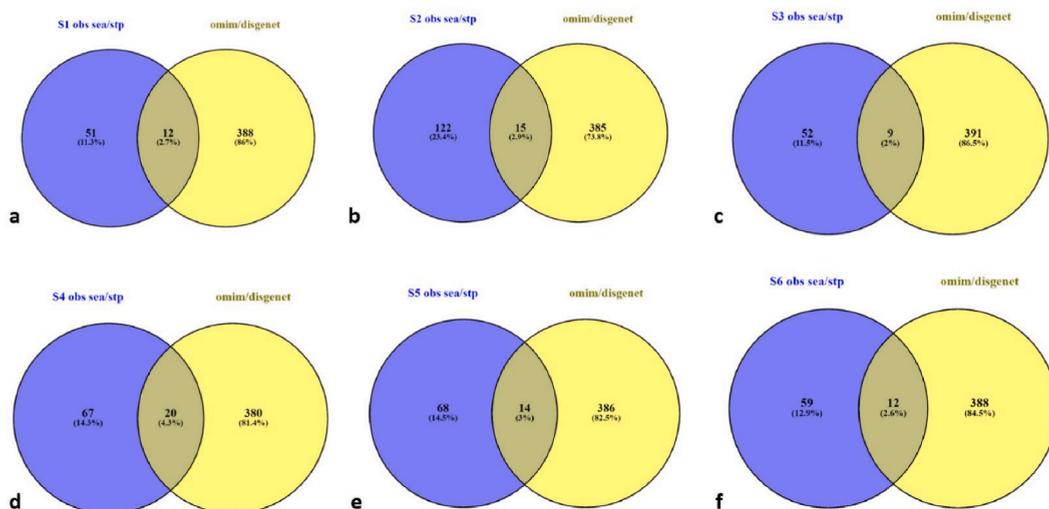
Fig. 2. Overlapping genes between compound related genes of, a: 100% aqueous extract; b: 100 % ethanolic extract; c: 50:50 hydroalcoholic extract; d: 25:75 hydroalcoholic extract; e: 75:25 hydroalcoholic extract; f: methanolic extract, and 400 Obesity related genes from two public databases, OMIM and DisGenet.
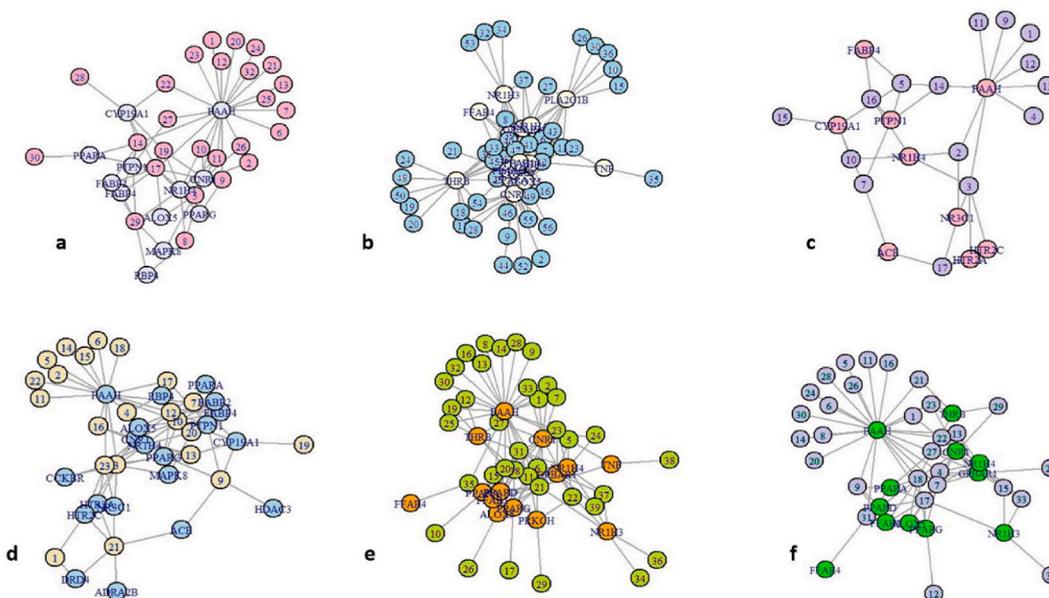
Fig. 3. Network linked to compounds in *L. edodes* and target genes related to Obesity; a: 100% aqueous extract; b: 100 % ethanolic extract; c: 50:50 hydroalcoholic extract; d: 25:75 hydroalcoholic extract; e: 75:25 hydroalcoholic extract; f: methanolic extract.

Biernacki et al., 2018 [29] demonstrated that fatty acid amide hydrolase inhibitor administration disrupts redox balance by lowering ROS levels and altering antioxidant capacity, resulting in oxidative modifications of proteins and lipids in the organs.

Collectively, we can predict that the extracts of *L. edodes* can be highly efficient for bioactivity of *L. edodes* against obesity due to the higher degree value of hub gene (FAAH) which might act on signaling pathways (endocannabinoid signaling, PPAR signaling, ROS signaling) that can activate insulin secretion, glucose uptake, lipid metabolism and other metabolic pathways responsible for management of metabolic syndrome and obesity.

3.5. In-vitro cell culture

3T3-L1 pre-adipocytes are undifferentiated fibroblasts that can be induced to differentiate into adipocytes, while adipocytes are mesenchymal cells that store energy in the form of lipids. Lipid buildup is a critical sign of adipogenesis. In fully developed cells,

Table 1

Degree value of genes related to different extracts of *L. edodes*.

Aqueous ext		Ethanolic ext		Hydroalcoholic ext (50:50)		Hydroalcoholic ext (25:75)		Hydroalcoholic ext (75:25)		Methanolic ext	
Gene	Degree Value	Gene	Degree Value	Gene	Degree Value	Gene	Degree Value	Gene	Degree Value	Gene	Degree Value
FAAH	21	FAAH	41	FAAH	10	FAAH	16	FAAH	26	FAAH	23
CNR1	9	CNR1	25	CYP19A1	4	CNR1	7	CNR1	14	CNR1	10
NR1H4	8	PPARG	17	PTPN1	4	NR1H4	7	GPBAR1	10	GPBAR1	9
CYP19A1	5	THRB	16	NR1H4	4	PTPN1	6	THRB	7	NR1H4	7
PTPN1	4	PPARA	15	NR3C1	3	PPARG	5	PPARG	7	PPARG	6
PPARG	3	PPARD	13	FABP4	2	CYP19A1	5	PPARA	7	PPARA	5
PPARA	3	PLA2G1B	13	HTR2A	2	ALOX5	4	ALOX5	6	THRB	5
FABP4	3	GPBAR1	13	HTR2C	2	FABP4	4	NR1H3	6	ALOX5	4
FABP2	3	NR1H4	13	ACE	2	FABP2	4	PRKCH	5	FFAR1	4
RBP4	2	FFAR1	12			HTR2C	4	FFAR1	5	PPARD	4
MAPK8	2	FABP4	12			PPARA	3	PPARD	5	NR1H3	4
ALOX5	2	ALOX5	12			HTR2A	3	TNF	3	FFAR4	1
		NR1H3	9			NR3C1	3	FFAR4	1		
		FFAR4	3			RBP4	2				
		TNF	3			MAPK8	2				
						DRD4	2				
						ACE	2				
						CCKBR	2				
						HDAC3	1				
						ADRA2B	1				

mushrooms prevented lipid droplets from forming, as demonstrated by microscopy (Fig. 4). At 490 nm, an ELISA reader was used to measure the amount of cellular lipids. The findings demonstrated that *L. edodes* extracts and metformin reduced lipid build-up compared to the control (untreated cells). It has been established that the aqueous and ethanolic extract of mushroom inhibited 3T3-L1 adipocytes' ability to differentiate (Fig. 5). The network pharmacology analysis showed that hexadecanoic acid methyl ester from the extracts interacts most with the overlapping genes. This was controversial to the study investigated by Lin et al., 2021 that revealed palmitic acid methyl ester enhances adipogenesis [30]. This seemingly paradoxical finding demands careful consideration and may open avenues for hypothesis regarding the complex interplay of compounds within the mushroom extract. It is essential to
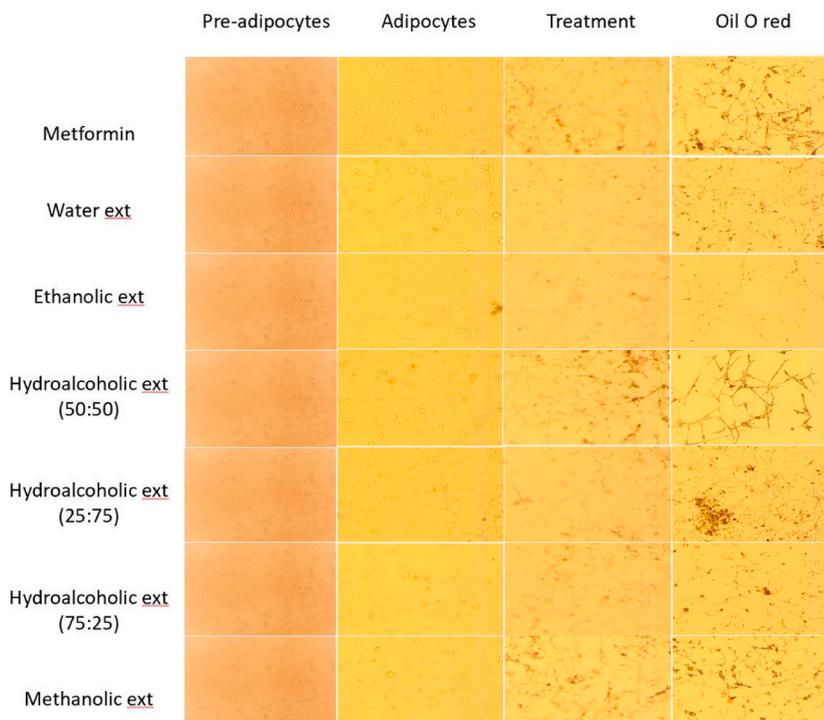
Fig. 4. Representative cell images demonstrating lipid accumulation in 3T3-L1 cells.

note that our study focused on the holistic network pharmacology perspective, and the observed anti-adipogenic effects may not be solely attributed to hexadecanoic acid methyl ester in isolation. One plausible hypothesis is that the observed anti-adipogenic effect of the mushroom extract could be the result of a synergistic interaction among various compounds present in the aqueous and ethanol extract. In a study by Drori et al., 2017, it was reported that vitamin-D enriched *L. edodes* were able to exhibit a synergistic anti-inflammatory effect in a diet-induced obesity murine model [31]. Similarly, in another study *Pleurotus eryngii* was reported to exhibit synergistic effects in suppressing ghrelin and improving postprandial glucose levels [32]. In addition, the regular consumption of the *L. edodes* as a dietary source is considered synergistic and has been reported to exhibit anti-cancer, anti-diabetic, anti-oxidant, anti-atherosclerotic, anti-microbial and hepatoprotective effects [33]. Hence, the overall anti-adipogenic effect may be due to the inhibition or interaction between obesity-related genes (FAAH, TNF, PPARG, PPARD, and PPARA, as stated in Supplementary Table S3) and chemical compounds. Despite its known pro-adipogenic effects, hexadecenoic acid methyl ester might be counteracted or modulated by other bioactive compounds, leading to an overall anti-adipogenic outcome.

Increased TNF-expression is correlated with adiposity and insulin resistance and is the first sign of obesity [34]. The PPARD plays a significant part in the emergence of abdominal obesity. Additionally, PPARG expression can be increased by the transmembrane protein 18 for the obesity-susceptibility gene to encourage adipogenesis [35]. N-acylethanolamines are mostly catabolized by FAAH, which activates G-protein-coupled receptors in the endocannabinoid system. When a person is obese, FAAH is linked to an increase in BMI, an increase in triglyceride levels, and a decrease in high-density lipoprotein cholesterol levels [36]. Therefore, the findings suggests that obesity increases the expression levels of TNF, PPARD, and PPARG while decreasing the expression levels of FAAH in mature adipocytes which may be significantly reversed by fruiting body of *L. edodes* extracts and metformin.

3.6. Effect of mushroom extracts on adiponectin levels

Adiponectin levels were tested to investigate mushrooms' impact on adipocytes in more detail. Because of its anti-inflammatory, anti-fibrotic, and antioxidant properties, the adipokine adiponectin, which is released by adipocytes, is a well-known homeostatic factor for controlling glucose levels, lipid metabolism, and insulin sensitivity. Since, a reduction in lipid build up in cells was seen in all the extracts, the adiponectin levels of these extracts were checked. The adiponectin levels were found to be significantly greater in aqueous, ethanolic and methanolic extract except for all the hydroalcoholic extracts of the mushroom, compared to the control cells (without treatment) (Fig. 6).

The endogenous cannabinoid (CB1) receptor ligand, anandamide, promotes increased food intake and energy storage. The hypothalamus and brain stem are two areas of the central nervous system where activation of the ECS affects the regulation of hunger and satiety [37]. Overactivation of the ECS is likely a factor in obesity because it is believed that the ECS's primary physiological purpose of the ECS is to change the energy balance in favor of energy storage [38]. The study results could be linked to the interaction between chemical compounds of mushroom extracts and FAAH gene in adipocytes. The primary FAAH enzyme when increased/elevated, helps in breakdown of anandamide, which in turn reduces calorie intake and suppresses the food intake. The aqueous, ethanolic and methanolic extract of *L. edodes* carries molecules such as hexadecanoic acid, linoleic acid, and methyl stearate, which may be responsible for decreasing anandamide signaling by boosting the activity of the FAAH enzyme and increasing adiponectin levels.

4. Conclusion

L. edodes is rich in valuable ingredients and is regarded as a potential source with miraculous biological properties. However, this study investigated the potential bioactive molecules of *L. edodes* and their interaction with obesity associated genes. The findings suggest that *L. edodes* might ameliorate the effects of obesity due to the presence of compounds responsible for breakdown of lipids. Moreover, network pharmacology can be widely used to predict bioactive compounds and their gene targets for alleviating the disease. Subsequently, an *in-vitro* cell-based experiment was carried out to verify the results of network pharmacology and it was discovered that the aqueous extract of *L. edodes* has been shown to exhibit lesser lipid accumulation and a greater adiponectin level, thereby indicating its potential application in regulating obesity. Future studies are geared towards isolating and identifying the myco-compounds responsible for the anti-obesity effects.

CRediT authorship contribution statement

Jasmeet Kaur: Writing – original draft, Methodology, Formal analysis, Data curation. **Humaira Farooqi:** Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Kailash Chandra:** Supervision, Resources, Project administration, Methodology. **Bibhu Prasad Panda:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
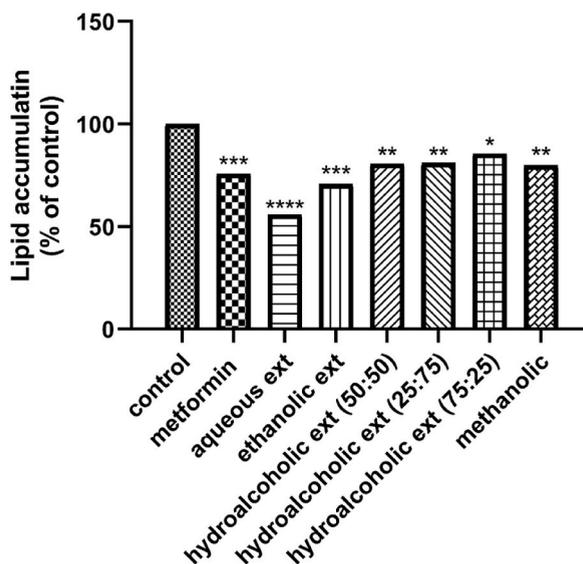
Fig. 5. Effect of *L. edodes* extracts on lipid accumulation. Data are presented as a percentage of control and metformin was used as a positive control for the experiment. Results are presented as ***p < 0.001, ****p < 0.0001, **p < 0.005, p < 0.05; * decreased in comparison to control.
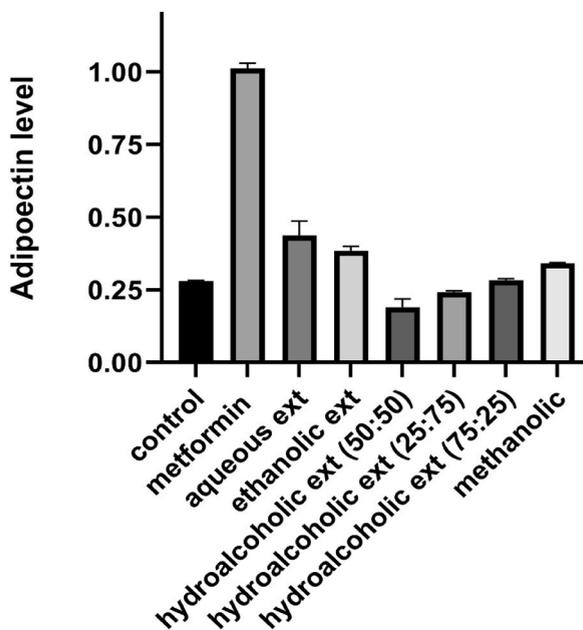
Fig. 6. Adiponectin level of the extracts in comparison to control. The metformin, aqueous and ethanolic extracts were found to be statistically significant while hydroalcoholic extracts and methanolic extract were found to be non-significant.

Acknowledgement

Abbreviations

GC–MS   Gas Chromatography–Mass Spectrometry
SEA   Similarity Ensemble Approach
STP   Swiss Target Prediction

AEA   Anandamide
FAAH   fatty acid amide hydrolase

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27363.

References

[1] M.U. Ukwuru, A. Muritala, L.U. Eze, Edible and non-edible wild mushrooms: nutrition, toxicity and strategies for recognition, J. Clin. Nutr. Metab. 2 (9) (2018) [Online]. Available: https://api.semanticscholar.org/CorpusID:76655494.

[2] Y. Ma, S.Q. Wang, W.R. Xu, R.L. Wang, K.C. Chou, Design novel dual agonists for treating type-2 diabetes by targeting peroxisome proliferator-activated receptors with core hopping approach, PLoS One 7 (6) (2012) e38546, https://doi.org/10.1371/journal.pone.0038546.

[3] Y.T. Tung, C.H. Pan, Y.W. Chien, H.Y. Huang, Edible mushrooms: novel medicinal agents to combat metabolic syndrome and associated diseases, Curr. Pharmaceut. Des. 26 (39) (2020) 4970–4981, https://doi.org/10.2174/1381612826666200831151316.

[4] S. Yang, J. Yan, L. Yang, Y. Meng, N. Wang, C. He, Y. Fan, Y. Zhou, Alkali-soluble polysaccharides from mushroom fruiting bodies improve insulin resistance, Int. J. Biol. Macromol. 126 (2019) 466–474, https://doi.org/10.1016/j.ijbiomac.2018.12.251.

[5] A. Dasgupta, K. Acharya, Mushrooms: an emerging resource for therapeutic terpenoids, 3 Biotech 9 (10) (2019) 369, https://doi.org/10.1007/s13205-019-1906-2.

[6] J. Wang, Z. Zhou, D. Dan, G. Hu, Physicochemical properties and bioactivities of Lentinula edodes polysaccharides at different development stages, Int. J. Biol. Macromol. 150 (2020) 573–577, https://doi.org/10.1016/j.ijbiomac.2020.02.099.

[7] H.J. Yang, M.J. Kim, D.Y. Kwon, D.S. Kim, T. Zhang, C. Ha, S. Park, Combination of aronia, red ginseng, shiitake mushroom and nattokinase potentiated insulin secretion and reduced insulin resistance with improving gut microbiome dysbiosis in insulin deficient type 2 diabetic rats, Nutrients 10 (7) (2018) 948, https://doi.org/10.3390/nu10070948.

[8] K.K. Oh, M. Adnan, D.H. Cho, Network pharmacology approach to bioactive chemical compounds identified from Lespedeza bicolor lignum methanol extract by GC–MS for amelioration of hepatitis, Gene Rep. 21 (2020) 100851, https://doi.org/10.1016/j.genrep.2020.100851.

[9] R. Li, Y. Li, X. Liang, L. Yang, M. Su, K.P. Lai, Network Pharmacology and bioinformatics analyses identify intersection genes of niacin and COVID-19 as potential therapeutic targets, Briefings Bioinf. 22 (2) (2021) 1279–1290, https://doi.org/10.1093/bib/bbaa300.

[10] A.L. Hopkins, Network pharmacology: the next paradigm in drug discovery, Nat. Chem. Biol. 4 (11) (2008) 682–690, https://doi.org/10.1038/nchembio.118.

[11] Y. Wang, B. Hu, S. Feng, J. Wang, F. Zhang, Target recognition and network pharmacology for revealing anti-diabetes mechanisms of natural product, J. Comput. Sci. 45 (2020) 101186, https://doi.org/10.1016/j.jocs.2020.101186.

[12] L.I. Shao, B. Zhang, Traditional Chinese medicine network pharmacology: theory, methodology and application, Chin. J. Nat. Med. 11 (2) (2013) 110–120, https://doi.org/10.1016/S1875-5364(13)60037-0.

[13] K.K. Oh, M. Adnan, D.H. Cho, Active ingredients and mechanisms of Phellinus linteus (grown on Rosa multiflora) for alleviation of Type 2 diabetes mellitus through network pharmacology, Gene 768 (2021) 145320, https://doi.org/10.1016/j.gene.2020.145320.

[14] J. Zhou, T. Feng, R. Ye, Differentiation of eight commercial mushrooms by electronic nose and gas chromatography-mass spectrometry, J. Sens. 2015 (2015), https://doi.org/10.1155/2015/374013.

[15] I. Marekov, S. Momchilova, B. Grung, B. Nikolova-Damyanova, Fatty acid composition of wild mushroom species of order Agaricales—examination by gas chromatography–mass spectrometry and chemometrics, J. Chromatogr. B 910 (2012) 54–60, https://doi.org/10.1016/j.jchromb.2012.10.025.

[16] A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (1) (2017) 42717, https://doi.org/10.1038/srep42717.

[17] M.J. Keiser, B.L. Roth, B.N. Armbruster, P. Ernsberger, J.J. Irwin, B.K. Shoichet, Relating protein pharmacology by ligand chemistry, Nat. Biotechnol. 25 (2) (2007) 197–206, https://doi.org/10.1038/nbt1284.

[18] A. Daina, O. Michielin, V. Zoete, SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules, Nucleic Acids Res. 47 (W1) (2019) W357–W364, https://doi.org/10.1093/nar/gkz382.

[19] Z. Xie, A. Bailey, M.V. Kuleshov, D.J. Clarke, J.E. Evangelista, S.L. Jenkins, A. Lachmann, M.L. Wojciechowicz, E. Kropiwnicki, K.M. Jagodnik, M. Jeon, Gene set knowledge discovery with Enrichr, Current protocols 1 (3) (2021) e90, https://doi.org/10.1002/cpz1.90.

[20] W. Xu, M. Tang, J. Wang, L. Wang, Identification of the active constituents and significant pathways of Cangfu Daotan decoction for the treatment of PCOS based on network pharmacology, Evid. base Compl. Alternative Med. 2020 (2020), https://doi.org/10.1155/2020/4086864.

[21] A.Y. Lee, W. Park, T.W. Kang, M.H. Cha, J.M. Chun, Network pharmacology-based prediction of active compounds and molecular targets in Yijin-Tang acting on hyperlipidaemia and atherosclerosis, J. Ethnopharmacol. 221 (2018) 151–159, https://doi.org/10.1016/j.jep.2018.04.027.

[22] Y.H. Park, M. An, J.K. Kim, Y.H. Lim, Antiobesity effect of ethanolic extract of Ramulus mori in differentiated 3T3-L1 adipocytes and high-fat diet-induced obese mice, J. Ethnopharmacol. 251 (2020) 112542, https://doi.org/10.1016/j.jep.2020.112542. Journal of ethnopharmacology. 2020 Apr 6;251:112542.

[23] S. Ilavenil, D.H. Kim, S. Srigopalram, M.V. Arasu, K.D. Lee, J.C. Lee, J.S. Lee, S. Renganathan, K.C. Choi, Potential application of p-coumaric acid on differentiation of C2C12 skeletal muscle and 3T3-L1 preadipocytes—an in vitro and in silico approach, Molecules 21 (8) (2016) 997, https://doi.org/10.3390/molecules21080997.

[24] O.M. Agunloye, G. Oboh, Blood glucose lowering and effect of oyster (Pleurotus ostreatus)-and shiitake (Lentinus subnudus)-supplemented diet on key enzymes linked diabetes and hypertension in streptozotocin-induced diabetic in rats, Food Frontiers 3 (1) (2022) 161–171, https://doi.org/10.1002/fft2.111.

[25] S. Yu, X. Wu, M. Ferguson, R.C. Simmen, M.A. Cleves, F.A. Simmen, N. Fang, Diets containing shiitake mushroom reduce serum lipids and serum lipophilic antioxidant capacity in rats, J. Nutr. 146 (12) (2016) 2491–2496, https://doi.org/10.3945/jn.116.239806, 2016.

[26] P.S. Dwivedi, V.P. Rasal, E. Kotharkar, S. Nare, P. Khanal, Gene set enrichment analysis of PPAR-γ regulators from Murraya odorata Blanco, J. Diabetes Metab. Disord. 20 (2021) 369–375, https://doi.org/10.1007/s40200-021-00754-x.

[27] S. Bátkai, P. Pacher, D. Osei-Hyiaman, S. Radaeva, J. Liu, J. Harvey-White, L. Offertáler, K. Mackie, M.A. Rudd, R.D. Bukoski, G. Kunos, Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension, Circulation 110 (14) (2004) 1996–2002, https://doi.org/10.1161/01.CIR.0000143230.23252.D2. Oct. 2004.

[28] E. Dainese, S. Oddi, M. Simonetti, A. Sabatucci, C.B. Angelucci, A. Ballone, B. Dufrusine, F. Fezza, G. De Fabritiis, M. Maccarrone, The endocannabinoid hydrolase FAAH is an allosteric enzyme, Sci. Rep. 10 (1) (2020) 2292, https://doi.org/10.1038/s41598-020-59120-1.

[29] M. Biernacki, W. Łuczaj, I. Jarocka-Karpowicz, E. Ambrożewicz, M. Toczek, E. Skrzydlewska, The effect of long-term administration of fatty acid amide hydrolase inhibitor URB597 on oxidative metabolism in the heart of rats with primary and secondary hypertension, Molecules 23 (9) (2018) 2350, https://doi.org/10.3390/molecules23092350.

[30] J.H. Lin, H.H. Chang, W.S. Lee, P.C. Ting, Y.P. Luo, K.T. Yang, Palmitic acid methyl ester enhances adipogenic differentiation in rat adipose tissue-derived mesenchymal stem cells through a G protein-coupled receptor-mediated pathway, Stem Cell. Int. 2021 (2021) 1–4.

[31] A. Drori, D. Rotnemer-Golinkin, S. Avni, A. Drori, O. Danay, D. Levanon, J. Tam, L. Zolotarev, Y. Ilan, Attenuating the rate of total body fat accumulation and alleviating liver damage by oral administration of vitamin D-enriched edible mushrooms in a diet-induced obesity murine model is mediated by an anti-inflammatory paradigm shift, BMC Gastroenterol. 17 (2017) 1.

[32] S.A. Kleftaki, S. Simati, C. Amerikanou, A. Gioxari, C. Tzavara, G.I. Zervakis, N. Kalogeropoulos, A. Kokkinos, A.C. Kaliora, Pleurotus eryngii improves postprandial glycaemia, hunger and fullness perception, and enhances ghrelin suppression in people with metabolically unhealthy obesity, Pharmacol. Res. 175 (2022) 105979.

[33] C. Ponnusamy, V.S. Uddandrao, S.P. Pudhupalayam, S. Singaravel, T. Periyasamy, P. Ponnusamy, P. Prabhu, V. Sasikumar, S. Ganapathy, Lentinula edodes (edible mushroom) as a nutraceutical: a review, Biosci. Biotechnol. Res. Asia 19 (1) (2022) 1.

[34] T. Tzanavari, P. Giannogonas, K.P. Karalis, TNF-α and obesity, TNF Pathophysiol. 11 (2010) 145–156, https://doi.org/10.1159/000289203.

[35] K. Landgraf, N. Klöting, M. Gericke, N. Maixner, E. Guiu-Jurado, M. Scholz, A.V. Witte, F. Beyer, J.T. Schwartze, M. Lacher, A. Villringer, The obesity-susceptibility gene TMEM18 promotes adipogenesis through activation of PPARG, Cell Rep. 33 (3) (2020), https://doi.org/10.1016/j.celrep.2020.108295.

[36] Y. Zhang, G.E. Sonnenberg, T.M. Baye, J. Littrell, J. Gunnell, A. DeLaForest, E. MacKinney, C.J. Hillard, A.H. Kissebah, M. Olivier, R.A. Wilke, Obesity-related dyslipidemia associated with FAAH, independent of insulin response, in multigenerational families of Northern European descent, Pharmacogenomics 10 (12) (2009) 1929–1939, https://doi.org/10.2217/pgs.09.122.

[37] P. Schulz, S. Hryhorowicz, A.M. Rychter, A. Zawada, R. Słomski, A. Dobrowolska, I. Krela-Kaźmierczak, What role does the endocannabinoid system play in the pathogenesis of obesity? Nutrients 13 (2) (2021) 373, https://doi.org/10.3390/nu13020373.

[38] J.M. Richey, O. Woolcott, Re-visiting the endocannabinoid system and its therapeutic potential in obesity and associated diseases, Curr. Diabetes Rep. 17 (2017) 1–7, https://doi.org/10.1007/s11892-017-0924-x.