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In-vitro activity of ethanolic extract of *Lentinus strigosus* mycelia in N2 wild strain *Caenorhabditis elegans* – An animal model for obesity and its chemical composition

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ABSTRACT

Lentinus strigosus is an edible and medicinal species of mushroom found in Central Luzon, Philippines. In the current study, the effects of ethanolic extract of *L. strigosus* mycelia (grown on coconut water in submerged culture) on the food intake and locomotion of *Caenorhabditis elegans* were investigated. Chemical compositions of the mushroom mycelia were likewise analyzed. Results showed that mycelial extract significantly reduced the food intake and increased the roaming and dwelling activities of extract-treated nematodes. The presently observed positive effects could not be accounted to the toxicity of the mycelial extract since the extract was confirmed safe in the acute lethality assay. In addition, mycelia of *L. strigosus* contained valuable mycochemicals such as flavonoids, alkaloids, terpenoids, steroids, phenols, saponins, athrones, anthraquinones, and coumarins, which have been reported to play important roles in the different mechanisms of anti-obesity activity. Accordingly, *L. strigosus* mycelium is a new addition of functional food resources, which has promising potential for the prevention of obesity and associated diseases.

1. INTRODUCTION

Mushrooms have been widely utilized as functional foods. They are an excellent source of physiologically beneficial bioactive compounds, including phenols, tocopherols, ascorbic acids, carotenoids, sugars, fatty acids, acylglycerols, triterpenes, steroids, anthraquinones, coumarins, anthrones, tannins, flavonoids, and alkaloids [1-6]. These compounds have been linked to the hypoglycemic, antibacterial, antifungal, antioxidant, antiviral, antitumor, and immunosuppressive properties of several mushrooms [7-10]. As nutritious foods, they contain valuable proteins, carbohydrates, crude fiber, vitamins, minerals, and crude fat [7,11]. Indeed, mushrooms could be considered as a high-value crop for nutritional and pharmacological purposes.

Lentinus strigosus, commonly known as *kabuteng balbon* among Filipinos, is a wood-rotting edible and medicinal mushroom that usually found growing on fallen logs, stumps, wood fence, and trunks of trees. The mycelial growth, fruiting body production, nutritional and mycochemical compositions, and some biological activities were studied and reported in our previous works [11-14]. With the significant chemical compositions elucidated [11], it is hypothesized that this mushroom could exhibit various biological activities such as antioxidant, antibacterial, anticancer, anti-diabetic, anti-cholesterolemic, anti-inflammatory, anti-obesity, and among others, which are of our interest, to establish its role in the nutraceutical and pharmaceutical industries. In this study, the effects of *L. strigosus* extract on the survival and behavior of a nematode *Caenorhabditis elegans*, an animal model for obesity, were investigated.

C. elegans, a free-living nematode, is an advantageous animal model because of its extensively studied genome, ease of handling and management, rapid life cycle, and a 3-week long lifespan [15]. Notably, *C. elegans* has a high degree of similarity with human in terms cellular processes such as cell signaling, metabolism, cell cycle, cell aging, and cell death [16-18]. Although an animal, *C. elegans* is not legally considered as an animal, which excludes it from other laboratory animals that are strongly protected by animal right laws [19]. This worm has been widely used in the evaluation of different parameters such as development, immune response, aging, reproduction, stress, and even neurological disorders such as dementia, Pankinson's, and Alzheimer's diseases [20-26].

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This current study aimed to evaluate the effects of ethanolic extract of *L. strigosus* mycelia on the survival, food intake, and locomotion of *C. elegans* with an intention to establish the potential of the extract in obesity management. The chemical compositions of the mycelial extract were also analyzed.

2. MATERIALS AND METHODS

2.1. Source and Mycelial Production

The culture of *L. strigosus* was obtained from the culture collections of the Bioassay Laboratory, Department of Biological Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Coconut water was used as a medium for mycelial biomass production under submerged culture conditions following the process described by Dulay *et al*. [27]. Mycelia were air-dried and pulverized before extraction.

2.2. Extraction

Powdered mycelia (10 g) were soaked in 1 L of 95% ethanol for 48 h. This was filtered using a Whatman filter paper No. 2 and the filtrate was subsequently concentrated to dryness in a rotary evaporator and yield of extract was recorded.

2.3. Source of Nematode and the Culture Medium

Culture of N2 wild strain of *C. elegans* was acquired from the College of Medicine, University of the Philippines, Ermita, Manila, Philippines. The ingredients of nematode growth medium (NGM) and its preparation were followed after Stiernagle [28]. Medium was pour-plated, solidified, and inoculated with *Escherichia coli* OP50 strain. The bacteria served as food for *C. elegans*. Plates were incubated at 28°C for 18 h.

2.4. Nematode Lethality Assay

The nematode lethality assay protocol of Quiao *et al*. [29] was followed with minor modifications. Fifteen L4-stage *C. elegans* were individually picked using a worm picker and transferred into each OP50-seeded NGM plate. A total of 18 plates were inoculated and each plate was treated with 300 µL of each treatment (different extract concentrations, 10 µg/mL L-carnitine tartrate, and 1% dimethyl sulfoxide [DMSO]). Each treatment was replicated 3 times. Assay plates were incubated at 20°C. The lethal effects of the different treatments were observed after 48 h under ×40 magnification using a stereomicroscope. Being unresponsive to external stimuli, lack of muscle activity, and appearing as a straight, rigid rod were observed as lethal effects [30]. The survival rate of *C. elegans* was determined.

2.5. Pharyngeal Behavior and Locomotion Assays

Pharyngeal pumping rate was measured by counting the number of contractions of the pharyngeal bulb within 1 min [31] under ×40 magnification using a stereomicroscope after 24 and 120 h post-treatment application. The nematode exhibits two modes of locomotion, the dwelling and roaming. The forward and backward movements (for dwelling) and body bends (for roaming) of the treated nematodes were counted per minute under ×40 magnification using a stereomicroscope after 24 and 120 h post-treatment application. All nematodes used in the assay were submerged in sodium hydroxide before disposal.

2.6. Chemical Composition Analysis

The screening of the different groups of compounds present in the mushroom extracts was employed following the methods of Guevara [32]. A thin-layer chromatography (TLC) was used to detect the presence of the different secondary metabolites of the mushrooms. TLC was performed in a vertical glass chamber with ethyl acetate. The different chemicals were detected as spots in TLC through the use of ultraviolet light, hot plate, and several reagents used for a typical visualization of the secondary metabolites. Vanillin-sulfuric acid was used to determine the presence of phenols, sterols, fatty acids, triterpenes, and essential oil. Methanolic potassium hydroxide was used to visualize anthraquinones, coumarins, and anthrones, while potassium ferricyanide-ferric chloride was used to test phenolic compounds and tannins. Alkaloids and flavonoids were detected using Dragendorff's reagent and antimony (III) chloride, respectively.

2.7. Statistical Analysis

Data were analyzed using analysis of variance and compared using Tukey's honestly significant difference at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1. *In-vitro* Activity of *L. strigosus* Mycelial Extract in *C. elegans*

Obesity is one of the major health problems worldwide, which is caused by multiple factors such as heredity, environment, diet, lifestyle, infectious agents, and societal determinants [33]. However, many researchers assumed that this metabolic disorder is a result of excessive caloric and fat intake and steady decline physical activity [34]. *C. elegans* is the commonly used relevant animal model because it is comparable to a mammalian model that elucidates the mechanism of obesity [35]. In this study, we used *C. elegans* to determine the effects of *L. strigosus* mycelial extract on the survival, food intake, and locomotion of this free-living nematode in our intention to establish the potential of the extract for obesity prevention.

Nematode acute lethality assay was performed first to confirm the safety of the extract. The nematodes treated with 1000 µg/mL recorded the lowest percentage survival [Figure 1]. This effect was found statistically comparable to the effect of the control L-carnitine. However, those exposed at 300 µg/mL and lower concentrations showed higher percentage survival and were found comparable to the DMSO-treated nematodes. Furthermore, none of the extract concentrations used showed a below 50% survival rate. Hence, the mycelial extract of *L. strigosus* would be considered safe for further assays.

Food intake in *C. elegans* is accomplished by pharyngeal movements through pumping. Pharyngeal pumping is a cycle of contractions and relaxations of the pharynx that joins the mouth to the intestines of *C. elegans*, which corresponds to food intake [36]. The food intake activity through the pharyngeal pumping rate of nematodes treated with varying concentrations of ethanol extract of mycelia is shown in Figure 2. The increase in the concentration of mycelial extract and the prolong treatment exposure significantly reduced the food intake of the nematodes, which suggest a loss of appetite and/or suppression of overeating. This observation is consistent with the recent study of Sheng *et al*. [37], who reported that water extract from mushroom *Pleurotus citrinopileatus* significantly decreased the food intake resulting to the reduction of weight gain and fat accumulation in high-fat diet-fed C57BL/6J mice. In addition, Khatun *et al*. [38] reported that feeding of edible mushroom Echigoshirayukidake supplemented chow (5% w/w) significantly reduced the food intake and prevented body weight gain, fat deposition in the viscera, and hyperlipidemia in genetically defined obese model rat.

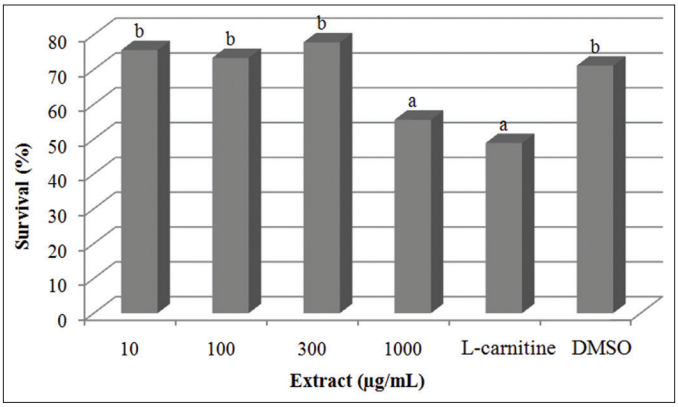

Figure 1: Effect of varying concentrations of *Lentinus strigosus* mycelial extract on the survival of *Caenorhabditis elegans* after 48 h of exposure. Means having the same letter of superscript in each time of exposure are not significantly different from each other at 5% level of significance.

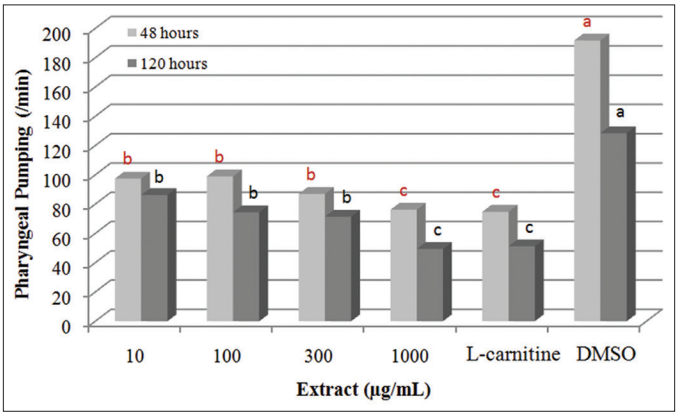

Figure 2: Effect of varying concentrations of *Lentinus strigosus* mycelial extract on the pharyngeal pumping behavior of *Caenorhabditis elegans* after 48 and 120 h of exposure. Means having the same letter of superscript are not significantly different from each other at 5% level of significance.

Several peptide hormones excreted from the gastrointestinal tract play a crucial role in controlling and stimulating appetite, which causes the feeling of satiety and hunger. Some of these include cholecystokinin, glucagon-like peptide-1 (GLP-1), peptide YY3-36, and ghrelin [39]. High level of ghrelin induces appetite, which stimulates feeding and increases weight gain and fat deposition [40]. On the other hand, elevated GLP-1 induces satiety, reduces food intake, and decreases weight gain and adiposity [41]. Wang *et al*. [42] reported that high-fat diets supplemented with 10% of either sugarcane fiber or psyllium significantly reduced stomach ghrelin mRNA levels in male mice (C57BL/6). Aside from released stomach hormones, alteration of neural signals in the brain may also cause loss of appetite [43]. In contrast, Mazidi *et al*. [44] disclosed the appetite-stimulating effect of hydroalcoholic extract of *Cannabis sativa* by increasing the total ghrelin level in male Wistar rats. In addition, *Zingiber officinale* improved food intake due to GLP-1 suppression [45,46]. Karri *et al*. [39] enumerated in their review paper the most commonly studied mechanisms of anti-obesity activity of natural agents and these include regulation of plasma lipid, gene expression, mRNA mediated lipid metabolism, and leptin levels, pancreatic lipase inhibition, adipose accumulation reduction, balancing energy intake and expenditure, and adipocyte differentiation suppression. The food intake reduction in nematodes observed in the present study could probably be attributed to any of the above-mentioned mechanisms, which warrants further investigations. In addition, presently observed suppressive effect of mycelial extract on the food intake of nematodes could not be accounted to the possibility of having a toxic effect because the extract was confirmed safe in nematode acute lethality assay.

Aside from suppressing appetite and reducing food intake, increasing calorie expenditure through physical activity is also one of the potential strategies in obesity management [47]. Physical activity helps to combat the occurrence of obesity by balancing energy intake and expenditure and by preventing adipocyte accumulation. In the present study, we found that both reversal and bending locomotions were significantly increased as the concentration of the mycelial extract increases [Figures 3 and 4]. Apparently, prolonged exposure of nematodes in the extract increased physical activity. Moreover, the effect of 1000 µg/mL extract was insignificantly different to L-carnitine in both locomotion assays. Our results suggest the positive effect of the mycelial extract on the roaming and dwelling activities of nematodes. This is in agreement with the observation of Kim *et al*. [48], who reported that increasing amount of taurine for the high-fat-diet N2 strain *C. elegans* significantly increased mobility and decreased deposition of fat.

3.2. Chemical Compositions of Mycelia

L. strigosus is a highly nutritive species of edible and medicinal mushrooms. Our results in chemical analysis showed that *L. strigosus* mycelia contain valuable mycochemicals, including flavonoids, alkaloids, terpenoids, steroids, phenols, saponins, athrones, anthraquinones, and coumarins, which have been reported to exhibit numerous biological properties. In the recent review of Karri *et al*. [39], the different natural compounds from various plants that have been linked to anti-obesity were listed and the most common phytochemical constituents responsible for the said activity were identified. These include flavonoids, phenols, terpenoids, alkaloids, saponins, carboxylic acids, other acids, glycosides, and tannins. However, in edible and medicinal mushrooms, polysaccharides, polyphenols, alkaloids, flavonoids, terpenes, sterols, and fibers were the identified bioactive compounds with anti-obesity potentials [33]. Interestingly, some of the above-mentioned chemicals were also detected in this study.

Previous works reported the significant physiological roles of these natural anti-obesity compounds. Phenolic compounds induced lipid metabolism by mRNA expression of mitochondrial uncoupling proteins 3 and inhibited alpha-amylase and pancreatic lipase [49,50]. Flavonoids decreased the mRNA levels of lipogenesis-related genes and exhibited pancreatic lipase inhibition activity and anti-adipogenic effect [51,52]. Saponins and terpenes prevented obesity by inhibiting pancreatic lipase activity [53,54]. Alkaloids suppressed the mRNA expression of neuropeptide Y and agouti-related peptide in the hypothalamus and decreased the food intake in rats [55].

According to literature, polysaccharides are the most common chemical constituents of mushroom with anti-obesity properties. For instance, polysaccharides extracted from edible mushroom *Tremella fuciformis* inhibited the differentiation of 3T3-L1 adipocytes by reducing mRNA expression of peroxisome proliferator-activated receptor-alpha, C/EBPα, and leptin, which suggests anti-obesity property [56]. Lentinan from *Lentinus edodes* reduced lipid accumulation and suppressed the formation of atherosclerotic plaques in mice, indicating that this polysaccharide helps in

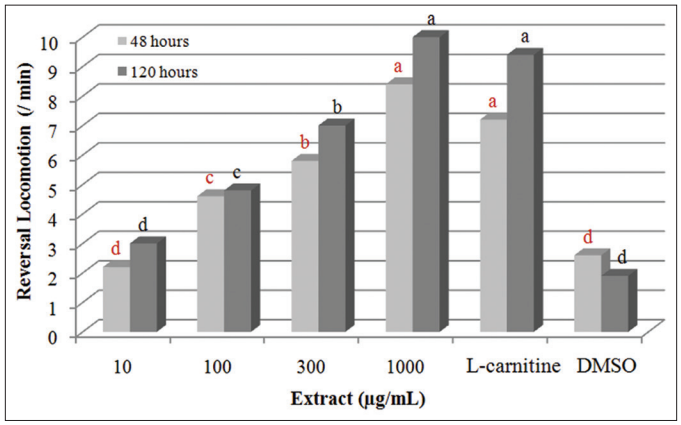

Figure 3: Effect of varying concentrations of *Lentinus strigosus* mycelial extract on the dwelling behavior of *Caenorhabditis elegans* after 48 and 120 h of exposure. Means having the same letter of superscript in each time of exposure are not significantly different from each other at 5% level of significance.

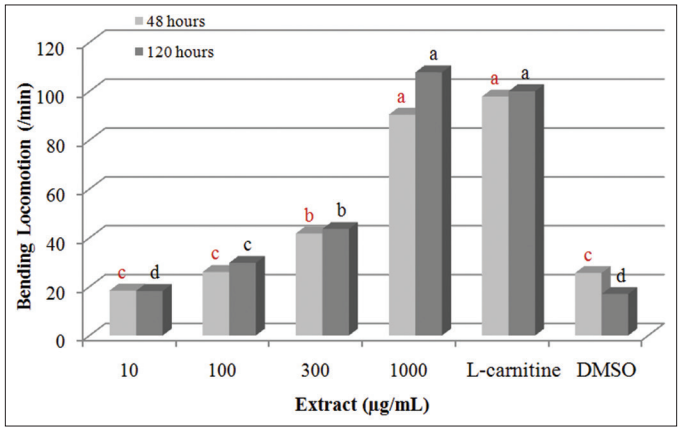

Figure 4: Effect of varying concentrations of *Lentinus strigosus* mycelial extract on the roaming behavior of *Caenorhabditis elegans* after 48 and 120 h of exposure. Means having the same letter of superscript in each time of exposure are not significantly different from each other at 5% level of significance.

preventing hypercholesterolemia [57]. Extract rich in β-glucan from *Pleurotus sajor-caju* prevented obesity by inducing lipolysis in high-fat diet-fed mice [58]. Moreover, residue polysaccharide of *Cordyceps militaris* SU-12 demonstrated the reduction of blood and liver lipid levels and the improvement of glutamate pyruvate transaminase and antioxidant activity in mice [59]. In addition, Huang *et al*. [60] reported the antihyperlipidemic and anti-hyperglycemic potential of *Pleurotus tuber-regium* polysaccharides in obese-diabetic rats. Aside from polysaccharides, fibers obtained from *Grifola frondosa*, *L. edodes,* and *Flammulina velutipes* showed cholesterol-lowering effects in rats [61]. Peptides derived from edible mushrooms, *Hypsizygus marmoreus*, *Pleurotus cystidiosus*, and *Tricholoma matsutake* exhibited inhibitory activities against angiotensin I-converting enzyme [62-64]. Our results and the results of earlier studies suggest that mushrooms are rich in nutritional value with numerous biologically active chemicals, which have significant impacts in controlling obesity and associated diseases such as diabetes, hypercholesterolemia, hypertension, and other cardiovascular diseases.

4. CONCLUSION

Overall, *L. strigosus* mycelial extract effectively reduced the food intake and significantly increased the dwelling and roaming activities of *C. elegans in vitro*. Chemical analysis revealed valuable bioactive compounds which have been reported to show significant impact in various mechanisms of anti-obesity activity. It is therefore concluded that mycelium of *L. strigosus* is a new resource of natural agents that could be used as a potential strategy for obesity management. However, comprehensive investigations are obviously required to understand fully the underlying mechanisms of anti-obesity activity of this medicinal mushroom.

5. CONFLICTS OF INTEREST

No conflicts of interest among authors.

6. FINANCIAL SUPPORT AND SPONSORSHIP

None.

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