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Proximate Analysis and Antimicrobial Activity of Cultivated Macrolepiota dolichaula Strain MFLUCC-13-0579

*Leela Maya Rizal[1,2], Kevin D. Hyde[1,2], Ekachai Chukeatirote[1,2], Pattana Kakumyan [1,2], Sunita Chamyuang[1,2]**

[1] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
[2] Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand
** Corresponding author: Sunita@mfu.ac.th*

Abstract

Macrolepiota dolichaula, an edible white spored gilled mushroom, is widely distributed in tropical and temperate countries. In this study, nutritional quality of *M. dolichaula* strain MFLUCC-13-0579 cultivated in compost was investigated. Proximate analysis of dry weight of *M. dolichaula* showed a composition of 25.53% protein, 44.37% carbohydrate, 14.17% fiber, 1.17 % lipid and 5.19% ash with energy of 299.06 kJ/g mushroom. In addition, the antimicrobial activities from fresh mushroom, dry mushroom and mycelial extracts showed significant activities against *Micrococcus luteus* and *Staphylococcus aureus*. Based on this study, it can be concluded that *M. dolichaula* is a good source of nutrients and posses some antimicrobial activities.

Keywords : *antimicrobial activity, edible mushroom, Macrolepiota, proximate nutritional data*

1. Introduction

Macrolepiota dolichaula (Berk. & Broome) Pegler & R.W. Rayner belongs to the family Agaricaceae (1). This mushroom is consumed as wild delicacy in China, north west India and northern Thailand (1, 2, 3). *M. dolichaula* was reported to contain significant amount of the vitamins A, B_1, B_2, B_3, C and D, including thiamine, riboflavin and has shown laccase activity (4, 5). Water soluble polysaccharides (PS-I & PS-II) have also been isolated from the hot aqueous extracts of this mushroom. The branched glucan obtained from PS-1, revealed splenocyte, thymocyte and macrophage activation (6). Apart from this, Kumari and Atri (2) reported its nutritional quality. It should be noted, however that the data were derived from the wild specimens. Our previous study showed that *M. dolichaula* could be cultivated using compost under controlled conditions (3). Based on this successful domestication, this present study was therefore carried out to determine its proximate analysis for comparative study. Besides, its antimicrobial activity was also determined.

2. Materials and Methods

2.1 Sample collection and preparation

100 g of spawn was manually inoculated in five kg of pasteurized compost at pH 7.5 and 60% moisture content in ten baskets. After 21 days following inoculation mycelia colonized 90% of compost. After application of casing soils over the mycelia, the fructification occurred at 25 °C at 92% relative humidity within 41 days after spawn inoculation. A total of 959g mushrooms from four baskets were harvested. Whole sporocarps (pileus and stipe) were cleaned to remove residual compost. Forty grams of sample was dried at 45 ºC for 24 hrs. The dried mushroom was ground in a mechanical grinder and powder was stored in a air tight plastic bag in a desiccator at room temperature.

2.2 Proximate nutritional analysis

The proximate nutritional analysis of mushroom powder was determined by using the method from association of Official Analytical Chemist (AOAC) (7). Moisture content was measured by drying 2 g of test sample at 105 ºC for 16 hrs in a hot air oven (UM500, Memmret). The ash content was determined by combusting 2 g sample in an electric furnace (Eurotherm 2416CG, Lento) at 505 ºC for 2 hrs. Fiber was evaluated by acid treatment and subsequently heating 1 g at 500 ºC, in the Fibertec system M10020 Extractor (Foss Tecator). The protein content was evaluated by using the macro-Kjeldahl method in which the protein content was estimated by multiplying with a conversion factor of (N × 4.38) (2, 8). Lipid content was analysed by the Soxtec 20055 Extraction unit (Foss Tecator) with petroleum ether. Total carbohydrate was evaluated by the difference method according to the equation 1 (9). Energy value was evaluated according to the equation 2, described by Kumari and Atri (2). All experiments were carried out in triplicates.

Carbohydrate (%) = 100 - (g moisture + g ash + g lipid + g protein + g fiber) (1)

Energy (Kcal) = 4 × (g protein) + 4.2 × (g carbohydrate) + 9.10 × (g fat) (2)

2.3 Preparation of crude extracts

For the preparation of crude extracts, 20 g of fresh mushrooms sample, 10 g of dried mushrooms sample and 14 days old mycelia from ten Petri dishes were used. Extracts were prepared using a modified solid phase extraction method (10). For this process, mushrooms sampels (fresh and dry) and the mycelia were macerated with 80 mL ethyl acetate (EtOAc) using a sterile stainless mechanical blender (Waring 800BU) separately. The mushrooms and agar slurry were soaked overnight, for the first EtOAc extraction. The solvent phase was then extracted and subsequently re-extracted twice. All the extracts were combined and filtered using Whatman no. 4 filter paper. Clear extracts were poured into a pre-weighed vials, then air dried for 24 hrs to yield a total amount of each crude extracts. Crude extracts were then kept at 4 °C in airtight vials for further use.

2.4 Antimicrobial activity assay

A 96-well microtire plate (MTT) bioassay was performed for the detection of antimicrobial activity (11). Crude extracts were diluted using HPLC grade absolute methanol (Sigma-Aldrich) to a concentration ranging from 0.5 – 10 mg/mL for bioassays. 10 µL aliquot of crude extract

was pipetted into individual well of a 96-well MTT plate. Subsequently positive controls; tetracycline (10 µL), chloramphenicol (10 µL) and negative controls; media (200 µL) and methanol (10 µL) were transferred prior to a preliminary quick assay. Crude extracts and controls on the MTT plates were then air dried in laminar flow and kept at 4 °C until required. For antimicrobial test, the testing microbes used were three Gram-positive bacteria (*Bacillus subtilis* TISTR 008, *Micrococcus luteus* TISTR 884 and *Staphylococcus aureus* TISTR 1466), two Gram-negative bacteria (*Escherichia coli* TISTR 780 and *Pseudomonas aeruginosa* TISTR 781) and a yeast *Candida albicans* TISTR 5779. 200 µL of the microbial suspension were prepared with an optical density (OD) 610 of 0.05 for Gram positive bacteria, and 0.04 for Gram negative bacteria and yeast and transferred to the prepared MTT plates. The solution in MTT plates were mixed using pipette and incubated at 37 °C for bacteria and at 30 °C for *C. albicans* for 24 hrs. The cell viability was determined calorimetrically by the addition of 30 µL of 0.02% resazurin solution into each well and the plates were further incubated for 15 mins (12). The antimicrobial activity of the extracts were analysed through the determination of the minimum inhibition concentration (MIC) in mg/mL. The MIC is defined as the lowest concentration of the tested sample to prevent the growth of microorganism from the calculated data obtained from MTT reader after 24 hrs.

3. Results and Discussion

3.1 Proximate nutritional analysis

The data of nutritional contents of *M. dolichaula* strain MFLUCC-13-0579 cultivated on compost are shown in Table 1.

Table 1. Nutritional composition (% dw), energetic value (kcal/100 g dw) of M. dolichaula strain MFLUCC-13-0579. The results are presented as mean ± SD (dw: dry weight)

Nutritional compositions	Amount
Ash	5.19 ± 0.00
Fiber	14.17 ± 0.25
Protein	25.53 ± 0.17
Lipid	1.17 ± 0.00
Moisture	9.57 ± 0.11
Carbohydrate	44.37± 0.07
Energy	299.06± 0.08

M. dolichaula is a good source of carbohydrate (44.37%), protein (25.53%), fiber (14.17%) and ash (5.19%). This mushroom has low lipid content (1.17%). Relatively low lipid content and high contents of fiber (14.17%) in this mushroom, make it to recommend for the people with cholesterol related ailments (13). Therefore, this mushroom can be categorized as a good edible mushroom because of its nutritional and culinary options.

3.2 Antimicrobial assay

The results of microorganism inhibitory were expressed as the percentage of the cell growth compared to normal cell growth at different concentration which showed significant activity in the antimicrobial quick screen (50% cell viability) are shown in Table 2.

Table 2. The minimal concentration of crude extracts of M. dolichaula strain MFLUCC-13-0579, which inhibited the cell viability by 50 % (NA: no activities observed)

Microorganisms	Crude extracts concentration (mg/mL)		
	Fresh	**Dried**	**Mycelium**
B. subtilis	2	6	NA
M. luteus	1	0.5	0.5
S. aureus	9	9	9
E. coli	NA	1.0	NA
P. aeruginosa	NA	1.0	NA
C. albicans	NA	NA	NA

In general, all the crude extracts (fresh, dry, mycelia) were able to inhibit the growth of *M. luteus* and *S. aureus* by 50% with the concentration of 0.5 – 1.0 mg/mL for *M. luteus* and 9.0 mg/mL for *S. aureus*. Furthermore, crude extracts from fresh and dry mushroom were able to inhibit the growth of *B. subtilis* by 50% at the concentration of 2.0 and 6.0 mg/mL respectively. None of the mushroom extracts were active against *C. albicans*. These results suggest that mushroom crude extracts (fresh and dry) are better in inhibiting bacterial growth than mycelia with the tested crude extracts concentrations. This may be because during the fruiting process of mushrooms, many enzymes are produced and for the resistance in the substrates, fungi secrete secondary metabolites such as antimicrobial compounds to compete other organisms (14). *M. dolichaula* being a saprobe has the potential to produce bioactive compounds having clinical significance.

4. Conclusion

Our study revealed the proximate nutritional contents and antimicrobial activities of

M. dolichaula cultivated in compost, for which there has been no report to date. This mushroom is edible, cultivable and thus nutritional data are important to introduce it as an additional mushroom for commercial production. *M. luteus* and *S. aureus* were the most susceptible bacteria Therefore, this mushroom should be further explored for other bioactivities such as antioxidant and characterization of chemical compounds.

5. Acknowledgements

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