Macrofungi diversity in Lawu Mountain Forests and their potential uses as medicinal mushroom for inducing immunity against Covid-19

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Abstract. Macrofungi is one of bio-medicinal sources containing various bioactive compounds, such as β-glucans, which are scientifically proven as immunity booster against coronavirus, including Covid-19. Lawu Mountain Forest has been reported as one of the macro fungi-rich ecosystems in Java. Due to its unique geography, each side of the mountain has a different climate with the southern slope is typically more suitable for various species of mushroom to grow. The aims of this study were to assess fungal diversity in the southern slope of Lawu Mountain Forest, and to ascertain their potential uses for medicinal purpose, particularly for boosting immunity against Covid-19. Cruise method was used to identify macroscopic fungi collected along the hiking trail of Lawu Mountain Forest at the elevation ranges of 1800-3100 m asl. Their morphological characteristics, including color, diameter, veil surface, lamella (ring and pore, type of lamella, and volva), stem shape, length and diameter, were observed. The study found 46 species from 15 families of macrofungi. Seven species potentially containing bioactive compounds as immunomodulator for boosting immunity were Auricularia auricula, Cerrena unicolor, Lentinus edodes, Pleurotus ostreatus, Stereum hirsutum, Schizophyllum commune, Trametes versicolor.

1. Introduction

Macrofungi play a significant role in the most mountain ecosystems due to their functions in supporting the ecological, economic, and cultural conservation [1]. The ecosystem of Lawu Mountain Forest, a dormant volcano and the third highest mountain in Java Island, is rich in biodiversity, particularly on the southern slopes of the mountain. According to [2], such condition occurs due to a higher rainfall (>2000 mm/year) specifically induced by heavy condensation process in the southern slopes. Temperature and rainfall are regarded as two main factors for macro fungal production [3]. Hence, southern slope of Lawu Mountain Forest hosts a diverse macro fungi which also provide a source of livelihood for upland community either used as medicinal mushroom or as an alternative source of food.

Mushrooms are packed with nutrients and bioactive compounds such as carbohydrates, fibers, proteins, vitamins, minerals and have enormous medicinal attributes such as antibacterial, antiviral, antioxidant, anti-cancerous, and hypcholesterolemia which are valuable for human health [4]. In recent years, the medicinal properties of mushrooms as well as their nutritional properties have been reported by many researchers. The interest in the research of the pharmaceutical potential of mushrooms is increasing [5, 6], even more at the moment of the Covid-19 pandemic [7].
During the Covid-19 pandemic, vaccination and medical treatment during the later stages of the disease are two steps of action which have been conducted up to now. However, due to the unavailability of effective drugs for killing the virus, except by increasing antibodies and immune, a healthy immune system becomes one of the most important weapons [8]. Antiviral effects of mushroom could be directly caused by viral enzymes inhibition, viral nucleic acids synthesis or viruses adsorption and uptake into mammalian cells [3]. While indirect antiviral effects are resulted from the immune stimulating activity of polysaccharides or other complex molecules, these direct antiviral effects are exhibited especially by smaller molecules. Therefore, mushrooms have a great potential as a successful antiviral treatment with a reduced chance of adverse side effects [9].

The aims of this study were: (1) to assess macrofungi diversity in the southern slope of Lawu Mountain Forest, (2) to ascertain the potential use of the macrofungi as medicinal purpose, particularly for boosting immunity against Covid-19.

2. Materials and Methods

2.1. Description of research site

The study was carried out on the Lawu Mountain Forest, a dormant 3265 m above sea level (asl) volcano located in the border area between the province of Central Java and the province of East Java, Indonesia in February 2020. The climate of the study site is humid tropical, with high rainfall and high humidity (up to 92%). The maximum and minimum of temperature during rainy season in February 2020, were ranging from 6°C to 10°C at night and 15°C to 20°C at the day, respectively. The macrofungi were collected in area of one meter right and left along the track, namely Cemoro sewu track (Figure 1), started from 1800 m asl until 3100 m asl.

2.2. Sample collection

All the fruiting bodies found in one meter left and right along the track were photographed in their natural habitat. As the morphological features might change with drying, these features such as size, colour, shape and texture of the sporocarp were recorded during field observation. Macrofungi was identified based on macroscopic and microscopic features. The observed macroscopic features were the cap size, shape, colour, surface texture and moisture, gill colour, attachment, spacing, lamellules, the stem size, and shape. In order to keep the fruiting body from damage, excess soil stuck have to be removed carefully using a tissue, and then kept into a labelled envelope. Each different fruiting body have to keep in different envelope in order to prevent spore contamination from different fungal species.

2.3. Identification and utilization as a medicinal mushroom

Identification of macrofungi were worked on the basis of macro and microscopic characteristic following available literatures. The three major literature that were used in the identification process were A Field Guide to the Larger Fungi of FRIM [10], a guidebook to the macrofungi of Tasik Bera [11], and a guide book to the macrofungi of Fraser’s Hill [12], and other related literature from google. The most literature used were from Malaysia, with consideration that Indonesia and Malaysia are tropical countries, which have high ecological similarities including the species of macrofungi. The utilization as medicinal mushroom, particularly for boosting immunity against corona virus has been documented from the available literature.
3. Results and Discussion

3.1. Macrofungi diversity

A total of 4758 specimens of macrofungi were collected along the track of Cemoro sewu, from 1800 to 3100 meters asl. These specimens represented 45 species, 26 genera, 15 families and 8 orders i.e., Agaricales, Auriculariales, Polyporales, Xylariales, Boletales, Pezizales, Dacrymycetales, and Sordariomycetales. The numbers of species in each family were presented in Figure 2. Based on the literatures, there were at least seven fungal species with immunomodulatory potential bioactive compounds (Table 1).

![Figure 1. Map of Cemoro sewu Track (indicated with blue line) at Lawu Mountain Forest, Java, Indonesia, altitude 1800 m asl to 3100 m asl.](image)

![Figure 2. Number of species in each family of macrofungi collected at the Cemoro sewu track of Lawu Mountain Forest, altitude 1800 to 3100 m asl.](image)
Table 1. Taxa of macrofungi collected at the Cemoro sewu track, Lawu Mountain Forest.

| No | Species               | Family               | Substrate                     | Potential Uses | Immuno-modulatory | Selected References |
|----|-----------------------|----------------------|-------------------------------|----------------|-------------------|---------------------|
| 1  | *Auricularia auricular* | Auriculariae         | Decaying wood, litter fall    | medicine       | (1→4)-α-D-glucan  | [13]                |
| 2  | *Cerrena unicolor*     | Polyporaceae         | Decaying wood, living trees   | medicine       | laccase (LAC), endopolysaccharides (c-EPL) | [14]                |
| 3  | *Clitocybe sp.*        | Tricholomataceae     | Living trees                  |                |                   |                     |
| 4  | *Coprinopsis sp.*      | Psathyrellaceae      | Decaying wood, humus          |                |                   |                     |
| 5  | *Dacrymyces sp.*       | Dacrymycetaceae      | Decaying wood                 |                |                   |                     |
| 6  | *Daldinia sp.*         | Xylariaceae          | Decaying wood                 |                |                   |                     |
| 7  | *Earliella sp.*        | Polyporaceae         | Decaying wood                 |                |                   |                     |
| 8  | *Earliella sp.*        | Polyporaceae         | Decaying wood                 |                |                   |                     |
| 9  | *Hygrocybe sp.*        | Hygrophoraceae       | Decaying wood, humus          |                |                   |                     |
| 10 | *Hygrocybe sp.*        | Hygrophoraceae       | Decaying wood                 |                |                   |                     |
| 11 | *Hygrocybe sp.*        | Hygrophoraceae       | Decaying wood                 |                |                   |                     |
| 12 | *Hygrocybe sp.*        | Hygrophoraceae       | Decaying wood                 |                |                   |                     |
| 13 | *Hygrocybe sp.*        | Hygrophoraceae       | Decaying wood                 |                |                   |                     |
| 14 | *Inocybe sp.*          | Inocybaceae          | Decaying wood, humus          |                |                   |                     |
| 15 | *Tricholoma sp.*       | Tricholomataceae     | Decaying wood                 |                |                   |                     |
| 16 | *Tricholoma sp.*       | Tricholomataceae     | Decaying wood                 |                |                   |                     |
| 17 | *Tricholoma sp.*       | Tricholomataceae     | Decaying wood                 |                |                   |                     |
| 18 | *Lentinus edodes*      | Lentinaceae          | Decaying wood                 | medicine       | Lentinan, KS-2    | [15, 16]            |
| 19 | *Marasmiellus sp.*     | Marasmiaceae         | Decaying wood                 |                |                   |                     |
| 20 | *Marasmius sp.*        | Marasmiaceae         | Decaying wood, litter fall    |                |                   |                     |
| 21 | *Melanoleuca sp.*      | Tricholomataceae     | Humus                         |                |                   |                     |
| 22 | *Mycena sp.*           | Tricholomataceae     | Decaying wood, litter fall    |                |                   |                     |
| 23 | *Mycena sp.*           | Tricholomataceae     | Decaying wood                 |                |                   |                     |
| 24 | *Mycena sp.*           | Tricholomataceae     | Humus                         |                |                   |                     |
| 25 | *Mycena sp.*           | Tricholomataceae     | Mossy rock                    |                |                   |                     |
| 26 | *Lentinus sp.*         | Lentinaceae          | Decaying wood                 |                |                   |                     |
| 27 | *Pleurotus ostreatus*  | Tricholomataceae     | Decaying wood                 | Medicine       | β-glucans and mannin | [17]                |
| 28 | *Polyporus sp.*        | Polyporaceae         | Decaying wood                 |                |                   |                     |
| 29 | *Polyporus sp.*        | Polyporaceae         | Litter fall                   |                |                   |                     |
| 30 | *Psathyrella sp.*      | Psathyrellaceae      | Mossy rock, humus             |                |                   |                     |
| 31 | *Psathyrella sp.*      | Psathyrellaceae      | Mossy rock                    |                |                   |                     |
| 32 | *Psathyrella sp.*      | Psathyrellaceae      | Mossy rock                    |                |                   |                     |
| 33 | *Psathyrella sp.*      | Psathyrellaceae      | Humus                         |                |                   |                     |
| 34 | *Resinomycena sp.*     | Tricholomataceae     | Litter fall                   |                |                   |                     |
| 35 | *Rhizopogon sp.*       | Boletaceae           | Humus                         |                |                   |                     |
| 36 | *Schizophyllum commune*| Schizophyllaceae      | Decaying wood, mossy rock     | Medicine       | Schizophyllan, Sonifilan, SPG, (1→3)-β-D-glucan | [18]              |
| 37 | *Schizophyllum sp.*     | Schizophyllaceae      | Living trees                  |                |                   |                     |
| 38 | *Schizophyllum sp.*     | Schizophyllaceae      | Decaying wood                 |                |                   |                     |
| 39 | *Scleroderma sp.*      | Sclerotormataceae    | Humus                         |                |                   |                     |
| 40 | *Scleroderma sp.*      | Sclerotormataceae    | Humus                         |                |                   |                     |
| 41 | *Scutellinia sp.*      | Pyronemataceae       | Living trees                  |                |                   |                     |
| 42 | *Stereum hirsutum*     | Stereaceae           | Decaying wood, living trees   | Medicine       | Monoterpenoids    | [19, 20]            |
| 43 | *Stereum sp.*          | Stereaceae           | Decaying wood                 |                |                   |                     |
| 44 | *Stereum sp.*          | Stereaceae           | Living trees                  |                |                   |                     |
| 45 | *Trametes versicolor*  | Polyporaceae         | Decaying wood                 | Medicine       | Krestin (PSK), PSP | [20, 21]            |
| 46 | *Trametes sp.*         | Polyporaceae         | Living trees                  |                |                   |                     |
3.2. The potential use of the macrofungi for medicinal purpose

Medical research in immunopotentiators is of great importance with the emergence of Covid-19. There are several mushroom species demonstrated great biological activity effects as immunomodulatory. In order to survive naturally, mushrooms themselves need antibacterial and antiviral compounds; consequently, they harbor a lot of those substances naturally. Mushroom have powerful constituents, called as beta-D-glucans, beta-glycosides, and other substances, that have been determined to having significant influence to stimulate immune system. *Auricularia auricula* fruiting bodies which have a soft, jelly-like texture, and are black-brown in color (Figure 3) commonly grow on decaying wood and litter fall around Cemoro sewu track of Lawu Mountain Forest. According to Zhang et al. [13], *A. auricula-judae* from submerged culture with the backbone of (1→4)-α-D-glucan could be explored as potential immunomodulatory agents for the application in complementary medicine or functional foods.

*Cerrena unicolor* -the wood degrading fungus- were commonly found on decaying wood and also living trees (Figure 4). Mizerska-Dudka et al. [14] reported that *C. unicolor* produce three bioactive fractions which are effective as antioxidant and antimicrobial for inducing additionally antiviral, immunomodulatory, and anticancer activities. The shiitake fungi with latin name *Lentinus edodes* (Figure 5), an edible mushroom which is commonly cultivated naturally at higher elevation around Lawu Mountain Forest, have been proved by many scientists as very excellent immunomodulatory. The fungus produces Lentina and KS-2 as promising stimulator of immunofunctions, strengthening the human immune system [15, 16]. *Pleuretus ostreatus* (Figure 6) with common name Oyster mushroom, according to Morris et al. [17] could potentially affects cell immunity, hence, are especially useful in the prophylaxis of tumours, immunodeficiency and as co-adjuvant in chemotherapy.

![Figure 3](image3.png)

**Figure 3.** The fruiting bodies of *Auricularia auricular* grown on the decaying wood (a) and on the litterfall (b) found at the Cemoro sewu track of Lawu Mountain Forest, altitude 1800 to 3100 m asl.

![Figure 4](image4.png)

**Figure 4.** The fruiting bodies of *Cerrena unicolor* grown at the living tree (a) and mossy maze polypore of lammefae (b) found at the Cemoro sewu track of Lawu Mountain Forest, altitude 1800 to 3100 m asl.
Figure 5. The fruiting bodies of *Lentinus edodes* grown on the decaying wood (a) with squamules stipe (b and c), found at the Cemoro sewu track of Lawu Mountain Forest, altitude 1800 to 3100 m asl.

Figure 6. The fruiting bodies of *Pleurotus ostreatus* grown on the decaying wood (a) with typicalle lamella (b) found at the Cemoro sewu track of Lawu Mountain Forest, altitude 1800 to 3100 m asl.

*Stereum hirsutum* (Figure 7) is one of the multicolour medicinal mushrooms belongs to Stereaceae. According to Doljak et al. [19], dry extract of *S. hirsutum* showed a significant inhibitory effect on thrombin, a compound involved in the physiology of clot-thrombus formation. Only small amount of extract can be used in the mixture for increasing the antioxidant and biomodulatory properties (synergistic effect). *Schizophyllum commune*, the cosmopolitan wood-loving mushroom (Figure 8), have been reported to produce polysaccharides, which performs structural elucidation and determines immune stimulation [18]. Overall, their study suggests that polysaccharides from *S. commune* might have potential as an ingredient of functional food or supplement to enhance immunity. *Trametes versicolor* (Turkey Tail) were abundantly growing on decaying wood at Lawu Mountain Forest (Figure 9). The fungus has been reported to possess a strong immunomodulatory effect due to a polysaccharide called Krestin (PSK) [21]. PSK, which are both proteoglycans with variations in the individual sugar compositions such as glucose, fructose, and galactose, influences the immune system activities through increased production of interleukin-2 (IL-2), interleukin-6 (IL-6) and interferon (IFN) on the humoral side and T-cells proliferation on the cellular side.
Figure 7. The fruiting bodies of *Stereum hirsutum* from under view (a) and upper view (b) found at the Cemoro sewu track of Lawu Mountain Forest, altitude 1800 to 3100 m asl.

Figure 8. The fruiting bodies of *Schizophyllum commune* from upper view (a) and under view (b) found at the Cemoro sewu track of Lawu Mountain Forest, altitude 1800 to 3100 m asl.

Figure 9. Variation of fruiting bodies of *Trametes versicolor* found at the Cemoro sewu track of Lawu Mountain Forest, altitude 1800 to 3100 m asl.

Mushrooms are considered as an untapped reservoir of several novel compounds of great value in industry and medicine [22]. During emergence of Covid 19 pandemic, medicinal research relating to immunopotentiators are of great importance. Since many of macrofungi found in Cemoro sewu track have a big potential as natural medicinal compound for boosting immunity, thus further investigation and research particularly with regard for inducing immunity against Covid-19 is needed.

4. Conclusion
A total of 46 species macrofungi were found on Cemoro sewu track in Lawu Mountain Forest. Based on previous research in the literatures, at least 7 species, i.e. *Auricularia auricula*, *Cerrena unicolor*, *Lentinus edode*, *Pleuretus ostreatus*, *Stereum hirsutum*, *Schizophyllum commune*, and *Trametes*
versicolor, have the potential as a source of immunomodulator compounds. It is recommended that medicinal mushroom from Lawu Mountain Forest should be developed as potential natural supplements against COVID-19.

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Acknowledgments
The authors are grateful to Mr. Suparman in guiding the collection of the macrofungi at Cemoro sewu track Mount Lawu. This research was partly funded by the Public Fund Fiscal Year 2020 Faculty of Forestry Universitas Gadjah Mada under contract No. 119/KS/2020. Technician and bachelor student contributed as enumerators on data collected.