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Wild Mushroom Identification through Morphological Approaches in middle of West Cost of Peninsular Malaysia

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Abstract

Mushroom has been considered as one of the choices of quality and functional food. Nutritional profiling of mushrooms has been well reported in different research. In Malaysia, most studies investigated secondary metabolite production, cultivation and health benefits of edible mushrooms. Research on wild mushroom has been very limited. Hence, the objective of this research project was to identify the morphology of wild mushroom species commonly found in middle of west coast of Peninsular Malaysia. Four different wild mushrooms were collected and characterised based on the morphology and growth appearance when cultured on potato dextrose agar. They were morphologically identified as *Tremella* genus, *Ganoderma* genus, *Phaeolus* genus and *Trametes* genus. However, the species identification was able to confirm up to genus level only as they are sharing many similar features. In conclusion, morphological characterization of mushroom was very time consuming and the accuracy is highly dependent on the researcher's experience. Therefore, molecular method is needed to further confirm the identity of the wild mushrooms.

Keywords

Wild mushroom, Morphological characterization, *Tremella, Ganoderma, Phaeolus, Trametes*

Introduction

Mushroom is one of the choices of quality and functional food (Abdullah & Rusea, 2009). In Malaysia, the studies on mushroom focuses on secondary metabolite production, cultivation, and health benefits of edible mushrooms, with a focus on commercially grown mushroom. There is limited information on wild mushroom (Raja et al., 2017; Samsudin & Abdullah, 2019). According to Abdullah and Rusea (2009), this could be due to the limited knowledge on the identification of edible wild mushroom and the lack of mushroom experts in Malaysia. Most of the reports have highlighted that Malaysians preferred the commercially grown mushroom (Amin & Harun, 2015) where the demand for commercially grown mushroom is increasing yearly because the weather in

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Malaysia is suitable for cultivation of mushroom throughout the year (Amin & Harun, 2015). Therefore, the identification of wild mushroom is needed to expand the new market.

According to the Malaysian Ministry of Natural Resources and Environment, in 2007, a high number of mushroom species, such as micro- and macro mushroom, have been discovered and reported from Malaysian ecosystems (Samsudin & Abdullah, 2019). Research also reported that various compounds obtained from mushroom species had the potential and distinctive properties to be utilized for nutritional and medicinal purposes (Amirullaha et al., 2018; Samsudin & Abdullah, 2019). Amin & Harun (2015) predicted that with the increase in the public's concern about health, mushroom demand in Malaysia would also increase. Research needs to be carried out to discover a new compound that can be extracted from wild mushroom which can be used for different purposes that are beneficial to human beings.

Common mushroom identification is through morphological methods, including observing the morphological characteristics such as cap, stalk or stipe, gills, annulus or ring, diameter, shape, and the color of mushroom (Yadav et al., 2017). Although there are some limitations to the morphological method, which is time consuming and the accuracy is highly dependent on the researcher's experience (Alsohaili & Bani-Hasan, 2018; Tekpinar & Kalmer, 2019), this method is affordable for the public and does not require complicated equipment. The objective of this study was to carry out the morphological identification of selected common wild mushroom species found in middle of west coast of Peninsular Malaysia.

Methodology

Sample collection

Four different types of mushroom samples were collected from the Traditional Chinese Medicine (TCM) garden in INTI International University, Nilai and the housing areas in Klang and Banting. The picture of wild mushrooms was taken on the spot in their natural habitat (Rubina et al., 2017), as shown in Figure 1. After that, the mushrooms were collected using a pocket knife to dig up the bases of the wild mushrooms, which included the soil sample (Kuo, 2019). On the other hand, the mushrooms growing on wood were collected along with small pieces of wood (Rubina et al., 2017). The wild mushrooms that were collected as samples were in good condition and medium-sized, and they were stored in a waxed paper bag with moist newspaper (Kuo, 2019).

All the morphology characteristics of the collected wild mushrooms were observed. The diameter of the fruit body of the collected wild mushrooms was measured with triplicate in centimetres using a ruler. The different parts of the fruit bodies, such as cap, stalk, gills, volva, annulus, shape, and colour, were recorded (Yadav et al., 2017). The samples of mushroom were cultured on the media as soon as possible because mushrooms will decay quickly, especially in warm weather (Kuo, 2019).

Figure 1. Habitat of different mushroom samples. (a) Sample 1 was found at the underside of a branch. (b) Sample 2 was found at the side of a dead tree. (c) Sample 3 was found under a tree. d) Sample 4 was found at the edge of a branch of a mango tree.

Mushroom culturing on media

Potato dextrose agar (PDA) was obtained from OXOID and prepared for mushroom cultivation to obtain the pure culture from mycelium. The wild mushroom was cut into half and a small portion of the inner part of the mushroom was cut and transferred to the center of the PDA plate using a sterilised scalpel (Shields, 2020). For each mushroom species, five replicas were made. All the culturing work was conducted in aseptic conditions. The fungi were identified by referring through online data provided by Mycobank Database (www.mycobank.org), Doctor Fungus (www.doctorfungus.org) and Mycology Online (http://www.mycology.adelaide.edu.au/).

Results and Discussion

Morphology identification

The identities of four different types of wild mushrooms were identified based on morphology observation. The morphological characteristics included cap, stalk or stipe, gills, annulus or ring, diameter of mushroom, texture of fruiting body, habitat of wild mushroom, shape of mushroom, and lastly, the color of mushroom as shown in Table 1. The mushrooms cultured on PDA were observed and recorded as shown in Figure 2.

Table 1. Morphological characteristics of four different types of collected wild mushrooms

Sample	1	2	3	4
Cap	+	+	+	+
Stalk/ Stipe	-	-	+	-
Gills	-	-	-	-
Annulus/ Ring	-	-	-	-
Scale	-	-	-	-
Diameter (cm)	6-16.5	5.5	8.6-15	6.2
Texture of fruiting body	Gelatinous	Hard and woody	Woody	Rubbery
Habitat	Underside of branch	At the side of dead tree	Under the tree	At the edges of dead tree
Shape	Shapeless	Bracket shaped	Overlapping rosette shaped	Fan shaped
Color	Yellowish	Brown with white border	Brown with yellow edges	Yellowish and brownish

Note: The symbol "+" represented the presence of the morphological characteristic while the symbol "-" will represented the absence of the morphological characteristic.

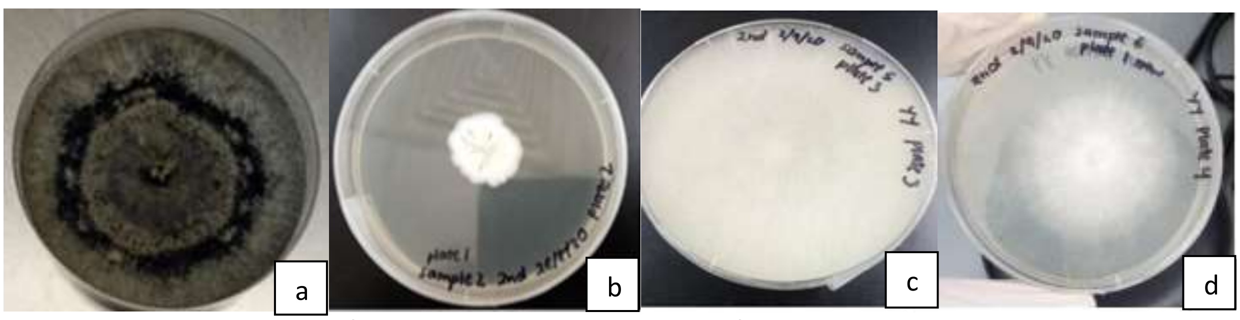

Figure 2 The appearance of mushroom culture on PDA after 7 days. (a) sample 1. (b) sample 2. (c) sample 3. (d) sample 4.

Tremella genus

The researchers reported that *Tremella fuciformis* was attached to the log surface in colony form (Ghosh et al., 2016), which is identical to the habitat for sample 1 (Table 1). Sample 1 was shapeless and yellowish in color, while the texture of the fruiting body was thin and gelatinous. According to Wang et al. (2015), ivory, translucent and gelatinous basidiocarp were the key features of *Tremella fuciformis*. Ghosh et al. (2016) also stated that the morphology of *T. fuciformis* from India had a jelly-like structure with white or snow colored fruiting body in the young stage and changed to creamy white in the maturity stage.

Sample 1 on the PDA produced semi-transparent white mycelium with cotton texture at the beginning of culturing. Eventually, the white mycelium became dark green with a cotton texture after one week (Figure 2a). The growth rate of sample 1 was the fastest where the mycelium started to form after two days and covered the whole petri dish in five days compared to other samples. According to the paper from Alice and Nian (2001), the growth of mycelia for *T. fuciformis* was about 7-10 days and the formation of whitish mycelium can be observed. Based on the morphological features observed from sample 1, sample 1 was identified as *Tremella* genus. Snow Fungus Facts and Health Benefits (2019) stated that snow fungus is normally grown in tropical climates such as the weather in Malaysia and is cultivated for medicinal properties. Snow fungus is an edible mushroom (Foo et al., 2018). It contains iron, vitamin C and calcium which can provide health benefits to body and can prevent diseases such as atherosclerosis. Therefore, in the future more research should be carried out to determine the beneficial compounds in this species.

***Ganoderma* genus**

Sample 2 was a brown bracket fungus with a white border (Table 1). Schwarze and Ferner (2003) reported that *Ganoderma* species have a bracket-shaped appearance and a crust-like upper surface of the basidiocarp. The texture of the fruiting body was hard and woody, and the underside was creamy white and full of pores. According to Rubina et al. (2017), most of the *Ganoderma* species in the National Botanical Garden, Dhaka, had a woody texture and contained white pores under the cap. Sample 2 was found at the side of a dead tree. This is because many *Ganoderma* species grow parasitically on a wide variety of woody plants (Tchotet Tchoumi et al., 2019). Schwarze and Ferner (2003) also mentioned that the presence of the *Ganoderma* species can usually be easily detected due to the formation of the perennial fruit bodies at the root of the tree. Sample 2 was morphologically characterized as *Ganoderma* genus. This is due to the high similarity of basidiocarp features. *Ganoderma* is the most difficult genus of polypores to accurately identify to species (Jargalmaa et al., 2017).

Sample 2 produced white mycelium with woody texture at the beginning of culturing on PDA. After three days, the white and irregular-shaped mycelium started to form. The size of the yellowish mycelium formed on PDA became bigger and wider (Figure 2b). The growth rate of sample 2 was the slowest compared to other samples. It was unable to cover the whole petri dish after one week. According to Ho and Nawawi (1986), the mycelium formed was white in color with a floccose structure and a thick dark brown at the bottom. One of the examples from the *Ganoderma* genus was *Ganoderma lucidum* which is also called reishi or lingzhi, "mushroom of immortality" (Loyd et al., 2018). The woody cap of lingzhi is tough and taste bitter, making it not suitable for culinary use, but the powder form of reishi can be used as a regular supplement. Tinsley (2018) reported that reishi has been used for thousands of years to accelerate vitality, boost the immune system, improve cardiovascular health and promote longevity. Hence, more studies need to be carried out to find out the essential compound in this species.

***Phaeolus* genus**

Sample 3 was brown in color with yellow edge. Sample 3 had a woody texture and yellow pores were found at the under-surface of the fruiting body (Table 1). According to Roehl (2017), *Phaeolus schweinitzii* is commonly known as dyer's polypore, and the underside of dyer's polypore was covered by a yellow pore surface. *Phaeolus schweinitzii* (n.d.) reported that the mature fruiting bodies of *P. schweinitzii* were reddish brown with tough flesh, and the pore surface was yellow. Kuo (2016) reported that the fruiting body of *P. schweinitzii* was loosely arranged with large lobes growing from a single stem-like structure that emerges from the ground. Kuo (2016) concluded that this species is usually found near the base of the tree's trunk, which appears terrestrial. At the overlapping part of the fruiting body of sample 3, small debris and sticks were observed. This type of growth pattern is known as indeterminate growth, which means this species engulfs sticks and other debris as it grows (Roehl, 2017). Hence, sample 3 was morphologically characterized as the *Phaeolus* genus.

Sample 3 produced white mycelium with smooth texture at the beginning of culturing on PDA. After one-week, a hairy structure attached to the mycelium can be observed (Figure 2c). Some black spores were found at the end of the hairy structure. The growth rate of sample 3 was average as it had covered the whole petri in one week. Roehl (2017) stated that dyer's polypore produces strong colors ranging from golden-yellow to forest green, depending on the age of the harvested mushroom. Lastly, Roehl (2017) reported that the woody texture of *P. schweinitzii* made it not suitable for eating and that it could be poisonous. However, *P. schweinitzii* can be used for dyeing wool.

Trametes genus

The upper surface of the fruiting body in sample 4 was fan-shaped with concentric zones of white and brownish colour and a yellowish edge, while the underside was white and covered with tiny creamy white pores (Table 1). This can be reviewed in *Trametes hirsuta* (n.d.) that stated that the pore surface of *Trametes hirsuta* was initially white turned into creamy white. Roehl (2018) reported that *T. hirsuta* normally has a fan-shaped upper surface with fewer rings of color compared to the turkey tail, which is *Trametes versicolor*. The cap surface of sample 4 was hairy with a rubbery texture and fused with the neighbouring caps. In the study reported by Kuo (2010), the key feature of *T. hirsuta* is a dense hairy cap surface with whitish and grayish coloration. *Trametes hirsuta* (n.d.) also mentioned that the adjacent caps of *T. hirsuta* sometimes merge and fuse together, which can be observed in sample 4. Therefore, sample 4 was morphologically characterized as the *Trametes* genus.

Sample 4 produced semi-transparent mycelium with cotton texture at the beginning of culturing on PDA. After three days, semi-transparent mycelium became opaque white mycelium (Figure 2d). The growth rate of sample 4 was average as it had covered the whole petri in one week. Lastly, Knežević et al. (2015) reported that *Trametes* species have been used for thousands of years as traditional and conventional medicine to treat different types of diseases. The researchers also concluded that the extracts from *Trametes* species may be considered as strong antigenotoxic agents and stimulate the genoprotective response of cells. In addition, another paper from Knežević et al. (2018) also claimed that the mycelial extracts from *T. hirsuta* contained the highest antifungal potential compared to *T. versicolor and Trametes gibbosa.* Hence, more studies should be carried out to discover the new beneficial compounds that can be extracted from *T. hirsuta.*

Conclusions

A total of four wild mushrooms species were collected from INTI Traditional Chinese Medicine (TCM) garden in Nilai and the housing areas in Klang and Banting. They were morphologically identified as *Tremella* genus, *Ganoderma* genus, *Phaeolus* genus and *Trametes* genus. However, the species identification was able to confirm up to genus level only as they are sharing many similar features Morphological characterization of wild mushrooms was time consuming and the accuracy is highly dependent on the researcher's experience. Therefore, molecular method is needed to further confirm the identity of the wild mushrooms.

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