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Short Communication

Edible Bitter Mushroom from Besut, Malaysia

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ABSTRACT

To date, the edible bitter mushroom that available in Besut known with the only name kulat gelam. Whether in Terengganu or Kelantan, the mushroom that provides bitter taste will be obtained in certain season especially raining season in habitat of gelam trees. However, people from communities in Besut, Terengganu and some from Kelantan called the name of pokok gelam to different species of trees. In Besut, the tree was referred to *Acacia mangium* while in Kelantan they referred to *Melaleuca cajuputi*. Even in Malaysian rain forest, there was found the tree that related to the gelam name, that is gelam bukit tree from the species of *Leptospermum* from Fagaceae family. Field survey had been done in the habitat of the bitter mushroom found, assisted by local people. Through this research, we had been observed for the relationship between the bitter mushrooms in Besut area with the so called gelam tree. Comparison had been made with the previous record of bitter mushroom in local and at the other countries. The nomination of this bitter mushroom was well-matched with the possible details accounts and found it significance to the name of *Tylopilus felleus* (Bull.) Karst., from Boletaceae family and some synonyms. There was ectomychorrizal relationship between kulat gelam and *Acacia mangium* trees. A simple experiment done to obtain the pH value of fresh juice found at pH 5, while the moisture content was 91.38 % and ethanolic extraction produced 26.09 % yield. Some macroscopic characters of the species had been recorded and the specimen deposited in herbarium. It is important to identify each fungus correctly for the status of its edibility and risks of poisonous, where it possible to cause fatal. Hence, this research may provide basic knowledge for the wild fungi identification and ecosystem management.

Keywords: Bitter mushroom*, Acacia mangium, Tylopilus felleus* (Bull.) Karst, Besut

ABSTRAK

Sehingga kini, cendawan pahit yang boleh dimakan dan terdapat di Besut hanya dikenali dengan satu nama iaitu kulat gelam. Sama ada di Terengganu atau Kelantan, cendawan yang memberikan rasa pahit ini boleh didapati pada musim tertentu sahaja terutama ketika musim hujan, di kawasan habitat pokok gelam. Walau bagaimanapun, masyarakat di Besut, Terengganu dan sebahagian di Kelantan memberikan nama pokok gelam terhadap dua spesies pokok yang berbeza. Di Besut, ia merujuk kepada *Acacia mangium*, manakala di Kelantan adalah merujuk kepada *Melaleuca cajuputi*. Malah di dalam hutan hujan tropika Malaysia terdapat juga pokok berkayu yang dikaitkan dengan nama gelam iaitu pokok gelam bukit dari spesies *Leptospermum* daripada keluarga Fagaceae. Kajian lapangan telah dibuat di habitat cendawan ditemui dengan bantuan penduduk setempat. Kajian yang dijalankan ini meneliti hubungan di antara kulat gelam di Besut dengan pokok gelam *Acacia mangium*. Perbandingan telah dibuat berdasarkan rekod-rekod terdahulu di dalam dan luar negara. Penamaan terhadap kulat gelam juga telah disuaipadankan dengan keterangan-keterangan yang munasabah dan mendapati ia signifikan dengan nama *Tylopilus felleus* (Bull.) Karst., daripada keluarga Boletaceae dengan beberapa nama sinonim. Terdapat hubungan ektomikorizal di antara kulat gelam dengan pokok *Acacia mangium*. Ujian ringkas terhadap nilai pH jus segar mendapatinya pada nilai pH 5, manakala kandungan lembapannya sebanyak 91.38% dan 26.09 % hasil didapati daripada pengekstrakan etanol. Beberapa ciri makroskopi yang telah dikenal pasti terhadap spesies ini telah pun direkodkan dan spesimen disimpan di herbarium. Oleh itu, kajian yang telah dijalankan boleh menyumbang maklumat asas dalam membuat identifikasi cendawan liar dan pengurusan ekosistem.

Kata Kunci: Cendawan pahit, *Acacia mangium*, *Tylopilus felleus* (Bull.) Karst, Besut

INTRODUCTION

Although the mushroom is very special to people in Besut district and part of Kelantan state, until now the only name to the species in Kelantan dialect is kulat gelam, meaning mushroom or toadstool from the area of gelam trees. However, the tree which called gelam by Besut people is not the actual *Melaleuca* sp. from Myrtaceae family, but was referred to *Acacia mangium*, Fabaceae, which not grows at the swamp area but available in forests area (Chan, 2010). This is in line with Ingold and Hudson (1995), who were reported about fungi associations

established with host plants including pine, conifers and mixed woods. Plants like beech, oak, pine and other woodland tend to have their own characteristics of agarics which producing their basidiocarps in the litter.

People may consume mushrooms for the purposes of nutritional on the reason of fungi contain high protein, carbohydrate, multivitamins and minerals. Hence, lot of scientific studies conducted to confirm the potential of well-known and commercial mushrooms like the abalone, rice straw, oysters, *Ganoderma* and shiitake for the nutrition and pharmaceutical values (Mohd Zaffrie *et al.,* 2014). However, less attention given for wild mushroom, like the edible bitter mushroom that found in Besut, Terengganu, Malaysia leads to lack of knowledge about the species of *Boletus*.

Lau *et al*., (2017) were identified the similar vernacular name of mushroom kulat gelam in peat swamp forests in Bachok, Kelantan as *Boletus griseipurpureus* and confirmed for its edibility, at low risk of toxicity level. The dominant vegetation in the area was *Melaleuca cajuputi*, which also called pokok gelam in local Kelantan dialect. However, the macroscopic figures of *B. griseipurpureus* were differ to kulat gelam that we had found in Besut, Terengganu. The similarities in this case were the local name kulat gelam, bitter taste and the occurrence of the mushroom, in the leaf litter areas.

For local people, the use of this mushroom is an exotic food especially among elderly. On the reason of it taste is bitter, the mushroom was neither to be eaten raw nor simply cooked as the commercial mushroom like oyster mushroom or shiitake. The preparation done before being edible was similar to the cooking method of *Lactarius necator*, Russulaceae, a toadstool that distributed in Europe, Asia and North America. The bitter substances can be removed by repeating boiling the mushroom in water for ten minutes and strained, before it to be cooked in usual way (Klan, 1981). Based on the general conversation with local people, kulat gelam that seasonally sold in local wet markets was the type of boiled and packed in plastic bag, maintained in good condition for only about three days in the ambient temperature but can be stored in freezer for many years. Some Besut people consumed kulat gelam as additional menu with staple food, and in the same time for treating hypertension and diabetic (Nor Asiah *et al*., 2016). However, proper and extended research needed to prove the effectiveness and the side effects if occurs.

Mushroom like kulat gelam does not contain the pigments like what higher plants have, such as chlorophyll, anthocyanin, carotenoid and terpenoid. Most pigments of higher fungi are quinones and other pigments that containing nitrogen, the products from the shikimate pathway (Velisek and Cejpek, 2011). In fungi, an antioxidant agent, diboviquinone may present (Kasuga *et al*., 1995). Quinine is a kind of alkaloids that possibly being a substance that produces bitter taste in most plants (Sequin, 2012). While ergot alkaloids are available in fungus like *Claviceps purpurea* and the derivatives medicinally used in certain controlled doses for anti-haemorrhage, treatment of migraine and pre-menstrual syndrome (Sim, 1970). However, despite of multiple benefits of the chemicals from fungi which appraised people's attention, the safety factor also to be cautious by consumers, because cases of poisonous may happened by mistaken consumption of poisonous mushroom, might cause death (Chew *et al*., 2008).

This bitter mushroom may only to be obtained in the season of March or July and little bit in December related to the rain season and humidity. Making the botanical descriptions of the local bitter mushroom is quite challenging due to lack of local sources and need to refer to any findings related to Boletaceae from other countries. In this case, we had been observed the population of edible bitter mushroom that found in the area of *Acacia mangium* trees in Besut, a district in Terengganu state. As to determine the class of the mushroom based on the botanical characters, we need to compare and match with previous reports. However, the possibly well-matched descriptions found far from Asian region and some details were confusing, such as the habitat and the way of it lives (Murrill, 1909).

Therefore, the objectives of this study were to identify the wild edible bitter mushroom from Besut in its ecosystem, to convey macroscopic observation, to compare with the previous botanical notes and to nominate the species into the correct identity. The obtained results hopefully may contribute as basic knowledge in making confirmation upon wild fungi and ecosystem management.

MATERIALS AND METHODS

Sampling

Field survey activity had been done in July 2017 by investigating the bitter mushroom, assisted by local people, who were well experienced in collecting the mushroom during its season. There were more rain and more humidity prior emergence time of the mushroom nearby the area of the *Acacia mangium* trees. The spotted area of bitter mushroom population was at the steep rock-soil face of hill in Kampung Beris Pak Abu (5.797737^{O} N, 102.514208^{O} E), about 10 kilometres from Kuala Besut town, at the land height of 6 m. Young mushrooms that protruding from dried leaves of *A. mangium*, collected in clustered and the mature mushroom collected as single. The mushroom had been identified, measured and photographed for documentation (Imes, 1990). Then, the mushroom samples had been taken into paper box and covered with envelops (Clarke, 1980) before undergone procedures for pH determination, herbarium specimen and to make dried form powder, for further experiments.

Mushroom identification

Comparison and justification were made to place the bitter mushroom into correct family. Observation on the macroscopic characterisations of the specimen was helpful for this preliminary study. The illustration made by Clarke (1980) and Klan (1981) had been referred for the morphology of the mushroom. The habit of the mushrooms had been observed to confirm the relationship of the species with the host plants. The general characters of mushroom based on main shape of the mushroom cap and the presence of reticulation-like pattern stipe surface had been investigated (Chan, 2010; Murrill, 1909) followed by confirmation by university botanists.

Herbarium

The whole fresh and undamaged fruit-bodies of mushroom were taken and cleaned for herbarium specimens. The procedures for herbarium were followed the steps by Klan (1981) and Imes (1990). Identification of the species had been confirmed by university botanists as the way done by Perevedentseva (2013). Descriptions for specimen of the species had been made, including the information of habitat, associated green plants, substrate in which the mushroom were found and the location. The fleshy fruit-body had been cut into sections and all the specimens then undergone air dried at 50° C. The dried specimens were treated with the suitable insecticide chemicals, stored in envelop with the herbarium label (Tomovic *et al*., 2002) and given the voucher number according to the system of herbarium institution (Wu *et al*., 2014).

Determination for pH value

The fresh whole mushroom fruit bodies were cleaned with a dry soft nylon brush and had been mashed and sieved in ambient temperature. The juice collected into a petri dish and immediately, the pH indicator paper strip (Whatman) dipped, to obtain the pH reading (Ben Meadows, 2017).

Dried powder samples and ethanolic extraction

The mushroom had been cut into small sizes, then put into paper envelop and dried in an electric dryer at 45 °C to a constant weight. An electric blender had been used to grind the materials to small particle. A 10 g dry weight dried powder had been put into cellulose extraction thimble and 150 ml 95% ethanol (Nacalai Tesque, Japan) had been used in the process, at 50 °C for 4 hours with soxhlet apparatus. The collected filtrate then had been filtered with filter paper (Whatman) and evaporated under vacuum at 50 °C, 174 mbar pressure, 100 rpm, using a rotary evaporator (Heidolph, Germany). The collected semisolid extract then had been stored in a glass bottle sample at 4 °C until to be used for further experiments (Grzybek *et al*., 1994; Nuttawan and Eshtiagi, 2013).

RESULTS AND DISCUSSION

Bitter mushroom in habitat

We believed, the forest floor with leaf-litters formed an ideal medium for fungal growth. Before the leaves fall, they supported varieties of organic substances such as simple sugars and organic amino-acids. It was rich in organic matter and well aerated. Sucrose was rapidly translocating to the roots and converted to the fungal carbohydrates for ectomychorrizal fungi. Hyphae later absorbed and conducted water and mineral ions over long distances while mycorrhizal plants benefit from their symbiotic fungi, especially in their water and minerals relations (Ingold and Hudson, 1995).

The uptake of nutrients by most plants was enhanced by association of the roots with fungi. Fungal-root associations give benefit to the plant by significantly increasing the volume of soil water accessible to the roots. Phosphorus is an example, less soluble in water and needed by plants. Hence, mycorrhizal fungi assisted the uptake phosphorus by extending the mycelia to the mycorrhizal plant roots. Apparently, mychorrhizal plants found it advantageous to expend their carbon resources supporting mychorrhizal growth and in the same time had extensive growth of its root system (Hopkins and Huner, 2004).

There were symbiotic associations between the vegetative mycelia of the fungi and the young roots of trees, for the continuity of fungi and host plants life, as in Figure 1. The factors as humid microclimate and rich diversity of host trees in our Malaysian forests support a wide diversity of fungi (Chan *et al.*, 2011).

Figure 1. Young basidiocarps of *Tylopilus felleus* (Bull.) Karst. with basal mycelia. At right, the root of young *Acacia mangium* seedling embedded in the basal part.

During our visit to the aimed area, we also found a group of long-tailed macaque, probably *Macaca fascicularis* (KPKKT, 2015) collecting and eating the mushrooms, then immediately ran away. They probably consumed the mushroom as for food or for treating illness like the chimpanzees in Tanzania. Huffman and Seifu (1989) had been observed the consumption of *Vernonia amygdalina*, a naturally bitter plant of known ethno-medicinal value, by an adult female chimpanzee in the Mahale Mountains, Tanzania. They were recognized the chimpanzee was sick, with signs of weak. Hence, this finding suggesting that consumption of bitter plant by primates was occurred for medicinal purposes. Wild mushroom like *Tylopilus felleus* may produced a high level of secondary metabolites, which also antioxidant agents including the ascorbic acid, terpenes, tocophenols and steroids, thus resulting the bitter taste (Heleno *et al.*, 2011).

In tropical forests, a fungus may form an ectomychorrizal relationship including the trees in the Fagaceae family and other hardwood. The *Leptospermum* species from Fagaceae had been found in Malaysian forest and called as gelam bukit tree (Premilla, 2002). However, there is no report about the relationship of the species with any kind of fungus in Malaysia.

Identified bitter mushroom

Chan (2010) had been provided the details and photographed two species of *Boletus* with bitter taste that were found in Pahang and Penang. The mushroom showed similar in general appearance and the information of both species had been matched and compared with the samples we collected. However, our comparison was unmatched with measurement and details of nearest morphological characters of the two boleti (*Tylopilus plumbeoviolaceus* and *Tylopilus rubrorunneus*); for the size of pileus, basal mycelium colour, size and pattern of stipe.

Taxonomic notes by Murrill (1909) had been used to strengthen the nomination of bitter mushroom into a specific placement. Because of the bitter taste being a distinctive identity of the mushroom, we later classified this bitter mushroom in line with the description by Klan (1981). The figures and the measurement of the mushroom size had been referred to identify our collected specimens and we found it well-matched, thus significant with the name *Tylopilus felleus* (Bull.) Karst. The specimens that were taken from the habitat had been photographed and the macroscopic characters on whole fruit body of *Tylopilus felleus* (Bull.) Karst. had been observed and shown in Figure 2.

Botanical descriptions

The botanical descriptions of the general macroscopic characters of bitter mushroom fruit-bodies had been shown in Table 1. Based on the physical characters, we were confident to place the bitter mushroom under family of Boletacae and genus of *Boletus*, as summarized in Table 2. The subdivision of the Basidiomycota is based on the form of the basidium, shape and morphology of the mature fruit body (Moore and Trinci, 2011). The specimens of *Tylopilus felleus* (Bull.) Karst. were given a voucher number (00389) and deposited in Universiti Sultan Zainal Abidin (UniSZA) Herbarium for reference in future.

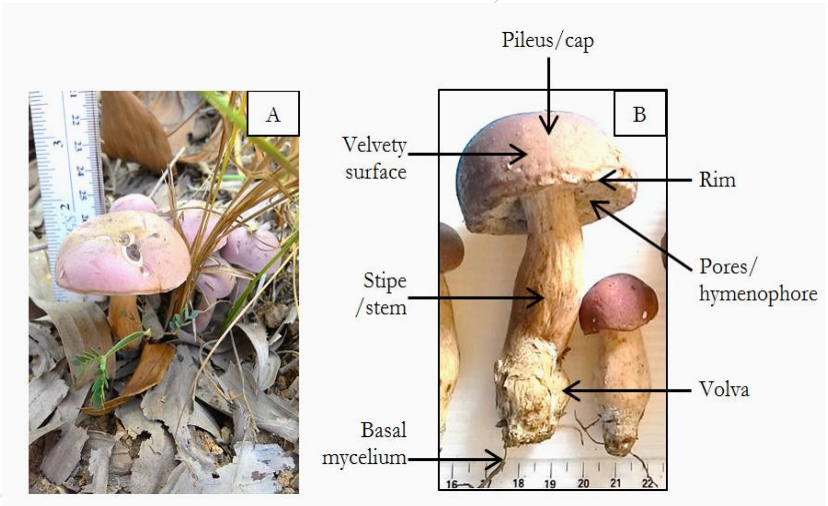

Figure 2. A) *Tylopilus felleus* (Bull.) Karst. in *Acacia mangium* dried leaves litter B) Young and mature basidiocarps of *Tylopilus felleus* (Bull.) Karst.

Table 1. The botanical descriptions of the bitter mushroom studied based on Clarke (1980), Klan (1981) and Murrill (1909)

General characters of pileus / cap	Stipe	Reticulation	Spore	Flesh	Aroma	Taste of context
Semi-globular, grey-brown, pinkish velvety cap 4 cm to 12 cm wide. Pileus finely tomentose, light pink or brownish pores, rusty brown when bruised	Brownish, 1 cm to 2 cm diameter, different at top, middle and bottom	Thin dark brown reticulations on the pale brownish background	Pink spore powder seen under hand-magnifier	White, not immediately changed to brownish after been cut	Fresh smelled fungoid, wilt smelled pungent	Very bitter

Table 2. The taxonomy of the bitter mushroom collected in Besut, based on Klan (1981), Moore and Trinci (2011) and Murrill (1909)

Kingdom	Phylum	Class	Order	Family	Genus	Species	Common name	Local name
Fungi	Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Boletus*	*Tylopilus felleus* (Bull.) Karst. Synonims: *Tylopilus felleus* P. Karst., *Tylopilus felleus* (Bull.: Fr.) P. Karst., *Tylopilus felleus* (Bull.ex Fr.) Karst.	Bitter boletus mushroom	Kulat gelam

Alexopoulos *et al.* (1996) were mentioned about the pigment of pulvinic acid derivatives had been diagnosed in the Boletaceae family. Hence, the pH value of the fresh extract that found at about 5, shown in Figure 3, might be the presence of weak acidic substance in mushroom such as polyporic acid, hyroxypulvinic acid, vulpinic acid and gomphidic acid that occur naturally in most high fungi (Velisek and Cejpek, 2011). The moisture content of the whole fruit bodies was 91.38 %, slightly higher than the moisture content of *Boletus auranticus* at 89.30 % as reported by Vidović *et al.* (2010). The estimation of pH value, percentages of moisture content and yield of the ethanolic extraction obtained, shown in Table 3.

Figure 3. Juice of *Tylopilus felleus* (Bull.) Karst., (labelled as *T.f*), at pH 5, while distilled water (labelled as dH_2O) as control at pH 7.

Table 3. pH value, moisture content and yield percentage of *Tylopilus felleus* (Bull.) Karst.

Estimated pH value of juice	Moisture content (7 days at 40 °C, moving air drying)	Percentage of yield under ethanolic extraction (in 95% ethanol)
5	91.38 %	26.09 %

Some inedible fungi were ignored in certain community, but well accepted and being exotic menu in other communities. The purpose of the mushroom is not limited as for food but also as an alternative medicine for treating common illness. Hence, nomenclature of the mushroom is most important prior to any extended research such as biochemical and bio-molecular. The ideas of previous researchers that embarked in mushrooms studies were widely diverse. Perhaps, this research will not be limited for only botanical descriptions however to reveal the symbiotic relationship occurs in the ecosystem of the mushroom habitat. Phytochemical and other derivatives that originated from wild mushroom may have high potential for medicinal purposes in future. It is hoped this preliminary study may contribute ideas for any extensive research and documentation about wild mushroom in this country especially related to the aspects of fungi conformation, economic values and conservation of *Boletus* species.

CONCLUSION

It is worthy if the diversity of large number of wild fungi in our country to be documented systematically. We have responsibility to identify every fungus correctly for the status of its edibility and risks of poisonous, where it possible to cause fatal. There was ectomychorrizal relationship between the *Tylopilus felleus* and the *Acacia mangium* in Besut, however in Kelantan; the host plant for the similar type bitter mushroom was the *Melaleuca cajuputi*. Every living organism is interrelated to each other and supports the continuity of life. The pH value 5 of *Tylopilus felleus* fresh juice showed it is acidic, while the moisture content as 91.38 % was high and common for any commercial mushroom. The ethanolic extraction produced high yield, 26.09 % by ethanolic extraction under soxhlet method. The presented macroscopic characters of the species will be useful for further research and the specimen that deposited in herbarium possible to be referred as a life document. Hence, this research may contribute for basic knowledge of the wild fungi identification and ecosystem management. Further, in Terengganu the deterioration of endangered species is on-going.

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