Journal of Sustainability Science and Management
Volume 20 Number 10, October 2025: 2142-2162

eISSN: 2672-7226

IDENTIFICATION AND NUTRITIONAL PROFILING OF AN EDIBLE GELAM MUSHROOM IN SETIU, TERENGGANU, MALAYSIA

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http://doi.org/10.46754/jssm.2025.10.009
Submitted: 18 September 2024 Revised: 6 February 2025 Accepted: 14 April 2025 Published: 15 October 2025

Abstract: A bitter bolete fungus locally known as "*kulat gelam*" was recorded near gelam trees (*Melaleuca cajuputi*) in Setiu, Terengganu, Malaysia. Despite being locally consumed, scientific information of the species remains limited. As such, this study aims to identify the species, assess its nutritional composition, and evaluate its antioxidant properties. Morphological characteristics and Internal Transcribed Spacer (ITS) markers were used for molecular identification. The mushroom's proximate composition (moisture, ash, crude fat, crude fibre, and crude protein) and antioxidant properties were analysed. BLAST analysis of the ITS region showed high genetic similarity (97%-100%) with *Tylopilus griseipurpureus* strains from Kelantan, Malaysia and Nakhon Si Thammarat, Thailand. Phylogenetic analysis revealed two clades, suggesting potential spore dispersal between locations. This suggests the existence of different strains or multiple clades of *Tylopilus* sp. Nutritional analysis showed that *T. griseipurpureus* contains significant crude protein (18.52%) and crude fibre (11.30%), followed by ash (10.85%) and crude fat (6.45%). The sample extract exhibited positive antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, with a radical scavenging activity of 81.4% at the highest concentration tested. These findings provide valuable insights for further study of this species and highlight the importance of ecological factors in understanding the distribution of ectomycorrhizal fungi.

Keywords: Bolete fungus, *Tylopilus*, gelam tree, gelam mushroom, bris soil.

Introduction

Boletes are macrofungi classified under the family Boletaceae within the phylum Basidiomycota (Pedneault *et al*., 2006). The Boletaceae family consists of macrofungi characterised by large fleshy fruit bodies featuring a tubular hymenium located on the underside of the cap (or pileus), where spore-producing cells known as basidia can be found (Smith & Theirs, 1971). These fungi possess fleshy fruit bodies and dry caps, which can be slightly viscid in wet weather. They have a wide, sturdy, reticulated stem that lacks an annulus or veil. Boletes play a crucial role in the nutrient cycle of tropical forests, forming ectomycorrhizal interactions—a symbiotic relationship between fungi and the roots of various plant species that benefits both parties within tropical forest ecosystems (Wu *et al*., 2014).

Fungal fruit bodies closely resemble those of *Tylopilus* sp., a group of bolete fungi known for their bitterness, which serves as a key identifying feature. These fungi have been found growing near Gelam trees (*Melaleuca cajuputi* Powell) in the Setiu Wetlands, Terengganu, Malaysia. Locally, this fungus is referred to as "*kulat gelam*" due to its association with Gelam trees land its species identification has yet to be determined in the area. Interestingly, this fungus is considered a delicacy among local communities and is traded on a small scale in local markets. In terms of habitat and fruiting pattern, the Gelam mushroom in Setiu thrives in shaded areas near *Melaleuca cajuputi* trees, specifically on bris soil. In Malaysia, bris soil (beach ridges interspersed with swales) is found only on the east coast of Peninsular Malaysia and the coastal areas of Sabah (Zahari *et al*., 1982). This soil type has a low water-holding capacity due to its sandy texture and is less fertile for plant growth. In a separate study conducted in the area, Lee and Mohammad (2020) interviewed selected respondents in the Setiu and Besut districts of Terengganu, who reported sightings of Gelam mushroom fruit bodies in early April 2017, following heavy downpours that marked the end of the dry season. They also noted the mushroom's availability in local fresh markets, where it is sold fresh or boiled between April and July (Figure 1).

Phillips (2006), in his comprehensive guide to mushrooms and other fungi, described the genus *Tylopilus* as inedible due to its bitter taste. However, the local people of Setiu and Besut, Terengganu consume it despite its bitterness and overpowering smell. According to interviews with local respondents in Setiu, Terengganu, the bitterness is mitigated by peeling the outer layer of the mushroom and washing it thoroughly before boiling. They also noted that inadequate washing of the mushrooms could lead to dizziness or nausea after consumption (Lee & Mohammad, 2020). However, no scientific studies have been conducted or documented regarding its potential side effects.

Several local studies have documented occurrences of identical species in Bachok (Muniandy *et al*., 2016; Lau *et al*., 2017; Yuswan *et al*., 2017) and Tok Bali (Yuswan *et al*., 2015), both of which are located in the state of Kelantan, north of Terengganu. These studies also analysed Gelam mushrooms for antiproliferative properties, DPPH scavenging activity, antidiabetic effects, and toxicity levels. They suggested that Gelam mushrooms could be a potential source of natural antioxidants, exhibiting low toxicity and the ability to delay complications in diabetic patients (Yuswan *et al*., 2015; Muniandy *et al*., 2016; Lau *et al*., 2017). In another study, Yuswan *et al.* (2017) documented the nutrient composition of Gelam mushrooms, noting their high potassium content. Lau *et al.* (2017) further explored the infestation of *Boletus griseipurpureus* Corner by *Megaselia scalaris* Loew, a Diptera insect, suggesting that *B. griseipurpureus* may serve as a potential selective host for *M. scalaris* in peat swamp forests in Kelantan.

Figure 1: Boiled Gelam mushrooms in packets sold in the local wet market

Awang *et al.* (2017; 2018) reported another occurrence of the Gelam mushroom along the east coast of Peninsular Malaysia, specifically in the Besut district of Terengganu. They identified the species as *Tylopilus felleus* based solely on macroscopic characteristics, lacking molecular evidence. They also noted discrepancies between their macroscopic descriptions and those reported by Lau *et al.* (2017) while acknowledging similarities in several aspects such as the local name, bitter taste, and habitat.

Moreover, several studies have documented the morphology of identical mushroom species found in southern Thailand (Seehanan & Petcharat, 2008; Horak, 2011; Aung-aud-chariya *et al*., 2012). Although similar descriptions have been recorded for related mushroom species in the neighbouring state, there is a lack of proper documentation regarding the occurrence of the edible bolete mushroom, particularly in the Setiu district of Terengganu. Therefore, this study aimed to (i) identify the wild edible bitter bolete mushroom through morphological observation and molecular markers and (ii) determine the nutritional values and antioxidant properties of the lesser-known bitter-tasting Gelam mushroom from Setiu, Terengganu.

Materials and Methods

Study Area and Field Activities

Fresh fruit bodies were collected from April to June in 2017 and 2018 in Gong Batu (GB) (N5.661686 E102.715489), Pengkalan Gelap (PG) (N5.661833 E102.728983), Telaga Papan (TP) (N5.538267 E102.9071), and Telaga Papan Baru (TPB) (N5.514733 E102.885167) in the Setiu district. This was done with the assistance of local representatives from the communities who have extensive experience in collecting and consuming mushrooms (Figure 2). The mushrooms were randomly collected from bris areas where *Melaleuca cajuputi* trees are abundant, both in shady and non-shady locations (Figure 3). The collected mushrooms were then placed in article containers and processed in the laboratory for further analysis.

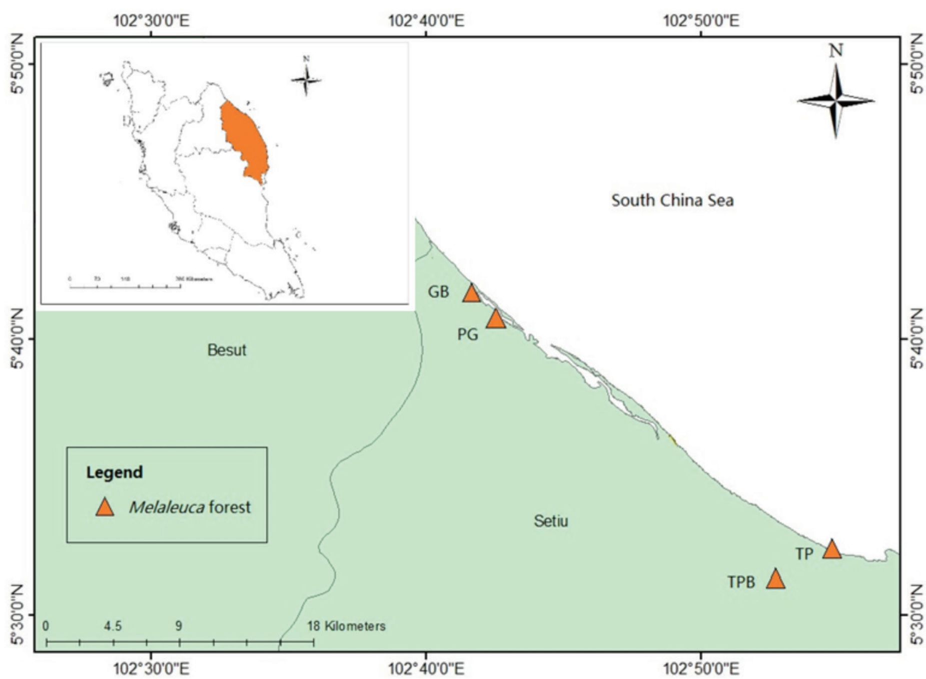

Figure 2: Sample collection sites in Setiu, Terengganu (GB = Gong Batu, PG = Pengkalan Gelap, TP = Telaga Papan, TPB = Kampung Telaga Papan Baru)

Figure 3: Vegetation area dominated by *Melaleuca cajuputi* in Setiu, Terengganu

Macroscopic and Microscopic Observations

Macroscopic and microscopic evaluations were conducted following the methodologies outlined by Corner (1972), Chandrasikul *et al.* (2008), and Horak (2011). Visible features, including the cap, stipe, and hymenophore sections were observed and detailed descriptions were recorded for taxonomic reference. The colouration of different fruit body sections was determined using the Flora of the British Fungi: Colour Identification Chart (1969, 1st edition). The scent and taste of the raw mushroom were also documented.

Microscopic observations focused on the spores and basidia of *Tylopilus* sp. Fresh specimens were carefully collected and thin sections were prepared for microscopic examination. Spores were scrutinised for colouration, shape, and surface characteristics using a high-resolution microscope (Primostar 3, Zeiss, Germany). Attention was directed to the basidia in the hymenophore area, focusing on their morphological features. Comparisons with existing literature, particularly Seehanan and Petcharat (2008) and Lau *et al.* (2017) facilitated the identification and confirmation of observed characteristics. Additionally, sensory aspects such as scent and taste were investigated. Fungal specimens were sniffed carefully to detect distinct odours and descriptions were noted, comparing them to familiar scents such as earthy, woody, or foul odours. For the taste test, a tiny portion of the fresh fruiting body was placed on the tongue briefly, without swallowing, to assess taste. The taste was categorised using descriptors such as mild, bitter, peppery, or sweet.

Molecular Identification

Molecular approaches offer the most accurate method for species identification (Lee *et al.*, 2006; Rajaratnam & Thiagarajan, 2012). Consequently, nuclear ribosomal internal spacer (ITS) markers were employed in this study. A total of five samples from each sampling site were analysed. The total genomic DNA of the mushroom samples was extracted using the Wizard® Genomic DNA Purification Kit (Promega, USA), in accordance with the manufacturer's instructions. The partial ITS region was amplified using the universal primer pairs ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3'; Gardes & Bruns, 1993) and ITS4 (5'- TCCTCCGCTT ATTGATATGC-3'; White *et al.*, 1990). Polymerase Chain Reaction (PCR) amplification was conducted in a Bio-Rad MyCycler thermal cycler (Bio-Rad, USA) with an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 35 seconds, annealing at 48°C for 30 seconds, extension at 72°C for 1 minute, and a final extension step at 72°C for 10 minutes. The PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostic GmbH, Germany) according to the manufacturer's protocol. A single band of approximately 600 to 700 bp was isolated from the PCR product and sent to a certified service provider for both forward and reverse strand sequencing using the same primers as those used in the PCR amplification. Agarose gel electrophoresis of the purified PCR products of the *Tylopilus*sp. (Lanes 1-5) is depicted in Figure 4.

For sequence alignment and dataset assembly, the DNA sequences were aligned, and any invalid or unwanted start and endpoints were removed before being subjected to the Basic Local Alignment Search Tool (BLAST) with sequences from the National Center for Biotechnology Information (NCBI) gene bank (https://www.ncbi.nlm.nih.gov).

All sample sequences were imported into Molecular Evolutionary Genetic Analysis Version 7.0 (MEGA 7) (Kumar *et al*., 2016), aligned, and a total of 1,000 bootstrap replications were performed to estimate the standard error, with *Leccinum scabrum* (AF454585) chosen as an outgroup for the analysis due to its broad range. To construct the phylogenetic tree, the evolutionary history was inferred using the Neighbour-Joining method (Saitou & Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is indicated next to the branches. The tree was drawn to scale, with branch lengths representing the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were then calculated using the maximum composite likelihood method.

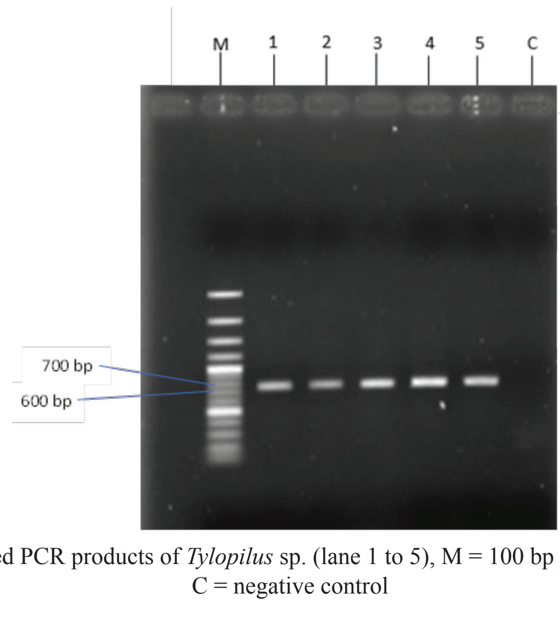

Figure 4: Purified PCR products of *Tylopilus* sp. (lane 1 to 5), M = 100 bp plus DNA marker, C = negative control

Nutrient and Antioxidant Analyses

Moisture Content

The moisture content of mushroom samples was determined using the method described by Liu *et al.* (2016) with some modifications. Samples collected were weighed and dried in an oven (Memmert UN110, Germany) at 105°C overnight until constant weight was obtained. The dried sample was weighed and the percentage of moisture was determined using the following formula:

$$\text{Percentage of moisture} = \frac{\text{The weight of (wet sample} - \text{dry sample) (g)}}{\text{The weight of original wet sample (g)}} \times 100\%$$

Ash Content

The ash content of the sample was obtained through the burning process. About 10 g of dried sample-filled crucibles were placed into a furnace overnight at 500°C before being placed in a desiccator to cool down (Ouzouni *et al.*, 2009). The remaining ash was weighed and its percentage was determined using the following formula:

$$\text{Percentage of ash (\%)} = \frac{Weight\ of\ ash\ (g)}{Weight\ of\ original\ sample\ (g)} \times 100\%$$

Crude Fat

The crude fat content of the Gelam mushroom was determined using Soxhlet's extraction method following AOAC protocol (1995) with slight modification based on Ouzouni *et al.* (2009). About 15 g of dried samples were cut into small pieces. Cheesecloth was used to wrap the cut sample before being inserted into the extraction thimble to prevent the sample from floating during extraction. The petroleum ether in the round bottom flask was heated to reflux, where its vapour flooded into the chamber. The process lasted about four hours until the solvent's colour turned colourless to light yellow. The concentrated petroleum ether was collected and evaporated using a rotary evaporator (Buchi R-100, Switzerland). The filtrate was transferred to a vial and concentrated again by using a rotary evaporator before the final crude weight was recorded. The percentage of crude fat was calculated using the formula below:

$$\text{Percentage of crude fat (\%)} = \frac{Weight\ of\ crude\ fat\ (g)}{Weight\ of\ sample\ used\ (g)} \times 100\%$$

Crude Fibre

The crude fibre content was determined using the Gerhardt Fibre Bag System, FB6, Gerhardt. The process was carried out according to its protocol. Firstly, an empty fibre bag was dried in an oven overnight. The cooled, dried, empty fibre bag was weighed (m1). About 1 g of the sample was placed into a fibre bag and weighed again (m2). Glass spacer was inserted into the fibre bag and the carousel. The sample was boiled in 360 ml of 0.13M sulphuric acid (H_2SO_4) (Merck, Germany) for 30 minutes. Acid removal was done by rinsing the sample with hot distilled water. The sample was then boiled in 0.31M of sodium hydroxide (NaOH) for 30 minutes. A similar procedure was repeated to remove sodium hydroxide by rinsing the sample with hot distilled water. Then, the fibre bag with an empty crucible was dried in the oven for 4 hours and cooled in the desiccator before being weighed (m3). The fibre bag was placed into the furnace overnight at 550°C. The crucible containing ash was cooled down in a desiccator and weighed (m4). The same procedures were repeated using an empty fibre bag to obtain the blank value (m5). The following formula was used to calculate the percentage of crude fibre.

$$\text{Percentage of crude fat (\%)} = \frac{(m3-m4)}{(m2-m1)} \times 100\%$$

m1 = empty fibre bag
m2 = fibre bag + sample
m3 = crucible +sample
m4 = blank

Crude Protein

The crude protein content of the Gelam mushroom was determined by applying the Kjeldahl method (AOAC 1995), using the Kjeltec^{TM} System consisting of a Digestor DS6 and a Kjeltec 2100 Distilling Unit. This analysis comprises three parts: Digestion, distillation, and titration. Firstly, a 1.0 g of powdered sample was placed into the digestion tube using filter paper. A catalyst tablet, Kjeltabs Cu-3.5 was added to each digestion tube. Accurately, 12 ml of concentrated H_2SO_4 (Merck Germany) was added to each digestion tube. All the digestion tubes were placed on pre-heated Digestor DS6 and the exhaust system was connected to every digestion tube. The digestion process was carried out for about an hour until the mixture turned into a light green solution. The digestion tubes were removed from the digestor and placed in a fume hood to cool down for about 10 minutes. Then, 75 ml of distilled water was added into the digestion tubes, followed by 50 ml of 40% NaOH. Prior to the distillation process, about 25 ml of 4% boric acid was added into the clean conical flask, together with five drops of Bromocresol green (indicator dye) were added into each conical flask. The receiver (conical) flask was placed into the distillation unit, Kjeltec 2100, followed by a digestion tube. This process was repeated with different digestion tubes and conical flasks. The receiver solution was titrated with 0.1M hydrochloric acid (HCl) until the green solution turned light grey. The volume of HCl required to change the colour of the receiver solution was recorded. Blank was prepared by following the same procedures as stated above. The following formula was used to calculate the percentage of protein:

$$\text{Percentage of nitrogen (\%)} = \frac{\textit{Vol. of}\ \text{HCl used (sample}-\text{blank) x N x 14.007}}{\textit{Sample weight (mg)}} \times 100\%$$

% of Protein = % of Nitrogen x 4.38 (Protein factor), where N = 0.1 (Normality of acid used)

DPPH Oxidation Assay

This assay was performed according to Clarke *et al.* (2013) with some modifications. A total of 20 μL of sample extract (10 mg/ml) was diluted in dimethyl sulfoxide (DMSO) entirely and mixed with 200 μL of DPPH in methanol in 96-well plates, where two-fold dilution was carried out. The plates were placed in the dark for 30 minutes. The absorbance of the solution was measured using an ELISA plate reader (BioTek800, Agilent, USA) at 517 nm. Lower absorbance of the mixture indicates higher radical scavenging activity. DMSO was used as control and quercetin was used as standard. The analysis was done in triplicate. The Radical Scavenging Ability (RSA) of the sample was calculated following the equation as shown below:

$$\text{RSA (\%)} = \frac{\textit{Absorbance (Control}-\textit{Sample)}}{\textit{Absorbance of control}} \times 100\%$$

Results and Discussion

Macro and Microscopic Features of the Gelam Mushroom

Morphologically, the pileus diameter measured ranged from 3.0 cm to 10.0 cm in width and 1.0 cm to 2.5 cm in thickness. It was convex to plane in shape [Figure 5 (a) and (b)] with a decurved-plane margin [Figure 5 (c) and (d)]. The pileus was grey to violet-white when young [Figure 6 (a)] and turned to brown or a close to reddish-orange colour with age [Figure 6 (b)]. Lau *et al.* (2017) documented the changing of pileus colour with age from light violet or violet-white to dull lilac, which differs from this present study. Meanwhile, Seehanan and Petcharat (2008), Horak (2011), Aung-aud-chariya *et al.* (2012), and Lau *et al.* (2017) also recorded similar observations on the shape of the pileus of the mushroom to this study with convex or plane shape while colour-ranged from grey to light purple, whitish violet. Some pileus cuticles appeared slightly covered with fine powder and pulverulent while others were smooth without scales. In addition, the surface was dry but turned slightly viscid when wet.

Figure 5: Morphological characteristics of Gelam mushroom's pileus, convex pileus (a), plane pileus (b), decurved margin (c), and plane margin (d)

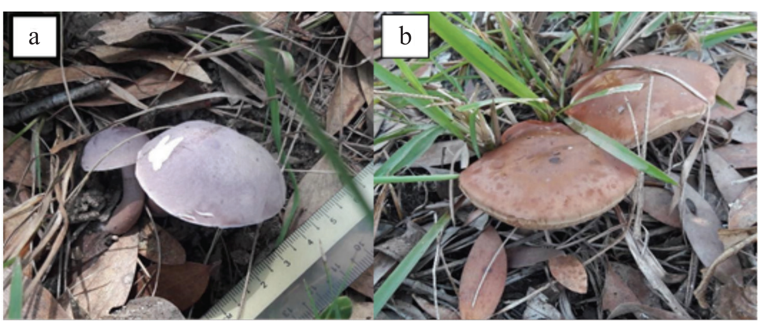

Figure 6: Young (a) and mature (b) fruiting bodies with different colours

The context in the centre of the mushroom stipe is white and spongy when cut into halves, similar to Horak (2011), who recorded a white context with a spongy texture in pileus and tough in the stipe.

Meanwhile, the hymenophore was composed of an adnexed arrangement of tubes [Figure 7 (a)], which was easily detached since it was soft. The pore surface was moist, mostly tubular pores, slightly elongated near the stipe [Figure 7 (b)]. The adnexed tube arrangement is similar to the description by Lau *et al.* (2017). However, sinuate notched tubes were not observed in this study. The colour of the hymenophore surface was white when young and later turned pale brown with age [Figure 7 (c)].

For the stipe, the length measured ranged from 3 cm to 6.5 cm, with a width of 0.5 cm to 1.0 cm. The stipe was attached to the centre of the pileus. Two different shapes of stipe were observed, namely clavate, slightly enlarged at the base area [Figure 8 (a)] and cylindrical, straight, and equal [Figure 8 (b)]. These descriptions are consistent with the observations by Seehanan and Petcharat (2008) while Horak (2011) recorded only sub-clavate stipes and Lau *et al.* (2017) documented straight and equal stipes, as well as flexuous and clavate stipes. The surface of the stipe was glabrous, dry, and with a brown net reticulated pattern [Figure 8 (c)] comparable to Horak (2011), but sometimes recorded as a white apex with a purplish tinge. The stipe colour ranged from light brown to dark brown or slightly purplish brown with a white rhizomorph at the base of the stipe [Figure 8 (d)]. However, Lau *et al.* (2017) recorded the stipe, which is concolourous with the pileus and paler at the upper part of the stipe.

Microscopically, only spores and basidia of *Tylopilus* sp. were observed. Spores were pale brown to olive-brown with an ellipsoid shape with a smooth surface with a length of 7µm to 8µm. The basidia observed in the hymenophore area were clavate-shaped, identical to the observation recorded by Lau *et al.* (2017) [Figure 9 (a) and (b)]. The spore and basidiospore shape observed from samples in this study was identical to Lau *et al.* (2017), which is ellipsoid in form and olive-brown in colour. Overall, the morphology of *Tylopilus* sp. observed in this study was close

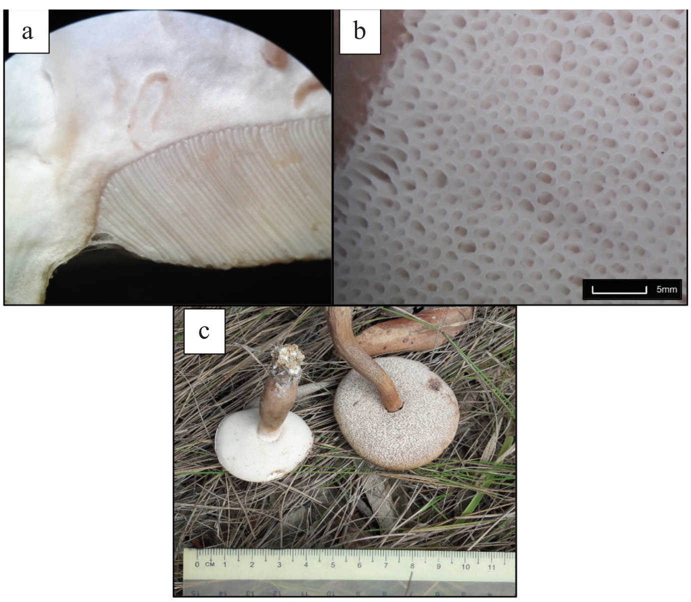

Figure 7: Morphological characteristics of Gelam mushroom's hymenophore, adnexed arrangement of the tube at the hymenium (a), pores surface (b), and hymenophore surface colour (c)

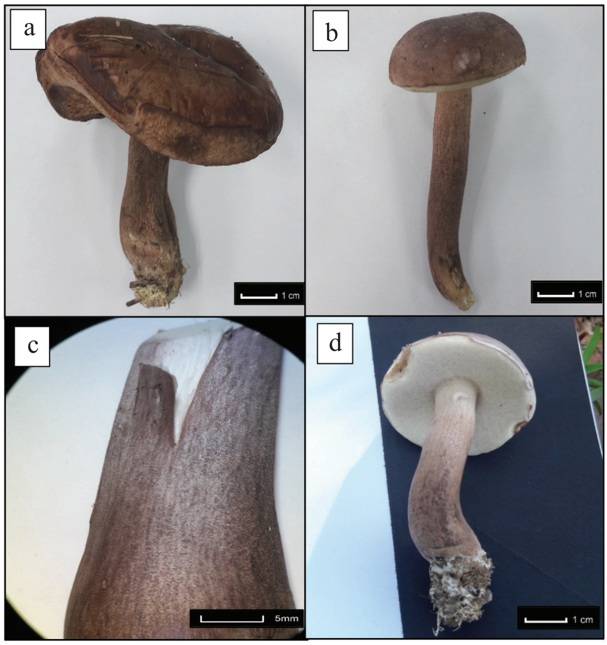

Figure 8: Morphological characteristics of Gelam mushroom's stipe, clavate stipe (a), cylindrical stipe (b), the reticulated pattern on the surface (c), white apex on top, and white rhizomorph at the base (d)

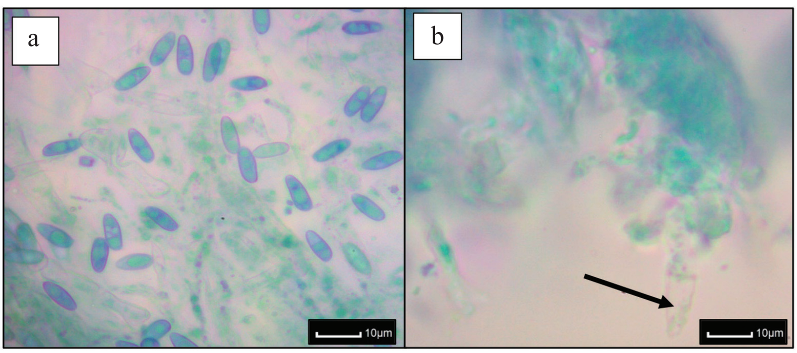

Figure 9: Micromorphology of Gelam mushroom spores (×1000 magnification) (a) and basidium, pointed by an arrow (×1000 magnification) (b)

to Seehanan and Petcharat (2008) and Lau *et al.* (2017). Scent-wise, it has a woody scent similar to Shiitake mushrooms when freshly picked but produces a rotten smell when mature and tastes bitter when cooked (boiled).

Molecular Analysis

Based on the results, the samples' sequences displayed 97% to 100% similarity to *Tylopilus griseipurpureus* (Corner) E. Horak (previous name: *Boletus griseipurpureus* Corner) strains from Bachok, Kelantan (KF442409.1) and Nakhon Si Thammarat, Thailand (JQ726594.1). The Neighbour-Joining (NJ) tree generated two main clades, with 12 samples grouped into the clade with the reference strains while the other eight samples were grouped in the sister clade. Both clades were grouped with 91% and 99% bootstrap values. Figure 10 shows a summary of the NJ tree.

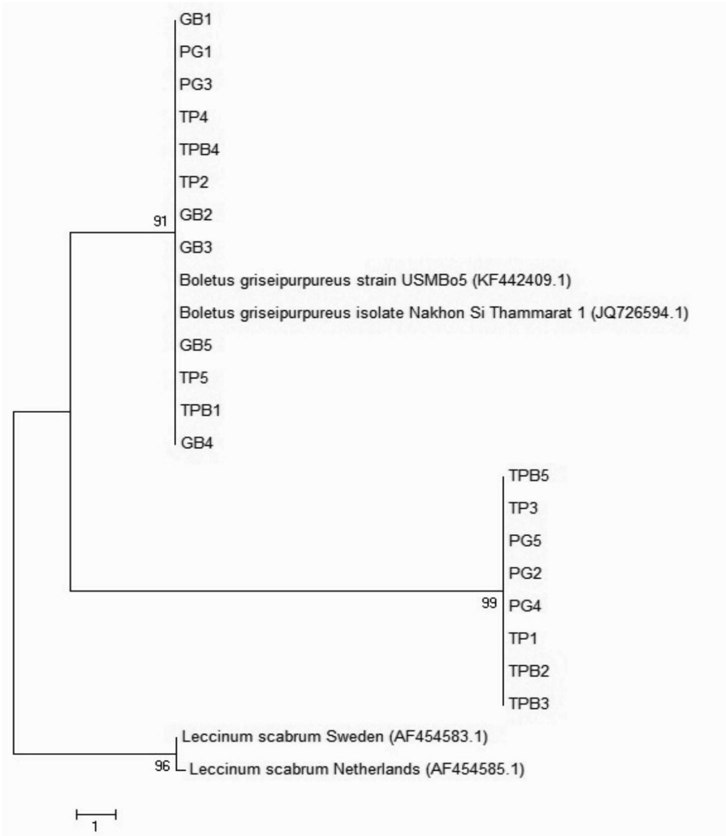

Figure 10: Phylogenetic tree *of Tylopilus griseipurpureus*, with *Leccinum scabrum* as the outgroup

Samples from four sampling locations; Gong Batu (GB), Pengkalan Gelap (PG), Telaga Papan (TP), and Kampung Telaga Papan Baru (TPB) were grouped within the same clade, suggesting potential spore dispersal among these sites. *Tylopilus griseipurpureus*, which is a basidiomycete and grouped as an ectomycorrhizal fungus is known to possess a specialised mechanism for generating dispersive airflow, where localised air currents beneath the pileus facilitate spore dispersal (Dressaire *et al*., 2016). This mechanism is consistent with the findings of the present study, which identified the proximity of the sampling sites as approximately 2.2 km between GB and PG and 3.8 km between TP and TPB as a strong indicator of potential spore dispersal. These distances suggest that airborne spore transport could play a significant role in the colonisation of *T. griseipurpureus* across these locations.

The observed clustering of samples within the same clade further supports that *T. griseipurpureus* spores were dispersed throughout the Setiu districts. This is consistent with the findings of Golan and Pringle (2017), who emphasised the influence of environmental factors such as distance, on the dispersal, and colonisation patterns of ectomycorrhizal fungi. Their research suggests that short-distance dispersal can aid in the successful establishment of fungal colonies, particularly for species with small spore sizes. The small spore size of *T. griseipurpureus* recorded in the present study (7-8 µm) likely contributes to its ability to disperse across these relatively short distances.

Additionally, the dispersal dynamics of ectomycorrhizal fungi have been extensively documented by Peay *et al.* (2012), who emphasised that spores can colonise new habitats even when separated by moderate distances. Their findings align with the present study's observations, where the proximity of the sampling sites further supports the idea that spore transport, possibly via air currents or other mechanisms could have contributed to the movement of *T. griseipurpureus* spores across Setiu district. Together, these studies reinforce the hypothesis that environmental conditions, the fungus's dispersal mechanisms, and small spore size played a crucial role in the successful dispersal and colonisation of *T. griseipurpureus* spores across the sampled locations.

On the contrary, the distance from GB and PG to TP and TPB is roughly 30 km for the other locations. Locals indicate that mushroom collectors often frequented these sites during the fruiting season. It was established that humans act as significant vectors in fungal dispersal. Therefore, this implies that mushroom collectors in these study areas may have inadvertently contributed to spreading spores (Golan & Pringle, 2017).

Although the similarity percentage for most samples reached 99%, there is a genetic distance between the strains of the sister clade. Still, the similarity of all recorded strains was relatively high (97%-100%). Both strains from Nakhon Si Thammarat, Thailand and Kelantan, Malaysia are high in similarity despite the considerable geographical distance, indicating that the samples of this study, which are geographically distant yet high in similarity, could be of different strains or multiple clades (Aung-aud-chariya *et al*., 2015).

However, the small number of DNA isolates for *Tylopilus* and the lack of information on the group's limit (Gelardi *et al*., 2015) do not allow more information to be gleaned from the current study. *Tylopilus griseipurpureus* (Corner) E. Horak is an ectomycorrhizal fungus with a limited conducive habitat to grow. In Thailand, *T. griseipurpureus* was associated with *Melaleuca leucadendron* and *Acacia mangium* (Aung-aud-chariya *et al*., 2012) while in Kelantan, Malaysia, it is present in peat swamp forests, which are dominated by *Melaleuca cajuputi* (Lau *et al*., 2017). Similar to Kelantan, the district of Setiu in Terengganu has a wetland area of mangroves with seasonal freshwater swamps dominated by *Melaleuca cajuputi* (Gelam) trees and coastal forests. Therefore, Setiu could be one of the sites favouring the growth of *T. griseipurpureus* (Corner) E. Horak in Malaysia.

However, the coastal ecosystem of Terengganu is currently facing multiple threats, including frequent occurrences of fires, especially during drought or non-monsoon months, illegal sand mining within the bris ecosystem, which disrupts natural vegetation such as *Melaleuca cajuputi* found on the dry parts (ridges) of BRIS soil ecosystems along the Terengganu coast and the issue of illegal dumping exacerbating environmental degradation (Salim *et al*., 2018).

However, considering these challenges, it is crucial to underscore the importance of conservation efforts to preserve the delicate balance of coastal ecosystems, particularly for the sustainability of the fungus reliant on its plant host to survive. By advancing scientific research, implementing sustainable management practices, and fostering public awareness, we can mitigate threats and ensure the long-term viability of the coastal environment and the fungal species dependent upon it for their growth and survival.

Proximate Analysis

The proximate composition of *Tylopilus griseipurpureus* is shown in Table 1. Based on the results, the nutrient components of *T. griseipurpureus* were arranged in the order of moisture (85.8%), crude protein (18.52%), crude fibre (11.30%), ash (10.85%), and crude fat (6.45%). Generally, all the percentages reported in this study were in the standard range of mushrooms' proximate composition documented by Wang *et al.* (2014). Thus, the results revealed that *T. griseipurpureus* could be a good source of protein and fibre on a dry weight basis, similar to other edible mushrooms (Amabye & Bezabh, 2015; Mocan *et al*., 2018).

The moisture content of *T. griseipurpureus* investigated was reported to be 85.8%, which

Table 1: The proximate composition of *Tylopilus griseipurpureus*

	Moisture	Proximate Composition[1] (%)			
		Ash	Crude Protein	Crude Fibre	Crude Fat
Tylopilus griseipurpureus	85.80 ± 0.45	10.85 ± 0.03	18.52 ± 0.12	11.30 ± 0.81	6.45 ± 1.92

[1]Each value is expressed as mean ± SD (n = 3).
Percentage of proximate composition on a dry weight basis.

aligns with the other studies that recorded higher moisture content ranging from 80% to 90% of the mushroom's fresh weight (Kalaĉ, 2009; Liu *et al*., 2012; Valverde *et al*., 2015). *Tylopilus griseipurpureus* showed higher moisture content than the wild edible mushrooms in Sabah, Malaysia, with a range of 58.2% to 84.75% (Chye *et al*., 2008). The difference in the moisture content of mushrooms is expected as it may be attributed to factors such as the temperature of the environment and relative humidity throughout the growth, harvest, and storage of mushrooms (Ouzouni *et al*., 2009; Guillamón *et al*., 2010). Fruiting bodies of fresh mushrooms have a short shelf life due to high moisture content. Therefore, a proper process is required for longer storage time (Guillamón *et al*., 2010).

Crude protein had the highest content (18.52%) among the other components on the dry weight basis (Table 1). This is consistent with the findings of Hung and Nhi (2012), who reported the range of protein from 7.2% to 36.5%. Meanwhile, wild edible mushrooms such as *Boletus edulis* (5.22%) and *Auricularia auricula-judae* (8.36%) have lower protein content (Gyar & Owaku, 2011; Afiukwa *et al*., 2015).

However, the crude protein level observed in this study is lower than wild *Pleurotus ostreatus* (32.8%), reported by Akyüz and Kirbağ (2010) and *Lentinus tuberregium* (25.0%) by Manjunathan and Kaviyarasan (2011). Another species of *Tylopilus*, *T. balloui* in Brazil was found to have a higher amount of protein (27.0%) than *T. griseipurpureus* (Lima *et al*., 2016).

In Malaysia, the authors reported the crude protein value of wild edible mushrooms to range from 1.24% - 14.95% (Chye *et al*., 2008), slightly lower than *T. griseipurpureus*. The crude protein content varies depending on the type or species of mushrooms, the development stage, and the part of the sample used (Hung & Nhi, 2012). Meanwhile, the substrate composition, pileus size, and time of harvesting could be the reason for the differences in protein level (Bernaś *et al*., 2006; Mshandete & Cuff, 2007).

Crude fibre is one of the essential components in edible mushrooms. *Tylopilus griseipurpureus* contained 11.3% crude fibre, the second highest on a dry weight basis (Table 1). This value is close to 10.84% in the *Schizophyllum commune*, as Chye *et al.* (2008) reported. A study by Okoro and Achuba (2012) showed several wild edible mushrooms with a lower value of crude fibre ranging from 3.24% to 8.7%, namely from *Lentinus squarrosulus*, *Volvariella volvacea*, *Coprinus micaceus*, *Lepiota procera*, and *Auricularia auricula*. Chong *et al.* (2007) revealed a higher range of fibre content (4.2% - 23.50%) in edible mushrooms in Sabah, Malaysia. As a potential source of dietary fibre, the cell wall of fungus contains chitin, mannans, other hemicelluloses, and beta-glucans, an interesting functional component that can reduce cholesterol levels and glycemic response (Chye *et al*., 2008).

In this study, *T. griseipurpureus* yielded a reasonable ash content (10.85%), which indicated an abundant mineral constituent. According to Ouzouni *et al.* (2009), the main components in the ash of mushrooms are potassium (K) and phosphorus (P). The ash content obtained in this present study is comparable with *Volvariella volvacea* (10.37%) and *Lentinus connatus* (10.14%) (Johnsy *et al*., 2011; Afiukwa *et al*., 2015). Crude fat level is the lowest among the proximate composition. The value of crude fat in *T. griseipurpureus* (6.45%) was higher than in wild mushrooms obtained by Johnsy *et al.* (2011), which ranged from 1.17% to 2.58%. Wang *et al.* (2014) reported that the crude fat content of mushrooms in China ranged from 1.0% to 6.7%, which is in agreement with the findings of this study. Although a fatty acid profile was not obtained in this study, it was proven that most edible mushrooms contain a polyunsaturated fatty acid that may promote cholesterol reduction (Valverde *et al*., 2015).

The nutritional values of the identical species, *T. griseipurpureus* were documented in Trang, Songkhla, Nakhon Si Thammarat and Ranong provinces in Thailand (Aung-aud-chariya *et al*., 2012; Sudjaroen & Thongkao, 2017). In the studies, the mushroom species reported was *Boletus griseipurpureus*. However, the name of this species has been updated from *Boletus griseipurpureus* to *Tylopilus griseipurpureus*. Meanwhile, Yuswan *et al.* (2017) documented the nutrients of Gelam mushrooms that were only identified as *Boletus* sp. in Bachok, Kelantan. Overall, the Gelam mushroom in their study was revealed to be high in protein and low in fat, as Aung-aud-chariya *et al.* (2012) recorded 31.4% of crude protein while Sudjaroen and Thongkao (2017) and Yuswan *et al.* (2017) recorded 4.28 g/100 g and 4.53 g/100 g, respectively.

Besides the type of mushroom, the availability of nitrogen and habitat may affect the crude protein level in mushrooms (Yuswan *et al*., 2017). The crude fat content of Gelam mushroom reported in Kelantan was 0.13 g/100 g (Yuswan *et al*., 2017) while in Thailand, it was 0.14 g/100 g (Sudjaroen & Thongkao, 2017) and 0.9% (Aung-aud-chariya *et al*., 2012). The crude fat content determined in this present study was higher than that of publications in Kelantan and several provinces in Thailand.

It has been proven that the fat found in mushrooms is dominated by unsaturated fatty acids (Wani *et al*., 2010), mainly oleic acid and linoleic acid, which are essential fatty acids for the human body (Pedneault *et al*., 2006; Kavishree *et al*., 2008; Valverde *et al*., 2015). The proximate composition of mushrooms varies among species and within the species itself, as the environment and maturity of the fruiting body can play a role. Therefore, the results suggested that *T. griseipurpureus* could be a healthy and nutritious food for locals who consumed it.

DPPH Free Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was used to assess the antioxidant activity of the sample. It is a rapid method widely and commonly used to characterise the antioxidant capacity of sample extract against oxidation due to free radicals (Mujić *et al*., 2010; Keleş Armed *et al*., 2011; Vamanu & Nita, 2013).

DPPH radical is a stable free radical that can accept an electron or hydrogen radical to form a stable diamagnetic molecule (Elmastas *et al*., 2007). The freshly prepared methanolic DPPH solution exhibits a deep purple colour; discolouration occurs when it fades from purple to yellow, indicating that an antioxidant is present in the medium (Elmastas *et al*., 2007; Ferreira *et al*., 2007; Woldegiorgis *et al*., 2014). The reduction capability of DPPH radicals was determined by the decrease in their absorbance at 517 nm. Thus, the faster the absorbance decreases, the higher the antioxidant activity owned by sample extracts.

The DPPH free Radical Scavenging Activity (RSA) of *T. griseipurpureus* was observed to be the highest (81.4%) at the highest concentration of sample extract, 10 mg/ml (Table 2). Figure 11 shows an increase in the RSA percentage with concentration. A similar pattern was recorded in other studies on DPPH assay (Elmastas *et al*., 2007; Wong & Chye, 2009; Woldegiorgis *et al*., 2014). Some medicinal mushrooms' RSA (%) was 67% in *Agaricus blazei* and 74% in *Ganoderma lucidum* in Korea (Kim *et al*., 2008).

Table 2: Radical Scavenging Activity (RSA) of *T. griseipurpureus* extract at different concentrations

Concentration (mg/ml)	0.16	0.31	0.63	1.25	2.5	5	10
RSA (%)	5.10 ± 0.92	6.7 ± 2.61	8.8 ± 1.57	16.3 ± 2.39	29.0 ± 1.94	56.3 ± 0.42	81.4 ± 0.91

Each value is expressed as mean ± SD (n = 3).

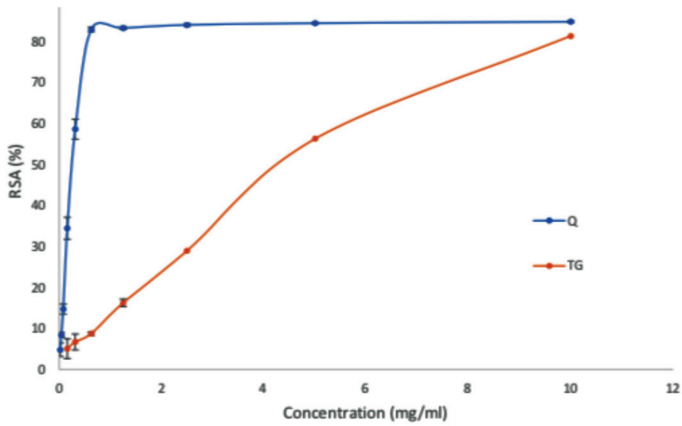

Figure 11: Percentage of DPPH free radical scavenging activity of the sample extract

In southern Vietnam, the RSA value of *Volvariella volvacea* was 82.9%, which is slightly higher than the present study (Hung & Nhi, 2012). A lower percentage of RSAs were documented from some tropical wild mushrooms, *Xerula furfuracea* (65.41%), and *Pleurotus* spp. (39.18%) (Wong & Chye, 2009). Meanwhile, several cultivated mushrooms in Malaysia had a percentage of RSA ranging from 15.09% in the *Schizophyllum commune* to 77.62% in *Agaricus bisporus* (Muhammad Ezzudin *et al.,* 2019).

The effective concentration for a sample to scavenge 50% (EC50) of radicals was calculated to determine the effectiveness of the sample extract in reacting with free radicals. Hence, a lower EC50 value indicates that the mushroom has a high amount of antioxidant activity. In this study, the value of EC50 of positive control, quercetin, was approximately 0.33 mg/ml. However, the sample extracts were less effective than the positive controls. The EC50 values in scavenging ability on DPPH radicals were 5.44 mg/ml in the methanolic extract of *T. griseipurpureus*. Higher EC50 values were observed in other studies, where Wong *et al.* (2013) reported the EC50 value of several commercialised mushrooms with a range from 31.58 mg/ml to 109.30 mg/ml while Carneiro *et al.* (2012) showed *Agaricus blazei* and *Lentinus edodes* with EC50 values of 6.77 mg/ml and 26.32 mg/ml, respectively.

Compared to previous studies on the same species, approximately 1.0 mg/ml of EC50 value was determined by Yuswan *et al.* (2015) from both methanolic and water extracts of *B. griseipurpureus* in Kelantan. However, a higher EC50 was reported from methanolic extract at 3.98 mg/ml, cold water extract at 1.97 mg/ml, and hot water extract at 1.79 mg/ml by Muniandy *et al.* (2016), with the RSA (%) ranging from 60.67% to 82.28%.

Meanwhile, in Thailand, the antioxidant activity of *B. griseipurpureus* determined by DPPH radical scavenging activity was 77.95 µg vitamin C/g sample (Angajchaariya *et al.*, 2017) and 136.5 µmol TEAC (Sudjaroen & Thongkao, 2017). There were differences in the antioxidant capacity of similar species, where environmental factors could be one of the elements affecting antioxidant properties. According to Hakkim *et al.* (2008), factors such as mean temperature, soil, wind speed, and UV radiation from sunlight influence the phenolic contents, which is in a linear correlation with radical scavenging activity. Thus, further studies need to be carried out to identify and characterise the antioxidant compound of this mushroom species.

This study documented the proximate composition consisting of moisture, ash, crude protein, crude fibre, and crude fat of *T. griseipurpureus*, as well as its antioxidant capacity. This mushroom species contained a significant amount of protein and fibre, low in fat content, and possessed antioxidant properties. Further studies should determine the other nutritional aspects and compounds responsible for the antioxidant activity. It is essential to understand the nutritional values of the Gelam mushrooms as they are one of the wild edible mushrooms collected and consumed by the locals in Terengganu.

Conclusions

This study successfully identified the Gelam mushroom from Setiu, Terengganu as *Tylopilus griseipurpureus* (previously known as *Boletus griseipurpureus)*, with genetic similarities to strains from Malaysia and Thailand. Phylogenetic analysis suggests potential spore dispersal across regions, indicating the presence of multiple clades or strains.

The mushroom demonstrated significant nutritional value, particularly in crude protein and fibre content, along with potent antioxidant properties. These findings enhance the understanding of the species' taxonomy and nutritional potential, paving the way for future research. Further studies should explore a broader molecular analysis to clarify strain diversity, investigate the bioactive compounds responsible for their antioxidant effects, and assess their ecological significance. Additionally, research into sustainable cultivation and commercial viability could unlock its potential as a valuable nutritional and medicinal resource.

Acknowledgements

We want to acknowledge the Malaysian Ministry of Education for Fundamental Research Grant Scheme (FRGS/1/2016/WAB13/UMT/02/2) and the facilities provided by Universiti Malaysia Terengganu for this study. Also, this study was only possible with the cooperation of local communities in Setiu and Besut. Therefore, we are dedicating this article to them as our token of appreciation, hoping the information may help ensure their livelihood. This transdisciplinary research is part of a thesis, which was submitted as partial fulfilment to meet the requirements for the degree of Master of Science at Universiti Malaysia Terengganu.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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