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Nutritional and Molecular Analysis of Wild Edible Gelam Mushroom (*Boletus* sp.) from Kelantan, Malaysia

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Authors' contributions

This work was carried out in collaboration between all authors. Authors WHL and FD designed the study. Authors MHY and YJT wrote the protocol and performed the experiments. Author JXS performed the statistical analysis. Authors ZZ and YYO wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To conduct nutritional and molecular analysis of Gelam mushroom which is believed to have contained medicinal properties.
Place and Duration of Study: School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, between 2012 and 2014.

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Methodology: The fruiting body of Gelam mushroom was collected from Bachok, Kelantan. Proximate composition and mineral content of the fruiting body of this mushroom was analyzed according to the guidelines of Association of Official Analytical Chemists and Alam et al. respectively. Fungal specific primers pairs ITS1-F and ITS4 were used in molecular analysis.
Results: Every 100 g of fruiting body containing 0.13 g fat, 4.53 g protein, 3.13 g fiber, 0.87 g ash, 5.1 g carbohydrate, 4.4 mg Ca, 297.13 mg K, 7.27 mg Mg and 24.4 mg Na. Molecular analysis using internal transcribed spacer (ITS) region of nuclear ribosomal deoxyribonucleic acid (DNA) on Gelam mushrooms collected from the state of Kelantan, Malaysia were identified to be from *Boletus* genus.
Conclusion: Present study demonstrated that Gelam mushroom could be a new *Boletus* species and is a potential source of food rich in minerals.

Keywords: Gelam mushroom; proximate analysis; minerals content; molecular analysis; Boletus sp.

1. INTRODUCTION

In recent times, mushrooms have dwelled as a greater importance in diet for human beings because they contain high nutritional values such as proteins, carbohydrates and fibers. In addition to containing essential components for metabolism in human being, they are low in fat and calories [1-4]. Furthermore, mushroom represents a rich source of bioactive compounds mostly belonging to several chemical groups, very often they are polysaccharides or triterpenes and these compounds usually present anti-microbial, anti-viral, anti-tumor and anti-allergic activities [5].

In addition to macro and micro nutrients, mushrooms also contain essential minerals such as calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) andiron (Fe) which play a crucial role in biological system [6]. For example, (i) Mg as co-factor for various enzymes involved in protein synthesis and energy metabolism; (ii) Fe acts as oxygen carrier in the red blood cell haemoglobin; (iii) K plays important role in relieving anxiety and stress as well as enhancing muscle strength and metabolism; (iv) Na an important electrolyte present in the extracellular fluid (ECF) essential for enzyme operation and muscle contraction and finally; (v) Ca important for strong bones and teeth [7].

According to Reis et al., more than 2,000 species of mushrooms exist in nature, but only around 25 species are widely accepted as food and commercially cultivated [8]. The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Lentinus edodes*, *Pleurotus* spp. and *Flammulina velutipes*. Lee et al. [9] reported the aborigines in West Costand Central of Peninsular Malaysia are utilising 31 species of wild edible mushrooms for personal consumption and/or for economic purposes, and 14 species from the total reported have been used as traditional medicines. However, there is scanty information on wild mushroom in East Coast of Peninsular Malaysia.

Therefore, present study is to investigate wild Gelam mushroom, a type of mushroom belonging to *Boletus* genus and is only available at coastal area of Bachok, Kelantan [10], where locals utilize the mushroom as both a food source and traditional medicine. Due to the growing interest for mushrooms as dietary source for human consumption and scanty information on this mushroom, this study was initiated to determine the nutritional value of this mushroom through proximate analysis; and to identify the genus and species of wild Gelam mushroom through molecular analysis.

2. MATERIALS AND METHODS

2.1 Mushroom Materials

The matured fruiting body of Gelam mushroom was collected from Bachok, Kelantan in November, 2012. The fruiting body was washed several times, blotted dried, lyophilized and grounded using blender into fine powder. The powder was stored at -20℃ until further analysis.

2.2 Proximate Analysis

The proximate analysis of Gelam mushroom for protein, fat, carbohydrates, moisture and ash content were carried out according to the Association of Official Analytical Chemists [11].

2.2.1 Crude protein

The crude protein was determined by using Kjeldahl method. 1 g of sample was placed in the

Kjeldhal flask, followed by addition of 10 g of potassium sulfate (K_2SO_4), 0.7 g of mercury (II) oxide (HgO) and 20 ml of sulfuric acid (H_2SO_4). The flask is then heated for 30 minutes and cooled down by gradually adding 90 ml of de-ionized water. Then, 25 ml of sodium sulfate (Na_2SO_4) was added and stirred followed by addition of one glass bead and 80 ml of sodium hydroxide (NaOH). After addition, two layers of solution are formed and the flask is then connected to the distillation unit. 50 ml of distillate is collected in the flask containing 50 ml of indicator solution and then the distillate was titrated with hydrochloric acid (HCl) solution. The nitrogen in sample (%) was calculated based on the formula:

$$100[\frac{AxB}{C}\ 0.014]$$

Where

A = HCl acid used in titration (ml)
B = normality of standard acid
C = weight of sample

Then, the crude protein (%) was obtained by using the nitrogen in sample (%) x 6.25

2.2.2 Crude fat

The crude fat was determined by using Soxhlet method. 3-5 g of sample were weighed in extraction thimble, and then was connected with the flask containing petroleum ether with 2/3 of total volume of flask. The extraction process was carried out by boiling the flask for 6 hours and the ether was evaporated in a rotoevaporator. The flask is cooled, dried and weighed. The crude fat was calculated with the formula:

$$100\ \frac{B-A}{C}$$

Where

A = weight of clean dry flask (g)
B = weight of flask with fat (g)
C = weight of sample (g)

2.2.3 Moisture content

The initial weight of mushrooms was weighed and recorded. Then, the mushrooms were dried in an air oven with 105 ± 2℃. After that, the moisture content was calculated by subtracting initial weight with constant dry weight.

2.2.4 Ash content

2.5 – 5 g of sample were placed in a crucible. The crucible was placed in a furnace at 600 ± 15℃ for 12 hours. It is then cool down and transferred to the dryer. The crucible together with the ash was weighed carefully.

2.2.5 Energy values

The energy values of each sample were calculated by using the equation:

Energy (kcal) = 4 (g protein + g carbohydrate) + [9 x (g lipid)] [12].

2.2.6 Crude fiber

The crude fiber contents were determined using slightly modified method of Alam et al. [13]. Sample was boiled for 30 minutes with 200 ml of 0.255 N H_2SO_4. Then the mixture was filtered and the residue were washed with hot water. The same procedure was repeated but 0.313 N NaOH was used instead of 0.255 N H_2SO_4. The residue was transferred to a crucible and dried overnight at 80 - 100℃ and weighed (W_e) by using balancer. The crucible was heated again in a muffle furnace at 600℃ for 5 – 6 hours, cooled and weighed again. The crude fiber was calculated based on following equation:

Crude fibre (g / 100 g of sample) = [100 + (moisture + fat)] x (W_e – W_a) / total weight of sample

2.2.7 Carbohydrate content

The carbohydrate content was calculated based on following equation [13]:

Carbohydrate (g / 100 g sample) = 100 – [(moisture + fat + protein + ash + crude fibre) / 100 g]

2.3 Mineral Contents Determination

The mineral contents was determined from total ash content in the sample according to Alam et al. [13]. 2 ml of concentrated nitric acid (HNO_3) were added to the sample and heated for 2 minutes. After that, the solution was transferred into a volumetric flask and one drop of hydrogen peroxide (H_2O_2) was added prior to deionize distilled water to make total volume 50 ml. The contents of calcium (Ca), iron (Fe), magnesium (Mg), potassium (K) and sodium (Na) was

determined by flame and graphite method with atomic absorption spectrophotometer.

2.4 Molecular Analysis

Extraction of DNA from fruiting body powder was conducted using Extract N. Amp. TM Plant kit (SIGMA). Fungal specific primers pairs ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) were used to amplify the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. Amplification parameters used was denaturation at 94℃ for 4 min followed by 35 cycles of 45 s at 94℃, 45 s at 54℃ and 1 min 30 s at 72℃, and a final extension at 72℃ for 2 min. The amplicons were analyzed using 1.0% agarose gel and then were purified. The purified products were sent to Macrogen (South Korea) for sequencing. The obtained sequence was subjected to Basic Local Alignment Search Tool (BLAST) to compare the similar sequence.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

Classification of mushrooms are basically made into four groups: Poisonous mushrooms, medicinal mushrooms, edible mushrooms and magic or hallucinogenic mushrooms [6]. In this context, edible mushrooms are ideal food for consumption and provide both macro and micro nutrients in our diet. Moreover, they are also potential source of carbohydrates, proteins, minerals and fat.

The wild Gelam mushroom was collected at coastal area of Bachok, Kelantan. It has a grayish brown cap, which appeared pale pink when it was young. Its stem surface has almost the same colour with its cap at all life cycles. The photos of Gelam mushroom specimens collected are as Figs. 1-4. In comparison with *Boletus variipes* which is closely related to it, has a young velvety and matured patchy cap, with tan to grayish brown colour, while its stem surface appear brownish.

Present study illustrates the content of moisture, total solids, crude protein, total lipids, available carbohydrates, dietary fiber, total ash, calorie values and minerals (Fe, Ca, Mg, Na and K) of Gelam mushroom (*Boletus* sp.). Generally, mushrooms have high moisture content, occupying approximately 90% of their fresh weight [6]. Proximate compositions of Gelam mushroom are presented in Table 1. Present results showed this mushroom has high moisture content and is slightly similar with *Boletus edulis* [14] but lower to *Boletus aereus* (91.65%) and *Boletus reticulatus* (91.10%) [6]. The high moisture content of mushroom indicates high perishability and promotes susceptibility to microbial growth and enzyme activity [15].

Fig. 1. Wild Gelam mushroom in its natural habitat

Fig. 2. Side view of the wild Gelam mushroom (whole fruiting body)

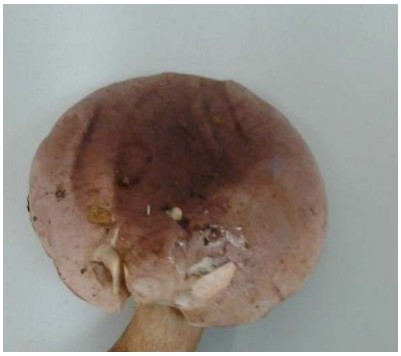

Fig. 3. Cap view of the wild Gelam mushroom (top)

Fig. 4. Cap view of the wild Gelam mushroom (grill)

Table 1. Nutritional values of wild Gelam mushroom (*Boletus* sp.)

Nutritional values	Gelam mushroom (GenBank Accession No.: KT582202)
Moisture (%)	89.5 ± 0.1
Protein (g/100 g)	4.53 ± 0.06
Ash (g/100 g)	0.87 ± 0.06
Fat (g/100 g)	0.13 ±0.06
Carbohydrate (g/100 g)	5.1 ± 0.2
Fiber (g/100 g)	3.13 ± 0.06
Energy (kcal/100 g)	45.67 ± 0.58

Mean ± S.D.

Evidence showed that mushrooms are good source of digestible proteins [6]. In our study, content of crude protein in wild Gelam mushrooms was found to be 4.53 g/100 g and is lower compared to *B. aereus* (17.86 g), *B. edulis* (21.07 g) and *B. reticulatus* (22.57 g) [16]. Variations of protein content in present study compared to other *Boletus* spp. in other countries still remain unclear. However, the type of mushrooms, the part sampled, the stage of development, availability of nitrogen and the habitat affect the protein contents of mushrooms [16].

Additionally, total lipids (fat) content in Gelam mushroom was found to be 0.13 g/100 g which is considered to be a very low. Mushrooms contain no cholesterol, low in fat and mostly all fresh mushrooms do not possess trans fats [6]. Unsaturated fatty acids were found mostly in edible mushrooms, which are less hazardous to the health as compared to saturated fatty acids [6]. Hence, the low content of fat in Gelam mushroom makes it a suitable health food. The second major nutrient component of mushrooms is carbohydrate [6] and current results yielded Gelam mushroom contains 5.1 g/100 g carbohydrate. Subsequently, fiber content in Gelam mushroom is 3.13 g while ash content is 0.87 g/100 g. It is important to note that the energy value obtained from proximate analysis of Gelam mushroom is 45.67 kcal.

Minerals content of Gelam mushroom are presented in Table 2. From the table it can be concluded that Gelam mushroom contains high concentration of potassium (K) which is 297.13 mg/100 g followed by sodium (Na), 24.4 mg/100 g, magnesium (Mg), 4.4 mg/100 g and calcium (Ca), 4.4 mg)/100 g. Present results yielded Gelam mushroom species are rich in K and are very low in Fe content.

Table 2. Mineral content of wild Gelam mushroom (*Boletus* sp.)

Mineral content (mg/100 g)	Gelam mushroom (GenBank Accession No.: KT582202)
Macro-mineral	
Calcium (Ca)	4.4 ± 0.10
Magnesium (Mg)	7.27 ± 0.15
Potassium (K)	297.13 ± 2.47
Sodium (Na)	24.4 ± 0.50
Micro-mineral	
Iron (Fe)	0.37 ± 0.06

Mean ± S.D

In addition, current results yielded this mushroom contains lower amount of Ca, K and Na compared to *B. edulis* [17]. However it has higher mineral content compared to some cultivated mushrooms like *A. bisporus*, *L. edodes*, and *P. ostreatus* [15]. The differences in the mineral contents among the mushrooms species might be due to the environmental factor such as organic matter, pH and habitat of the mushroom itself [18].

3.2 Molecular Analysis

Deoxyribonucleic acid (DNA) sequence of the polymerase chain reaction (PCR) products of Gelam mushroom (GenBank Accession No.: KT582202) was analyzed and it yielded 91% similarity to *Boletus griseipurpureus* (GenBank Accession No.: JQ726594.1), *Gyrodontium* sp. (GenBank Accession No.: KR349658.1), *Omphalina* sp. (GenBank Accession No.: KR155038.1), uncultured *Piloderma* sp. clone (GenBank Accession No.: KM008624.1), and uncultured *Agaricales* sp. clone (GenBank Accession No.: FJ475680.1). Present results

could not identify the exact species of Gelam mushroom because these matches are well below the 97% cutoff value for species identification [19]. However, these results demonstrated the genus of Gelam mushroom is *Boletus*. *Boletus* is a genus of mushrooms that consist of over 100 species. In central-European countries, *Boletus* sp. are most frequently harvested among wild edible mushrooms, and their popularity in the market is because of their sensory qualities, like aroma, taste and texture [20]. In Malaysia, *Boletus* spp. can be frequently seen during the month of March to April and August to September. This is due to the raining season and the fruiting season of the Basidiomycetes [21]. Most of the *Boletus* spp. were collected from lowland dipterocap forests from West Peninsular Malaysia [21].

4. CONCLUSION

Present results demonstrated that wild Gelam mushrooms collected from the state of Kelantan in Malaysia for this study were from *Boletus* genus and could be a new species. It contained high levels of K, which indicated the mushroom as a potential mineral supplement source. Current findings add substantially to our understanding of nutritional and mineral contents of wild Gelam mushrooms (*Boletus* sp.).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hung PV, Nhi NY. Nutritional composition and antioxidant capacity of several edible mushrooms grown in Southern Vietnam. International Food Research Journal. 2012;19(2):611-615.
2. Manjunathan T, Kaviyarasan V. Nutrient composition in wild and cultivated edible mushroom, *Lentinus tuberregium* (Fr.) Tamil Nadu., India. International Food Research Journal. 2011;18:784-786.
3. Pereira E, Barros L, Martins A, Ferreira ICFR. Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats. Food Chemistry. 2012;130:394-403.
4. Racz L, Papp L, Prokai B, Kovacz Z. Trace element determination in cultivated mushrooms: An investigation of manganese, nickel and cadmium intake in cultivated mushroom using ICP atomic emission. Microchemical Journal. 1996;54*:* 444-451.
5. Lindequist U, Niedermeyer THJ, Jülich WD. The Pharmacological potential of mushrooms. Evidence-Based Complementary and Alternative Medicine. 2005;2(3):285–299. Available:http://doi.org/10.1093/ecam/neh107
6. Zahid M, Barua S, Haque S. Proximate composition and mineral content of selected edible mushroom varieties of Bangladesh. Bangladesh Journal of Nutrition. 2009;22-23.
7. World Health Organization (WHO), Food and Agriculture Organization (FAO) of the United Nation. Vitamin and mineral requirements in human nutrition; 2004. Available:http://apps.who.int/iris/bitstream/10665/42716/1/9241546123.pdf?ua=1 (Accessed 6 September 2016)
8. Reis FS, Barros L, Martins A, Ferreira ICFR. Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An interspecies comparative study. Food and Chemical Toxicology. 2012;50:191-197.
9. Lee SS, Chang YS, Noraswati MNR. Utilization of macrofungi by some indigenous communities for food and medicine in Peninsular Malaysia. Forest Ecology and Management. 2009;257: 2062-2065.
10. Yuswan MR, Al-Obaidi J, Rahayu A, Sahidan S. New bioactive molecules with potential antioxidant activity from various extracts of wild edible gelam mushroom (*Boletus* spp.). Advances in Bioscience and Biotechnology. 2015;6:320-329.
11. AOAC. Official methods of analysis of the association of official's analytical chemists. 17th ed. Virginia: Association of official analytical chemists; 2003.
12. Manzi P, Marconi S, Aguzzi A, Pizzoferrato L. Commercial mushrooms: Nutritional quality and effect of cooking. Food Chemistry. 2004;84:201-206.

13. Alam N, Amin R, Khan A, Ara I, Shim MJ, Lee MW, Lee TS. Nutritional analysis of cultivated mushrooms in Bangladesh: *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybe indica*. Mycobiology. 2008;36:228-232.
14. Heleno SA, Barros L, Sousa MJ, Martins A, Santos-Buelga C, Ferriera ICFR. Targeted metabolites analysis in wild *Boletus* species. Food Science and Technology. 2011;44(6):1343-1348.
15. Adejumo TO, Awosanya OB. Proximate and mineral composition of four edible mushroom species from South Western Nigeria. African Journal of Biotechnology. 2005;4(10):1084-1088.
16. Flegg PB, Maw G. Mushrooms and their possible contribution to world protein needs. Mushroom Journal.1977;48:395–403.
17. Caglarlrmak N, Unal K, Otles S. Nutritional value of edible wild mushrooms collected from the black sea region of Turkey. Micologia Aplicada International. 2002; 14(1):1-5.
18. Uzun Y, Genccelep H, Kaya A, Akcay ME. The mineral contents of some wild edible mushrooms. Ekoloji. 2011;20(80):6-12.
19. Sarwar S, Khalid AN. Diversity and phylogeny of *Suillus* (Suillaceae; Boletales; Basidiomycota) from coniferous forests of Pakistan. International Journal of Agriculture and Biology. 2014;16: 489–497.
20. Jaworska G, Bernaś E. The effect of preliminary processing and period of storage on the quality of frozen *Boletus edulis* (Bull: Fr.) mushrooms. Food Chemistry. 2009;113:936-943
21. Horak E. Revision of Malaysian species of *Boletales* s. l. (*Basidiomycota*) described by E. J. H. Corner (1972, 1974). Malayan Forest Records. 2011;51:1–28.

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