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Taxonomic Study and Bioprospecting Indonesian *Termitomyces eurrhizus*

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Abstract

Termitomyces are known as wild edible mushrooms that are commonly consumed in Africa and Asia. *T. eurrhizus* can be easily found in many areas in Indonesia. However, the recent collection and proper documentation of this species has not been done in Indonesia. In addition, the potential utilization of this species needs to be revealed. This research aims to identify, investigate the proximate value, and test the antibacterial activity of *T. eurrhizus*. The exploration was carried out at the IPB University campus forest using an opportunistic sampling method. Fresh basidiomata were used for morphological identification. Proximate analysis was done following AOAC method. Mushroom extract was subjected to antibacterial test against Enteropathogenic *Escherichia coli.* Our specimen was identified as *T. eurrhizus* based on pileus characters (macroscopically) and spore features under an electron microscope (microscopically). The proximate result showed that the moisture content was 13,70 %, the ash 13,66 %, total fat content 2,05 %, total protein content 27,73 % and fiber content 8,49 %. The investigated antibacterial was evaluated based on size of antibacterial zone for concentration 200 mg/ml extract (3,5 mm), 100 mg/ml (6,5 mm), 50 mg/ml (3,83 mm), 25 mg/ml (2,5 mm), and 12.5 mg/ml 6,33 mm. Our result showed the potential utilization of *T. eurrhizus* as nutraceutical food and antibacterial. Further research is expected to collaborate with various cultivation or product development fields.

Keywords: Antibacterial activity; identification; Proximate analysis, *Termitomyces eurrhizus*.

INTRODUCTION

Sources of macronutrients for humans can be obtained from foods that contain carbohydrates, fats, proteins, and fiber. It is also important to balance these macronutrients with micronutrients found in foods that contain vitamins and minerals. Incorporating edible mushrooms into the diet is an excellent option for promoting health. Edible mushrooms have the potential to serve as both a source of essential nutrition and functional food (Tharu et al., 2022). Their taste and texture characteristics are similar to those of meat, which increases their appeal and versatility for processing (Paloi et al., 2023). Edible mushrooms have been popularly consumed in the past and continue to be chosen by many as part of their daily diet (Mursito et al., 2009).

Currently, every year the demand for food mushrooms continues to increase. In this case, they can be used as a promising source of income (Koné et al., 2013). There is a significant demand for mushrooms and estimates of future food supplies cannot meet the needs. Therefore, the alternative is to use wild mushrooms. One of the edible wild mushrooms often found in Indonesia is *Termitomyces*. The genus *Termitomyces* R. Heim is a group of paleotropic fungi that can be harvested well (Mossebo et al., 2017). *Termitomyces* is classified in the family Lyophyllaceae, has a unique symbiosis with termites, the fruit bodies of this fungus grow to penetrate termite nests to the ground surface (Putra et al., 2020). Enzymes produced by *Termitomyces* allow termites to process plant structural components that are difficult to degrade such as cellulose or lignin (Mossebo et al., 2017). This fungus prefers open places such as in the forest or grasslands/rice fields adjacent to the forest) without prominent termite advice (Karun & Sridhar, 2013). However, its use as food is still rare because there are only a few harvests per season, and no one cultivates it in Indonesia. Furthermore, Augustinus and Putra (2021) also point out that many people do not know that wild mushrooms can be consumed. This is due to a lack of education regarding the types of wild mushrooms that are safe to eat.

In 1847, Berkeley (1847) identified a type of fungi known as *Agaricus eurrhizus*, which was later reassigned and renamed *Termitomyces eurrhizus* by Heim in 1940. The most recent classification of *Termitomyces* can be found in the publication "*Termitomyces of Asia*" by Pegler and Vanhae (1994), which includes detailed comparisons with related species. This fungus is typically found in the paleotropical regions of Africa, Asia, and Oceania (Tharu et al., 2022), where it grows in scattered groups. The pileus of *T. eurrhizu*s is characterized by a grayish-brown color, slightly slimy texture when dry, and distinctly slimy when wet (Augustinus & Putra, 2021). This organism has a unique ecology, often found in symbiosis with termite colonies and their nests. Despite previous studies on spore characteristics, there is a lack of recent SEM images from Indonesia. This study seeks to analyze the morphology and microscopic features of the fungus *T. eurrhizus*, as well as evaluate its nutritional content and antibacterial properties as an edible mushroom.

MATERIALS AND METHODS

Study area

T. eurrhizus was obtained at IPB University, Dramaga, Bogor, West Java, in January - February 2024. Basidiomata were collected from Soka Field, CPWF+H43, Soka Street (Fig. 1). The mushrooms obtained were preserved in 70% ethanol for herbarium. The fruiting bodies were documented in situ, ecological conditions were noted.

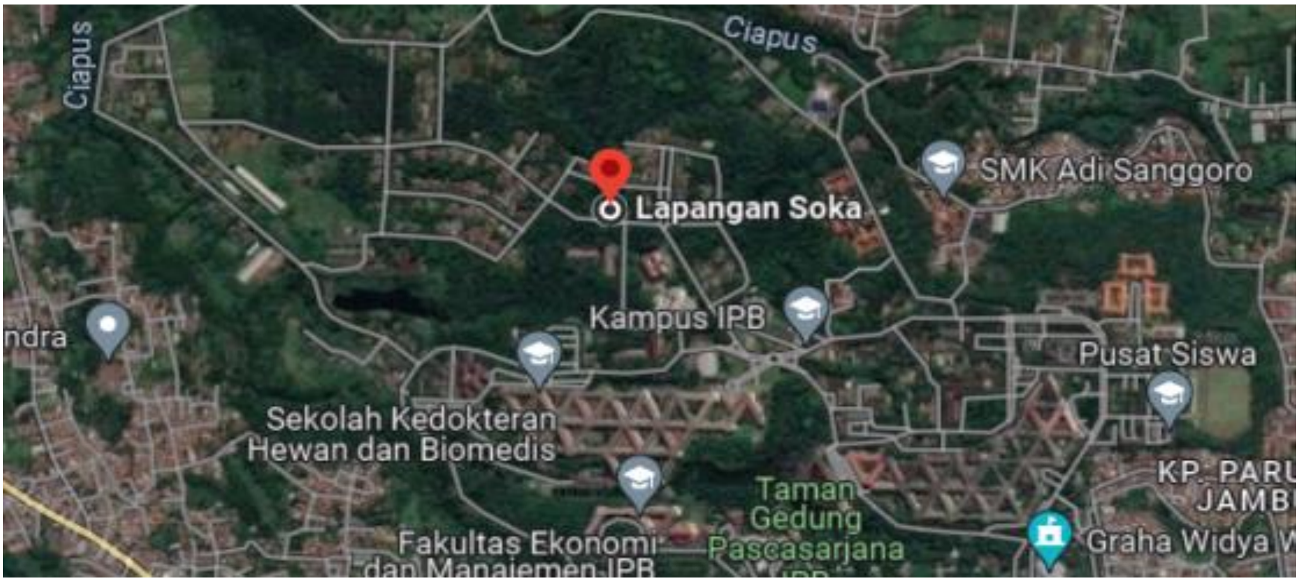

Figure 1. Sampling collection at Lapangan Soka (coordinate point/red dote: 6.553770120925562, 106.7222765279091.

Morphological Identification

Identification was carried out based on the morphological characteristics of fungi. Macroscopic parameters recorded include growth method, shape of the fruit body, changes in wetness level, color of the cap when the fruit body is young and old, diameter of the cap, shape of the top and bottom of the cap, surface of the cap, edge of the cap, margin of the cap, and blade type. which includes how to stick to the stipe, length, distance between rows, and margins. Other characters observed were the shape of the stipe, the color of the stipe (when young and old), the surface of the stipe, the position of attachment to the hood, the type of attachment of the stipe to the substrate, the cross-section of the stipe, partial veil and universal veil, texture of the fruit body, smell and taste (Pegler & Vanhae, 1994), (Watling, 2013). The hymenium was analyzed using scanning electron microscopy (SEM) following the methodology described by Goldstein et al. (1992) at iLaB, BRIN, Bogor, Indonesia. Small hymenium layers (5 × 5 mm) were treated with glutaraldehyde and tannic acid before being dehydrated, infiltrated with t-butanol, and freeze-dried. The samples were then mounted on an aluminum stub, coated with gold, and examined using a JSM IT 200 SEM system (JEOL, Tokyo, Japan).

Proximate Analysis

Test components such as water, ash, fat, protein and fiber content were analyzed using the AOAC (Association of official analytical chemistry) method. Fruiting bodies were dried using a dehydrator at a temperature of 50^0 C. A drying, a crispy texture is obtained, followed by grinding until it forms a powder. Water content and ash content are determined using the gravimetric method (AOAC). Sample fat content was detected in the "Soxhlet" device type extraction. The crude fiber content test uses the method, with alpha-amylase thermostable; protease and amyloglucosidase. Determination of total protein content using the Kjeldahl method.

Fruiting bodies Extraction

The extraction was conducted over 3 successive days by blending the powder sample. A 10 g sample was blended at a ratio of 1:10 using 100 ml of 99% methanol solvent. Subsequently, the samples were soaked in a shaker for 24 hours at 30°C (100 RPM). Once the filtrate from the sample was collected, it was evaporated at 50°C until a paste-like sample was obtained, at which point the weight of the paste was measured.

Antibacterial activity test

Antibacterial activity was conducted to assess the inhibitory effect of the T. eurrhizus extract on the growth of EPEC (Enteropathogenic *Escherichia coli*) bacteria. The isolate used in the study was obtained from Mr. Aji, Microbiology Program at IPB University. *E. coli* was inoculated onto the agar using the spread method, with 20-100 ml of extract aseptically placed onto a disk with a diameter of 6-8 mm. The extract of *T. eurrhizus* was placed onto the disk, along with a combination of DMSO 5% at five concentrations (12.5, 25, 50, 100, 200 mg/ml). A positive control using the antibiotic chloramphenicol and a negative control using DMSO 5% were also included. The agar plates were then incubated under optimal conditions for the test microorganisms. The *T. eurrhizus* extract acted as an antibacterial agent, diffusing into the agar and inhibiting the growth of the microbial strains tested.

Antibacterial activity was analyzed through the results of inhibition zone measurements with the following equation:

$$\frac{(\boldsymbol{DH}-\boldsymbol{DC})+(\boldsymbol{DV}-\boldsymbol{DC})}{\mathbf{2}}$$

Notes:
DH : Horizontal diameter
DC : Disc diameter (6mm)
DV : Vertical diameter

Data analysis

The antibacterial activity test results were analyzed using the SPSS statistical software (Statistical Product and Service Solutions). An ANOVA test (Analysis of Variance) with a significance level of α 0.05 was performed. If the results were significant, a further DMRT (Duncan Multiple Range test) was carried out. The data is presented in table form along with descriptions of the results of the statistical analysis results.

RESULTS AND DISCUSSION

Taxonomy

Termitomyces eurrhizus (Berk.) R. Heim, Archives du Muséum National d'Histoire Naturelle 18: 140 (1942)

Synonym:
Termitomyces eurrhizus (Berk.) R. Heim, Archives du Muséum National d'Histoire Naturelle 18: 140 (1942)
Agaricus eurrhizus Berk., London Journal of Botany 6: 483 (1847)
Rajapa eurhiza (Berk.) Singer (1945)
Armillaria eurhiza (Berk.) Sacc. (1887)
Collybia eurhiza (Berk.) Höhn. (1908)
Collybia eurrhiza (Berk.) Höhn., Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften *Math.-naturw. Klasse* Abt. I 117: 992 (1908)
Rajapa eurrhiza (Berk.) Singer, Lloydia 8: 143 (1945)
Armillaria eurrhiza (Berk.) Sacc., Sylloge Fungorum 5: 85 (1887)
Mastoleucomyces eurrhizus (Berk.) Kuntze, Revisio generum plantarum 2: 861 (1891)
Termitomyces eurhizus (Berk.) R. Heim (1942)
Agaricus eurhiza Berk. (1847)
Agaricus eurhizus Berk. (1847)

Figure 2. Morphology *Termitomyces eurrhizus.* A. Pileus with pointed umbo. B-C. Immature fruiting body. D. Gills. E. Stipe. F. Pseudorhiza.

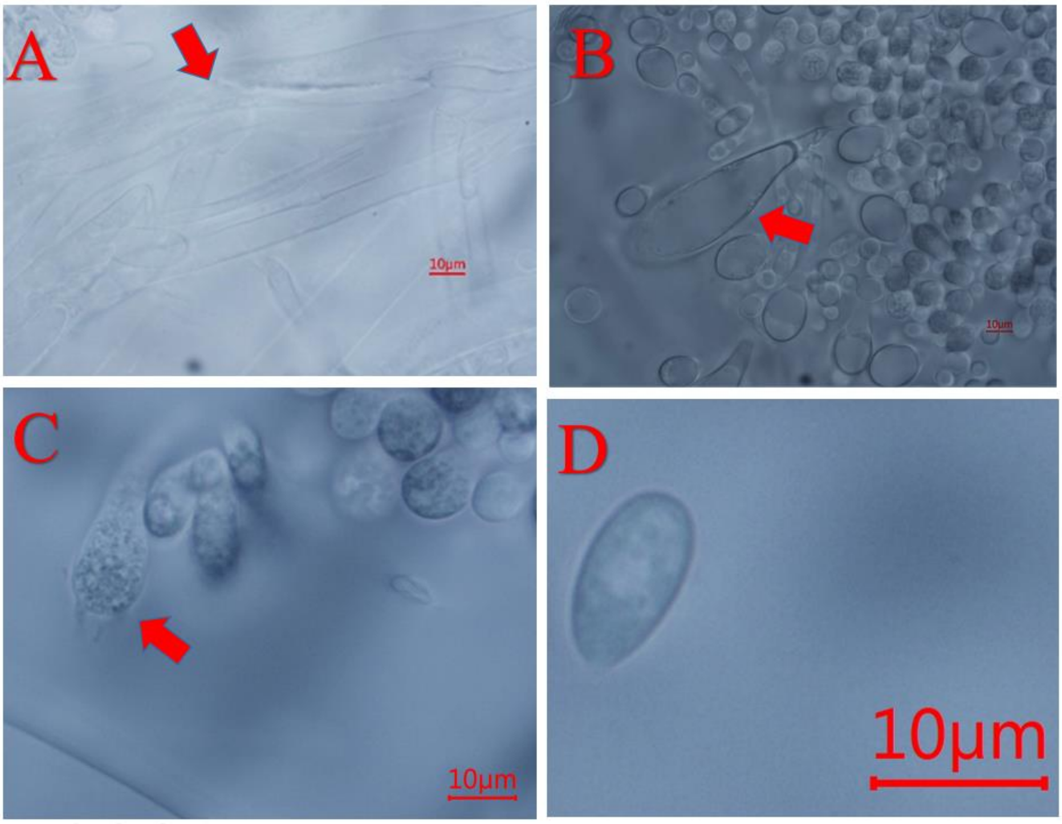

Figure 3. Microscopic characters of lamella. A. Trama (arrow). B. Cystidia (arrow). C. Basidia (arrow). D. Spore.

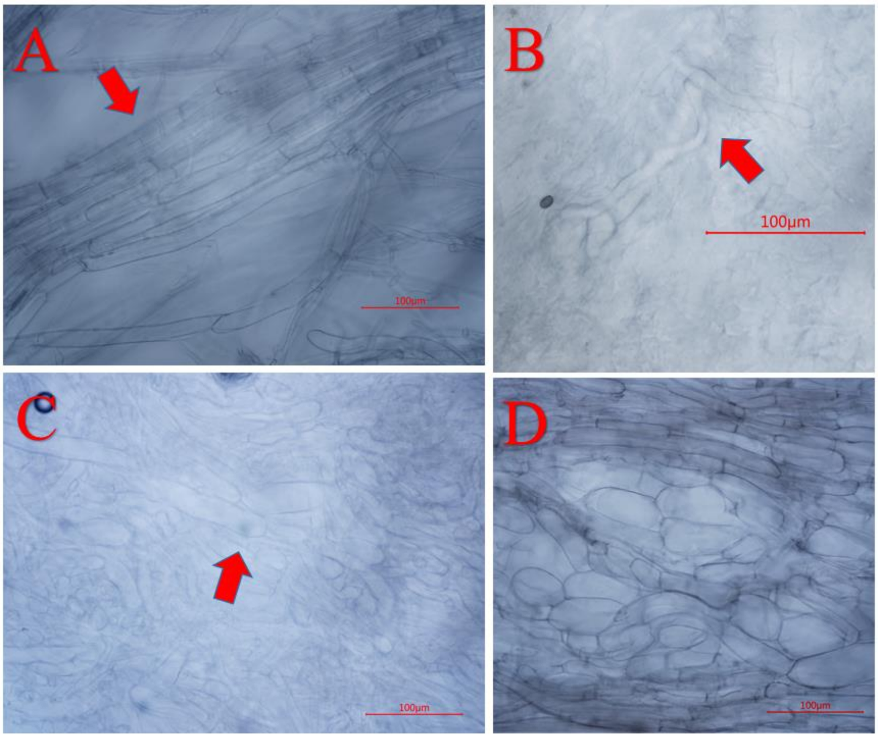

Figure 4. Microscopic characters of stipe and pileipellis. A. Hypahe on stipe (arrow). B. Perforatorium oleiferous hyphae (arrow). C-D. Perforatorium hyphae (arrow).

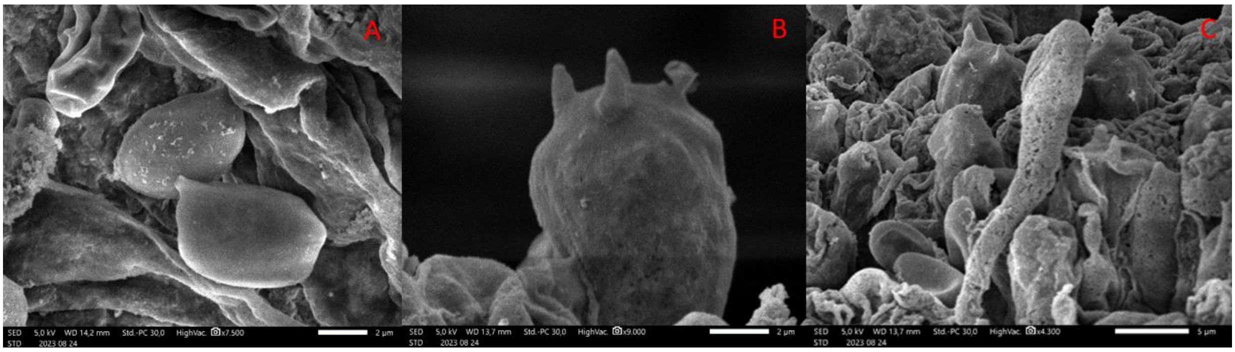

Figure 5. SEM images of specimen. A. Spores. B. Side view of Basidia. C. Cystidia.

Basidiomata were found on the soil's surface and litter, solitary, scattered. **Cap** diameter 16 cm, the shape of the fruit body is a stem covered in lamellae, fleshy. When immature the cap is split at the edges (Fig. 2C), with a pointed tip darker than the pile. **Surface of the cap** is smooth and moist. The characteristics of a mature cap are blooming and breaking easily (Fig. 2A), and the edges are regular, easier to color than the surface of the cap. **Lamellae** are white to cream colored, up to 1 mm wide, classified as free attachment lamellae to adherent, densely packed. **Stipe** 11,5 cm, cylindrical, at the apex it is white and becoming brownish below, fibrillose-striate. **Pseudorhiza** is 3 cm long, 1,5 cm in diameter, fusoid above, with a cartilaginous, dark crust. Fresh fruiting this body wild edible mushroom has a savory taste, and unique flavor like dried fish. **Spore** of *T. eurrhizus* 5.8-6 x 3.8-4 µm. The shape of spores is ellipsoid (Fig. 4 C), subhyaline, with a thin to slightly thickened wall, and refractive contents. **Basidia** 25-30 µm x 9-10 µm, clavate, bearing four sterigmata. **Lamella-edge** sterile, with crowded cheilocystidia. **Cheilocystidia** 30-36 µm x 5-9 µm, obovoid to pyriform, thin walled, with few contents. **Pleurocystidia** 70-76 µm x 21-23 µm, resembling cheilocystidia in shape. Types of trama were irregular (Fig. 4 A) and (Fig. 6 C). Pileipellis with oleiferous hyphae.

T. eurrhizus is taxonomically an edible mushroom originating from the Division Basidiomycota, Class Agaromycetes, Order Agaricales, Family Lyophyllaceae, and Genus Termitomyces. Morphologically, this mushroom is similar to the species *T. umkowaan,* differing only in the size and shape of its basidiospores. *T. eurrhizus* has larger basidiospores compared to *T. umkowaan* and has clavate to pyriform-shaped cystidia (Tibuhwa et al., 1998). Additionally, other references state that *T. eurrhizus* has a sharper umbo compared to the blunter umbo of *T. umkowaan* (Purkayastha & Chandra, 1975). Based on the obtained morphological data, the description of the fruiting body matches Pegler (1994), which includes *T. eurrhizus* having a pseudorhiza with a blackish crust, large basidiomata that are grayish to brown, and rarely with blackish spots. The pileus size is larger compared to the identification key. No annulus was found on the stipe, presumably because the mushroom was no longer in its early stage. Recent reports show *T. eurrhizus* found in Yunnan Province, China, and Nakhon Si Thammarat Province, Thailand, described as having a greyish-brown pileus color (Tang et al., 2020). The same type of fungus is also found in Sheikhupura, Punjab, Pakistan, with a broadly umbonate perforatorium (Izhar et al., 2020), and in Malaysia with the same description (Sathiya Seelan, 2020).

Augustinus & Putra (2021) documented a variety of *Termitomyces* in Indonesia, especially in the urban area of Gunung Pati subdistrict, Semarang city, Central Java. One of their specimen is *T. eurrhizus,* which has a long pseudorhiza than this research. In Indonesia, precisely in the Mount Tukung Gede area, *T.eurrhizus* is also commonly known as mushrooms "moon supa" and "horn mushrooms" (Khastini et al., 2022). The discovery of exploration results in Lore Lindu National Park, Central Sulawesi also explains *T. eurrhizus* substrat decomposing litter and soil (Yusran et al., 2021). Another description is also described by Ye et al. (2019) the same type of mushroom is found in forests in Xishuangbanna, Yunna, China with soil substrate, tree leaf blades known as "big jizong" or "chicken mushroom".

Proximate Analysis

The proximate value describes the composition contained in the fruit bodies of the fungus. Calculating the total content of water, ash, fat, protein and fiber can be used as a reference in post-harvest handling. In addition, the proximate value also provides reasons for choosing what type of method will be used to maintain post-harvest mushroom quality. The percentage of dried fruiting bodies of the *T. eurrhizus* contains as much water content as 13.70 %/ 100 g. Like other fleshy mushrooms, this water content is relatively high, because the storage of fresh fruiting bodies after harvest does not last long. Water content data in previous research shows a higher figure than the water content results in Table 1, as much as 92.08% / 100g (Gunasekara et al., 2021). It is recommended that earlier processing or drying treatment or packaging of *Termitomyces* products be carried out if they are to be used longer.

Table 1. Proximate composition of *Termitomyces eurrhizus.*

Proximate Analyses	Composition (%)
Water content	13,70
Ash content	13,66
Fat content	2,05
Protein content	27,73
Fiber content	8,49
Carbohydrate	34.37

The percentage of ash content from the mushroom is 13.66, this ash depends on the characteristics of the sample. Ash content is useful for distinguishing the type of purity of a product, by looking at acid insoluble ash, for example in fruit vinegar products and synthetic vinegar. Other components such as minerals in the sample also affect the shelf life of *T. eurrhizus* fruit bodies. In the previous study, the amount of ash content was higher with the amount of 11.52% / 100 g DW, the mineral content was also included with the amount of calcium 100, iron 50, magnesium 160, and potassium 2360 mg / 100 g DW (Gunasekara et al., 2021). In this case, these minerals can be greater than the total ash content obtained in this study.

The total fat content of *T. eurrhizus* is 2.5 / 100g (Table 1), which is lower when compared to the results obtained by Gunasekara (2021), as much as 6.27% / 100g DW. The study also states that unsaturated fat (UFA) is higher than total saturated fat (SFA) in *T. eurrhizus,* as UFA is found to play an important role in the physiology of the human body. As in molecular observations there is an oleiferous perforatorium (Fig.3) which can produce oil.

The highest percentage of proximate content is 27.73% of total the protein content. This amount is close to the protein content of *T. eurrhizus* found in Sri Lanka as much as 29.40% (Gunasekara et al., 2021). Purkayastha and Chandra (1975) explain that the amino acids of the fungus *T. eurrhizus* are histidine and arganine. In detail, the protein content (Table 1) contains essential amino acid (EAA) types of glutamate, aspartate, and leucine (Gunasekara et al., 2021). The elaboration of this protein content can explain the umami taste in mushrooms.

By comparing the fiber of wild mushroom fiber, it has a low fiber percentage of 8.49%, while Gunasekara (2021) has 26.64%. Srikram and Supapvanich (2016) showed that the content of wild mushrooms is higher than that of cultivated mushrooms. Mushrooms have both soluble and insoluble fiber, there are about 50-65% carbohydrates based on dry mass which includes monosaccharide sugars, derivatives and oligosaccharides (Santhosh et al., 2023). Zhao (2023) the type of fiber in the mushroom fruit body, namely polysaccharides, has a complex structure so that digestion has a good effect on health. type of polysaccharide D-glucose with fraction water-soluble polysaccharide (PS I and PS II) (Paloi et al., 2023), the fiber content in this mushroom is suitable for consumption by dieters (Alim et al., 2023).

According to the results above, nutrients in these reports are by no means toxic to humans. Growing wild does not mean this mushroom is poisonous, based on its genus, it is still safe to consume (Agustinus & Putra, 2021). For a long time, this species has been one of the foods favored by people of east and central Africa (Koné et al., 2013). The genus *Termitomyces* is primarily consumed by Indian, Chinese, Lao, and Nepales people and is also consumed in several countries such as Malaysia and Thailand (Paloi et al., 2023). Nutritional in Table 1. Represent potentially food which has relatively high protein and fiber content. The content can be an alternative for vegan groups, but the yield of one harvest is only in small quantities so it is rarely traded (Koné et al., 2013).

Antibacterial Activity

The results of the antibacterial activity test of methanol extract of *Termitomyces eurrhizus* showed positive results inhibiting the organization of gram-negative bacteria *E. coli* (Table 2).

Table 2. Antibacterial activities from *Termitomyces eurrhizus* crude extract to enteropatogenic *Escherichia coli.*

Concentration	Inhibiton Zone (mm)
Chloramphenicol (30µg/disc)	$26{,}66 \pm 0{,}763^{c}$
12.5 mg/ml (20 µg/disc)	$6{,}33 \pm 2{,}020^{b}$
25 mg/ml (20 µg/disc)	$2{,}50 \pm 0{,}500^{ab}$
50 mg/ml (20 µg/disc)	$3{,}83 \pm 2{,}843^{ab}$
100 mg/ml (20 µg/disc)	$6{,}50 \pm 5{,}678^{b}$
200 mg/ml (20 µg/disc)	$3{,}50 \pm 0{,}866^{ab}$
DMSO (20 µg/disc)	$0{,}00 \pm 0{,}0000^{a}$

Data represented as Mean ± SD with triplicate. The number followed by a different superscript letter in the same column indicates a significant difference in level ($p < 0.05$) based on Duncan's multiple range tests (DMRT) 5%. EPEC: Enteropathogenic *Escherichia coli*.

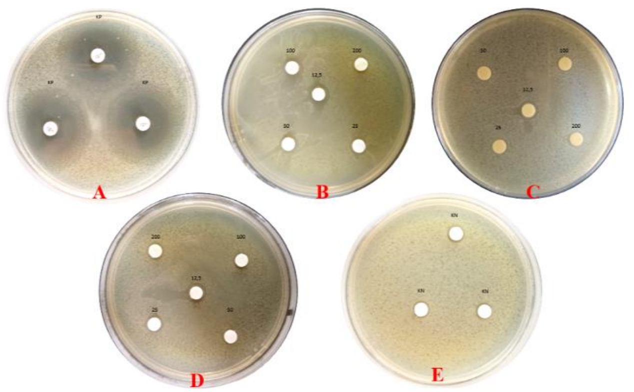

Figure 6. Antibacterial activity of *Termitomyces eurrhizus* crude extract against enteropathogenic *Escherichia coli* **at** five concentrations (12.5, 25, 50, 100, 200 mg/ml). A. chloramphenicol. B. Replication 1 (5 concentration). C. Replication 2 (5 concentration). D. Replication 3 (5 concentration). E. Negative control.

The inhibitory zone formed has different values for each type of concentration. The highest inhibitory zone value was found at a concentration of 100 mg / ml with an average diameter of 6.5 ± 5.67 mm, while the smallest inhibitory zone was formed in an extract concentration of 25 mg / ml with an average zone diameter of 2.5 ± 0.5000 mm. The difference in the inhibitory zone formed shows a difference in sensitivity to *E. coli* bacteria. This sensitivity was evident in the results of a positive control (Chloramphenicol) that had a very high inhibitory zone (Fig 7A). In addition to *E. coli,* antibacterial activity testing has been carried out on bacteria *Staphylococcus aureus, B. cereus, B. subtilis, Proteus vulgaris* but received negative results (Giri et al., 2012). *E. coli* can live in facultative anaerobic conditions, commonly found in warm-blooded animals in the lower intestine. In addition, its role in the digestive tract under normal conditions can be constructive, but when it reaches abnormal levels, it can be parasitic (Abdul Hussein et al., 2023). These properties underlie its use in this study, in vitro the content of secondary metabolites of extract. Based on Kumar and Sagar (2019), the fungus *T. eurrhizus* has antibacterial activity against *L. monocytogenes*, *E. coli*, *S. aureus*, and *P. aeruginosa* with a fairly strong inhibition zone. Differences in activity may be influenced by variations in the strains of both the test bacteria and *T. eurrhizus* itself.

Extract of *T.eurrhizus* has secondary metabolites such as tannins, anthocyanins, saponins, flavonoids, steroids and alkaloids. This is because the strong antioxidant content has the potential to inhibit reactive oxygen species (ROS) so that exposure to free radicals can be avoided (Tharu et *al.,* 2022). These secondary metabolites' content significantly impacts various biological activities (Paloi *et al.,* 2023). The fungus *Termitomyces* contains polysaccharides, which have additional benefits such as antioxidant, hypolipidemic, and hepatoprotective activities, as well as antineoplastic, ulcer health and analgesic, antidiabetic, and immunomodulatory effects (Liu et al., 2021). The fungus *T. eurrhizus* has properties as an ethnomedicine, including treating fever and measles outbreaks (Paloi et al., 2013), treating chickenpox (Manna et al., 2014), managing rheumatism, diarrhea, and lowering high blood pressure (Sachan et al., 2013). When mixed with *Cynodon dactylon*, it can treat skin diseases (Aryal & Budathoki, 2016). For sustainability,there is a need to cultivate this fungus as an additional variety of nutritional sources. This research can be the basis for the community in utilizing *T. eurrhizus* mushrooms for functional food and possibly breakthrough drugs. It is expected to form collaborations with various fields in cultivation for the development of *T. eurrhizus*-based products.

CONCLUSION

In conclusion, our study successfully identified *Termitomyces eurrhizus* in Indonesia, providing valuable documentation of this species. The proximate analysis revealed significant nutritional content, highlighting its potential as a nutraceutical food source. Additionally, the antibacterial activity demonstrated by *T. eurrhizus* against Enteropathogenic *Escherichia coli* suggests its potential application in antibacterial treatments. These findings underscore the importance of further research and collaboration across various fields to explore the cultivation and product development of *T. eurrhizus*, maximizing its utilization and benefits.

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Authors' Contributions: IPP and ODN designed the study. SMA and WAM performed macroscopic analyses. ODN conducted the SEM observation. SMA performed the proximate analyses. WAM performed the antibacterial assay. SMA provides the specimen description. IPP performed morphological identification. All authors wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

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