

**Chemical Profiles and Bioactivities of Ethanol and Water Extracts of Sclerotium and Fruiting Body of *Lignosus rhinocerotis* (Cooke) from Banggai Islands, Indonesia**Kris H. Timotius^{1,2}, Adit W. Santoso^{2,3,4}, Adelina Simamora^{1,2*}¹Department of Biochemistry, Faculty of Medicine and Health Sciences, Krida Wacana Christian University, Jakarta, Indonesia, 11510²Centre for Enzyme Research in Health and Diseases, Krida Wacana Christian University, Jakarta, Indonesia, 11510³Department of Biology, Faculty of Medicine and Health Sciences, Krida Wacana Christian University, Jakarta, Indonesia, 11510⁴Research Unit, Faculty of Medicine and Health Sciences, Krida Wacana Christian University, Jakarta, Indonesia, 11510

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ABSTRACT

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Lignosus rhinocerotis (tiger milk mushroom), a sclerotium-forming mushroom, has been shown to have various pharmacological properties. However, its chemical constituents were not much investigated. In addition, its enzymes' inhibitory potentials are not yet described. Hence, the study aimed to investigate the chemical compositions of its sclerotium and fruiting body. The UPLC-QTOF-MS/MS was used to analyze ethanol and water extracts of both sclerotium and fruiting bodies. The ethanol extracts of the sclerotium and fruiting body were investigated for their antioxidant (DPPH and phosphomolybdenum) and enzymes (α -glucosidase, xanthine oxidase, and trypsin) inhibitory activities. The UPLC-QTOF-MS/MS analyses showed the presence of adenosine, chelidimerine, and pingpeimine B in the water extracts of the sclerotium and the fruiting body. Whereas cytidine, feroxin A, hirsuteine, mangiferin, neomangiferin and sophoraisoflavone A were identified in ethanol extracts of the sclerotium and the fruiting body. The fruiting body showed higher phenolic content than the sclerotium. The fruiting body also exhibited relatively higher radical scavenging and reducing activities, as well as α -glucosidase inhibition activities, when compared with the sclerotium. However, both the sclerotium and the fruiting body showed slight xanthine oxidase (compared to allopurinol) and trypsin inhibition activities. Findings provide a basis for further exploration of phytotherapeutic applications of *L. rhinocerotis*.

Keywords: α -glucosidase inhibition, *Lignosus rhinocerotis*, Polyporaceae, UPLC-QTOF-MS/MS, xanthine oxidase inhibition.

Introduction

Lignosus rhinocerotis (Cooke) Ryvarden (tiger milk mushroom), a giant mushroom (Polyporaceae), can be found in several areas in Southeast Asia. In Indonesia, Banggai Archipelago is known for *L. rhinocerotis*, locally called as "benalu batu". *L. rhinocerotis* is an important medicinal mushroom in China and Southeast Asia, including in Indonesia, Malaysia, and the Philippines. It has been consumed frequently by the natives to enhance general wellness or to cure a variety of illnesses, such as respiratory ailments, cancers, and fever.¹

The fruiting body of *L. rhinocerotis* consists of pileus (cap) and stipe (stalk). These woody parts are attached to a sclerotium which is a hardened mass of mycelium buried underground. Information on the chemical composition of the sclerotium and the fruiting body of *L. rhinocerotis* is still limited. Its sclerotium was found to be non-toxic and rich in carbohydrates, proteins, and dietary fibers with small amounts of fat and sugar.² The amino acid composition of the protein contained all essential amino acids. In addition, the aqueous extract of the sclerotium contained phenolic, terpenoid, and glucan.³ However, very limited information was found on the phytochemicals in the fruiting body.

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Previous studies have reported some pharmacological activities of *L. rhinocerotis*, including antioxidant,² immunomodulatory,⁴ antidiabetic,⁵ and antibacterial⁶ activities. Its cytotoxic and apoptosis activities against different cancerous cell lines have also been reported, for instance against leukemic, breast, lung, and colon cell lines.³ However, no study has reported on inhibition action against enzymes related to metabolic disorders, such as type 2 diabetes mellitus, gout diseases, and obesity.

The present study aimed to identify the phytochemicals in the water and ethanol extracts of the sclerotium and the fruiting body by UPLC-QTOF-MS/MS. In addition, this study investigated the antioxidant and enzymes (α -glucosidase, xanthine oxidase, and trypsin) inhibition activities of the ethanol extracts of *L. rhinocerotis*.

Material and Methods*Chemicals and Fungus Material*

All solvents and chemicals used in the experiments were of analytical grade. Folin & Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, *p*-nitrophenyl- α -D-glucopyranoside, α -glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), acarbose, xanthine, xanthine oxidase from bovine milk (EC 1.17.3.2), allopurinol, and porcine pancreatic trypsin (EC 3.4.21.4) were purchased from Sigma-Aldrich (St. Louis, USA). Gallic acid was obtained from Santa Cruz Biotechnology (Dallas, USA). Ammonium molybdate and sodium carbonate were purchased from Merck (Darmstadt, Germany).

L. rhinocerotis (Figure 1) was collected from Momotan Village, District of South Banggai, Peling Island, Indonesia on April 2020. The identification was carried out by one of the authors (KHT).

Methods

Extract preparation

The sclerotium and the fruiting body of *L. rhinocetis* were cut to small pieces before extraction, either with water or ethanol 96%. Water extracts were prepared by decoction. Five grams of the sclerotium or the fruiting body were added into 100 mL water. Each mixture was stirred at 95°C until the volume was left to 50 mL, then filtered through a vacuum filtration (nylon filter 0.47 µm). Ethanol extracts were obtained by maceration, by soaking 5 g of the sclerotium or the fruiting body in 100 mL ethanol. Each mixture was left for 3 days, followed by filtration, then concentrated to dryness by a rotary evaporator (Buchi R3, Labortechnik AG, Switzerland). The four different extracts were then analyzed with UPLC-QTOF-MS/MS. Ethanol extracts of the sclerotium and the fruiting body were analyzed for their *in vitro* bioactivities.

UPLC-QTOF-MS/MS analysis

UPLC-QTOF-MS/MS analysis of the sclerotium and the fruiting body extracts of *L. rhinocetis* was performed using an Acquity I class ultra-performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA, USA), coupled to a tandem MS/MS detector. The chromatographic separation of phytochemicals was achieved using a Waters Aquity HSS T3 column (100 mm x 2.1 mm, 1.8 µm). The mobile phase consisted of solvent A (0.1% formic acid in acetonitrile) and solvent B (0.1% formic acid in water) at a flow rate of 0.6 mL/min at 40°C and injection volume of 2.5 µL. MS detection was conducted using an MS/MS detector of XEVO G2-S QTOF model, equipped with an ESI source, operating in both positive and negative ionization modes. The following conditions were used: capillary voltage (2.0 kV), cone voltage (40 V), source temperature at 120°C, desolvation gas flow (1000 L/h) and desolvation temperature at 550°C. The collision energy varied between 15 and 40 V and the acquisition range was set at 50 to 1200 Da. Identification of the mass spectra were carried out by comparing with the UNIFI library.⁷

Total phenolic content assay

Total phenolic content of ethanol extract of *L. rhinocetis* was determined using a spectrophotometric method using Folin-Ciocalteu reagent, as reported previously.^{8,9} Gallic acid (12.5 – 200 µg/mL) was used to generate a standard curve. The total phenolic content of the sample was calculated from the liner regression equation obtained from the plot ($y = 0.008x + 0.034$, $R^2 = 0.9976$). Results were expressed as mg gallic acid equivalent/ g biomass.

Assay of DPPH radical scavenging activity

Radical scavenging activity of ethanol extract of *L. rhinocetis* was evaluated using DPPH radicals according to a spectrophotometric method as reported before.^{10,11} Percentage of scavenging activity was calculated based the following formula: $(A-B)/A \times 100$, where A is absorbance of negative control and B is absorbance of sample. Antioxidant activity was expressed as IC₅₀ which was calculated from the regression equation obtained from percentage of inhibition versus sample concentrations. Ascorbic acid was used as a standard for comparison purpose.

Assay of total antioxidant activity/phosphomolybdenum

Reducing capacity of ethanol extracts of *L. rhinocetis* was evaluated based on a colorimetric method using a phosphomolybdenum reagent as reported previously.¹² The method is widely employed to measure antioxidant activity in plant extracts, which is based on the reduction of Mo(VI) to Mo(V) in acidic condition, resulting in the formation of green color complex. Increased absorbance at 695 nm indicates increased reducing activity. Trolox (40 – 1000 µg/mL) was used to generate a calibration plot and reducing activity was expressed as mg trolox equivalent/gram biomass.

Assay of α -glucosidase inhibition activity

Ethanol extracts of *L. rhinocetis* were evaluated for their inhibition activity on α -glucosidase based on an *in vitro* method reported previously.¹³ In this method, α -glucosidase from *Saccharomyces cerevisiae* was used and *p*-nitrophenyl- α -D-glucopyranoside served as

a substrate. Inhibition activity was measured on a spectrophotometer at 405 nm which was due to the release of *p*-nitrophenol from the substrate. Inhibition activity was presented as IC₅₀ and values were compared with acarbose as a positive control.

Assay of xanthine oxidase inhibition activity

The ability of extracts of *L. rhinocetis* to inhibit xanthine oxidase activity was evaluated according to a reported method.¹⁴ Xanthine oxidase (0.2 U/mL, 100 µL) from bovine milk was added to extracts (100 µL) of different concentrations. The reaction mixture was preincubated for 5 mins at 37°C. Xanthine (0.3 mM, 200 µL) served as a substrate was added to start the reaction. After incubating at 37°C for 30 mins, HCl (100 mM, 200 µL) was added to terminate the reaction. Absorbance was read against blank solution at 290 nm on a spectrophotometer. Inhibition percentage was calculated based on $(A-B)/A \times 100$, where A is absorbance of negative control and B is absorbance of sample. Inhibition activity was presented as IC₅₀ value. Allopurinol was used as a positive control.

Assay of trypsin inhibition activity

Inhibition on trypsin was evaluated qualitatively based on a reported method¹⁵ with some modifications. An agar plate (1.5%, w/v) was prepared added with skimmed milk (5%, w/v). Circular wells of 5 mm diameter were made on the plate. Test solutions were prepared by mixing in a 1:1 ratio of each sample and trypsin solution (1 mg/mL in 100 mM tris buffer HCl pH 7.6). Each test solution (50 µL) was suspended in each well and left overnight at 37 °C. A decrease in diameter zone was used as an indication of a trypsin inhibition activity.

Statistical analysis

All experiments were performed in triplicates and results were presented as mean \pm SD. Statistical analysis was carried out using an SPSS software v25.0 (IBM Corp., New York, USA). Statistical significance of experimental findings was analyzed using one-way analysis of variance (ANOVA) and Tukey test. Values were considered significance when *p* value < 0.05.



Figure 1: The sclerotium (A), the young sprout (B), and the fruiting body (C) of *L. rhinocetis* (tiger milk mushroom) from Banggai Archipelago.

Results and Discussion

Phytochemical studies of *L. rhinocetis*

Tiger milk mushroom (*L. rhinocetis*) has been reported for its medicinal potential including for anti-coagulation, anti-inflammatory, anti-microbial/anti-viral, anti-obesity and antioxidant.¹ To the best of our knowledge, the present study is the first to describe the chemical constituents of water and/or ethanol extracts of sclerotium and fruiting body of *L. rhinocetis*.

The LC-QTOF-MS/MS analysis of water and ethanol extracts of both the sclerotium and the fruiting body is shown in Table 1 and a representative of the chromatograms is shown in Figure 2.

Based on results in the LCMS-QTOF-MS/MS analysis, some flavonoids were also identified in the ethanol extracts of both the sclerotium and the fruiting body, such as kushenols H and M,

epicatechin 5-*O*- β -D glucopyranoside, sophoflavescenol, and sophoraisoflavone A, as also xanthone derivatives, such as mangiferin and neomangiferin. Some alkaloids were also identified such as picrasidine, hirsuteine, and piperine. The flavonoids, xanthone, and alkaloids may explain some of bioactivities found in the extracts, as will be described later in this study. Other components were characterized, namely adenosine, pingpeimine B, and chelidimerine. It is not yet clear their relationship with the efficacy of tiger milk mushroom in treating various diseases.

Adenosine is widely distributed in most body fluid. Adenosine is an important regulator for various physiologic and pharmacologic processes. It has been reported that adenosine regulates immune and inflammatory response by activating 4G protein coupled receptors (A1, A2A, A2B, and A3).¹⁶ These adenosine receptors are present in various parts of the body, including tissues, cartilage, brain and heart. Modulation of adenosine level has been key target in the treatment of rheumatic diseases and psoriasis.¹⁷ Adenosine is also known to exert anti-seizure and anticonvulsant effects and associated with the prevention of epilepsy, probably through activation of adenosine A1 receptor.¹⁸ In addition, blockade of A2A adenosine receptor has been reported to reduce Parkinson's disease symptoms.¹⁹

Limited studies were reported on the pharmacological activities of chelidimerine and pingpeimine B. Both are steroidal alkaloids that were considered to have potential antitumor and antiviral activities.²⁰ Chelidimerine isolated from *Fumaria* and *Corydalis* species was previously proved to have antimicrobial and antiviral activity against parainfluenza-3.²¹ So far, there is no previous study on the relationship of chelidimerine and pingpeimine B to the efficacy of *L. rhinocerotis* in the treatment of health problems.

Antioxidant activities

Results in Table 2 shows that total phenolic content of sclerotium is significantly different from that obtained in the fruiting body ($p < 0.05$). Compared with the sclerotium, the TPC of the fruiting body was twice more concentrated. Two different approaches were employed to assess antioxidant activity of the sclerotium and the fruiting body, namely radical scavenging activity (DPPH assay) and reducing capacity (phosphomolybdenum assay). DPPH assay measures the proton donating ability of antioxidant compounds in *L. rhinocerotis*.²² Donation of protons neutralizes the odd electrons present in free radicals, thus changing them into unreactive species. Both the sclerotium and the fruiting body exhibited radical scavenging activity. In both cases, addition of extracts increased the percentage of DPPH scavenging activity in a concentration-dependent manner (data not shown). However, the IC₅₀ of the sclerotium was significantly different from the fruiting body ($p < 0.05$). Although the fruiting body showed higher concentration in phenolic than the sclerotium, the sclerotium showed stronger radical scavenging activity, almost twice to that of obtained for the fruiting body. This observation suggests that other secondary metabolites in the sclerotium might contribute to the DPPH radical scavenging activity, such as those of alkaloids and xanthones. Phosphomolybdenum assay directly measure the capacity of *L. rhinocerotis* to transfer electron to Mo(VI) ion to reduce it into Mo(V). The reducing property of plant extract is a significant indicator of antioxidant activity. The reducing capacity between the sclerotium and the fruiting body is significantly different ($p < 0.05$). Findings are parallel to that found in the TPC, where the fruiting body exhibited almost twice stronger reducing activity in comparison with the sclerotium.

Table 1: Compounds identified in the water and ethanol extracts of the sclerotium and fruiting body of *L. rhinocerotis* identified by an LC-QTOF-MS/MS.

No	Compound	RT (min)	ESI	Molecular weight	Main fragments ion (m/z)	Molecular formula
<i>Identified in the fruiting body</i>						
1	Chelidimerine	0.425	(-)	720.72	719.19; 470.15	C ₄₃ H ₃₂ N ₂ O ₉
2	Sophoraisoflavone A	0.485	(-)	352.33	377.08; 191.02	C ₂₀ H ₁₆ O ₆
3	Epicatechin 5- <i>O</i> - β -D glucopyranoside	0.49	(+)	452.41	381.08; 360.15	C ₂₁ H ₂₄ O ₁₁
4	Mangiferin	0.505	(+)	422.34	258.11; 163.06;	C ₁₉ H ₁₈ O ₁₁
5	Kushenol H	18.44	(+)	472.5	393.21; 141.98	C ₂₆ H ₃₂ O ₈
<i>Identified in the sclerotium</i>						
6	Adenosine	1.56	(+)	267.24	268.10; 136.06	C ₁₀ H ₁₃ N ₅ O ₄
7	Hirsuteine	9.91	(+)	366.45	740.47; 379.25; 133,09;	C ₂₂ H ₂₆ N ₂ O ₃
8	Sophoflavescenol	16.64	(+)	368.4	312.36; 184.07	C ₂₂ H ₂₄ O ₆
9	Piperine	16.65	(+)	285.34	340.39; 113.06	C ₁₇ H ₁₉ NO ₃
10	Picrasidine K	17.25	(-)	313.39	381.23; 297.15	C ₁₈ H ₂₃ N ₃ O ₂
11	Feroxin A	18.46	(+)	356.37	393.21	C ₁₇ H ₂₄ O ₈
12	Kushenol M	18.46	(+)	508.6	340.39; 281.05	C ₃₀ H ₃₆ O ₇
<i>Identified in the fruiting body and the sclerotium</i>						
13	Cytidine	0.420	(-)	243.22	387.11; 181.07	C ₉ H ₁₃ N ₃ O ₅
14	Neomangiferin	0.505	(-)	584.48	439.08	C ₂₅ H ₂₈ O ₁₆
15	Pingpeimine B	16.96	(+)	479.6	429.32; 256.27	C ₂₇ H ₄₅ NO ₆

Excessive free radicals in human body induce damages to various biomolecules, in particular lipid, protein and nucleic acids. Oxidative stress gives rise to lipid peroxidation of the cell membrane, cellular protein denaturation, and DNA strand breakage, causing disruption of cellular functions. Cellular damages have been associated with the onset progression of diverse chronic and degenerative diseases, such as diabetes mellitus, cancer, Parkinson's diseases, atherosclerosis. Antioxidant have been evidenced to be useful in preventing harmful effect of oxidative damage. Natural antioxidants contained in plants offer a good candidate.

The present study confirmed the antioxidant activity of *L. rhinocerotis* *in vitro*, although the activity was weaker than the synthetic antioxidant ascorbic acid. The antioxidant components in the *L. rhinocerotis* may act through proton donation to free radicals, as shown in DPPH assay. In addition, they can also act through electron donation, as shown in phosphomolybdenum assay. Reducing capacity was previously reported for methanol and aqueous extracts of the sclerotium of *L. rhinocerotis*. In this report, reducing activity evaluated by FRAP assay was shown to be concentration-dependent, with methanol extract showed stronger activity than aqueous extract.²³ DPPH radical scavenging activities were also reported for the aqueous-methanol extract of the mycelium and the sclerotium of *L. rhinocerotis*, with IC₅₀ of 0.9 - 3.6 mg/mL.²⁴ Flavonoids, xanthone, and alkaloids derived from plants have been well known to have antioxidant activity,²⁵⁻²⁷ including those found in this study such as epicatechin, kushenols H and M (flavonoids), mangiferin (a xanthone derivate) and piperidine (an alkaloid).

Enzymes inhibition activities

In order to investigate antidiabetic properties of *L. rhinocerotis*, ethanol extracts of the sclerotium and the fruiting body were evaluated

for their inhibition activity on α -glucosidase. Results in Table 2 evidenced that both the sclerotium and the fruiting body inhibited α -glucosidase activity. Both showed a concentration-dependent activity in accordance with increasing concentration (data not shown). Findings unveiled that the sclerotium exerted stronger inhibition, more than twice stronger than the fruiting body, as can be seen from the IC₅₀ values. However, the activities are significantly lower than acarbose ($p < 0.05$), a reference used in this study.

One of effective strategies in the management of type 2 diabetes is by maintaining blood glucose level. This can be achieved by inhibition on α -glucosidase which catalyzes the hydrolyzes of α -(1-4)- linkage glycosidic bond of polysaccharides or oligosaccharides to release glucose. Inhibition on this enzyme would reduce intestinal glucose absorption, thus reducing post prandial glucose level. Acarbose is one of the first line prescribed inhibitors. However, the use of acarbose has been associated with gastrointestinal disturbances that interfere with its use. Plant extracts can be considered for screening potential candidates for antidiabetic agents. The present study found that the sclerotium and the fruiting body of *L. rhinocerotis* can inhibit α -glucosidase. Antidiabetic activity of *L. rhinocerotis* was reported previously using streptozotocin induced diabetic rats.⁵ Findings in the present study may explain in part that hypoglycemic activity of *L. rhinocerotis* is possibly from inhibition on α -glucosidase. This observation was supported by previous study in which mangiferin, epicatechin and piperidine, which were identified present in the sclerotium of *L. rhinocerotis*, were previously reported to exert inhibition on α -glucosidase. For instance, hydroxy piperidine derivatives was shown to have 87.4 and 54.7% inhibition on α -glucosidase.²⁸ In another report, epicatechin gallat was reported to inhibit α -glucosidase with IC₅₀ of 4.03 \pm 0.01 μ g/mL²⁹ and mangiferin with IC₅₀ of 36.84 μ g/mL³⁰

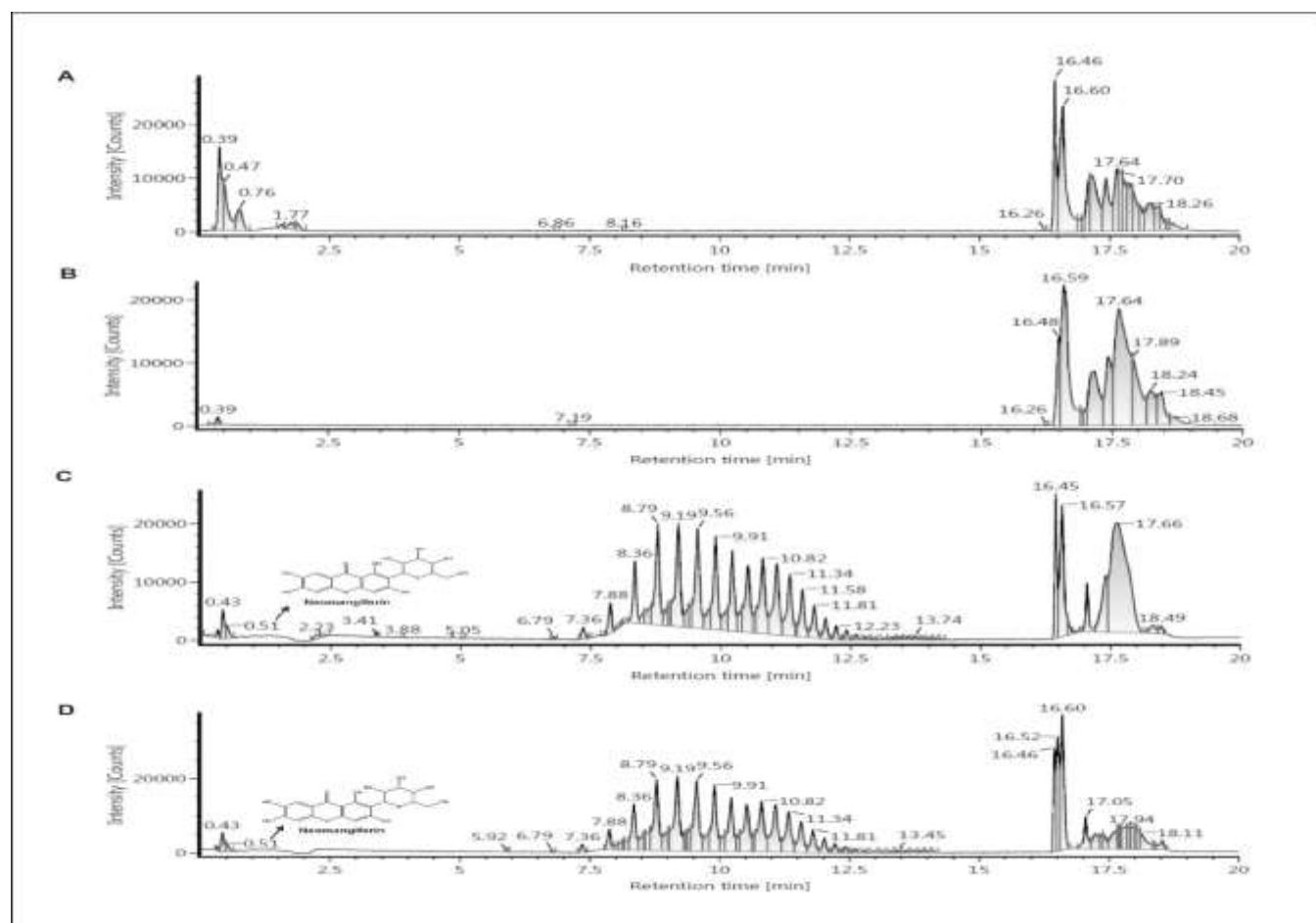
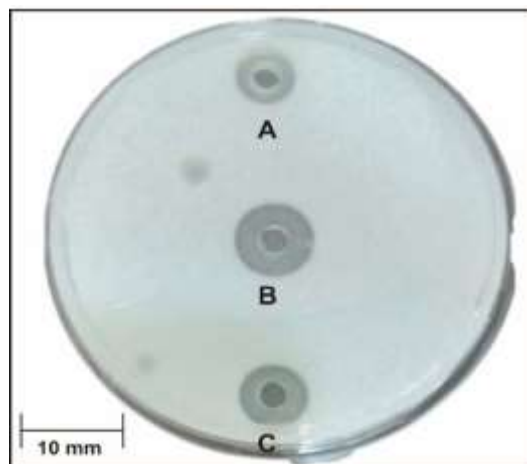


Figure 2: Representative chromatograms of water and ethanol extracts of the sclerotium and fruiting body of *L. rhinocerotis* in positive mode by an LC-QTOF-MS/MS.

Table 2: Antioxidant and enzyme inhibition activities of *L. rhinocerotis* extracts

Assays	Sclerotium	Fruiting body	Ascorbic acid	Acarbose	Allopurinol
Total phenolic content (mgGAE ^a /g biomass)	0.042 ± 0.00	0.088 ± 0.00	-	-	-
DPPH scavenging (IC ₅₀ , mg/mL)	240.57 ± 4.22	109.35 ± 0.95	0.053 ± 0.00		
Phosphomolybdenum (mgTE ^b /g biomass)	2.92 ± 0.00	4.77 ± 0.00			
α-Glucosidase (IC ₅₀ , mg/mL)	0.53 ± 0.00	1.16 ± 0.00		0.10 ± 0.00	
Xanthine oxidase (IC ₅₀ , mg/mL)	24.56 ± 1.41	21.73 ± 2.41			0.0057 ± 0.00

^amgGAE (mg gallic acid equivalent) ^bmgTE (mg trolox equivalent) *Values expressed as mean ± SD (n=3)

**Figure 3:** An agar plate showing trypsin inhibition activity by the sclerotium (well A) and the fruiting body (well C) of *L. rhinocerotis*. Well B was without inhibition.

In addition to T2DM, gout disease is also a common metabolic disorder, which is characterized by hyperuricemia. Sustained hyperuricemia leads to deposition of monosodium urate crystals in joints, that cause gouty arthritis. Serum urate is formed as a product of catalytic hydroxylation by xanthine oxidase of hypoxanthine to xanthine, then to urate. In addition, this catalytic oxidation generates superoxide radicals, that contribute to oxidative stress condition. Thus, inhibition on xanthine oxidase is a target strategy in controlling hyperuricemia (lowering serum urate). Allopurinol, an urate lowering drug, is known to cause side effects, such as hypersensitivity and renal toxicity.³¹ Thus, it is important to search for safe xanthine oxidase inhibitors from natural resources. The ethanol extracts of the sclerotium and the fruiting body of *L. rhinocerotis* were evaluated for their inhibition effect on xanthine oxidase. The sclerotium and the fruiting body showed inhibition effect on xanthine oxidase, with similar activity ($p = 0.320$), Tabel 2. However, both extracts showed weak inhibition as indicated by large IC₅₀ values, compared with allopurinol, a standard drug for treating hyperuricemia. Previously, various mushrooms were reported to exhibit xanthine oxidase inhibition activity,³² such as *Agaricus brazillensis* and *Pleurotus salmoneostramineus*, and *Phellinus baumii*. In particular, a tripeptide phenylalanine-cysteine-histidine has been isolated from *Pleurotus ostreatus* and shown to have antigout activity in a dose dependent manner using animal model.³³ Flavonoids have also been reported to have inhibition activity on xanthine oxidase, including epicatechin (IC₅₀ of > 100 μM) and maringenin (IC₅₀ of > 50 μM).³⁴ Both of these flavonoids were identified in the ethanol extract of the sclerotium. In the present study, a qualitative test for trypsin inhibition activity was also performed for both ethanol extracts of the sclerotium and fruiting body. Figure 3 shows antitrypsin activity of the sclerotium (well A) and fruiting body (well C), in comparison with well B (no inhibition). It is evident that the presence of ethanol extracts led to a reduction in the zone diameters, indicating trypsin inhibition activity.

Using the same concentration, the sclerotium (well A) is seen to cause stronger inhibition compared with the fruiting body (well C). Trypsin inhibition activity has a promising application for obesity treatment. It has been shown that trypsin inhibitors induce the release of cholecystokinin, a hormone responsible to signal the reduction of hunger. Limited studies have appraised the potential of plant extracts as trypsin inhibitors. Previously, potato extract containing trypsin inhibitor³⁵ and trypsin inhibitor isolated from *Tamarindus indica*³⁶ have been shown to suppress food intake using animal models. Previously, *L. rhinocerotis* was reported to have anti-obesity effect using hamster model.¹ It was reported that the aqueous extract of *L. rhinocerotis* prevented weight gain in hamsters fed by high fat diet. The qualitative observation in the present study supported the previous study and may offer a therapeutic importance of *L. rhinocerotis* for the treatment of obesity. However, more works are required involving more *in vitro* and *in vivo* studies.

Conclusion

Phytochemical analysis of the sclerotium and the fruiting body of *L. rhinocerotis* led to the identification of various flavonoids, xanthenes, and alkaloids in the ethanol extract of *L. rhinocerotis* which is likely to be associated with bioactivities observed in the present study. In addition, LC-MS analysis also identified adenosine, chelidimerine and pingpeimine B in the water extract of the sclerotium, whereas the fruiting body is characterized by pingpeimine B. *L. rhinocerotis* proved to be a good source of natural agents as demonstrated by its antioxidant properties and inhibition activities on α-glucosidase and xanthine oxidase.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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