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Artificial Cultivation Anti-tumor Activity of *Ganoderma mbrekobenum*

(Penanaman Buatan dan Aktiviti Anti-barah *Ganoderma mbrekobenum)*

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ABSTRACT

Different varieties of Gandoerma *have different functional effects. In this study, identification, cultivation, anti-tumor activity characterization and active constituent determination of* Ganoderma *specimen collected in Tanzania were carried out. The fungi specimen was identified as* Ganoderma mbrekobenum *by morphological and molecular methods, which was found in Ghana and was firstly reported in 2016. The fruit body was formed in artificial culture using sawdust as the main substrate at the suitable fruiting temperature of 27-30 ℃, relative humidity 85-90%, and daily processed lighting for 10 h (300-500 Lux). The fruit body of* G. mbrekobenum *is hard and has ordinary-yield production, uniform shapes, and high stability. The* in vitro *experiments showed that the extract from* G. mbrekobenum *fruit body inhibited cancer cell proliferation of various cells, such as HepG2, MDA-MB-231 and U87. The water extract of the fruit body was tested, and the content of crude polysaccharides was 1.12%. The data showed that* G. mbrekobenum *was a potential valuable variety. This is the first study that reports the cultivation method and the anti-tumor activity of* G. mbrekobenum.

Keywords: Ganoderma*; medical fungi; polysaccharides*

ABSTRAK

Varieti Ganoderma *yang berbeza mempunyai kesan kefungsian yang berbeza. Dalam kajian ini, pengenalpastian, penanaman, pencirian aktiviti anti-barah dan penentuan sebatian aktif terhadap spesimen* Ganoderma *yang diperoleh dari Tanzania telah dijalankan. Melalui kaedah morfologi dan molekul, spesimen kulat tersebut telah dikenal pasti sebagai* Ganoderma mbrekobenum*, yang sebelum ini ditemui di Ghana dan pertama kali dilaporkan pada tahun 2016. Jasad buah telah terhasil dalam kultur buatan menggunakan serbuk gergaji sebagai substrat utama pada suhu untuk berbuah yang sesuai antara 27-30 °C, kelembapan relatif 85-90% dan pencahayaan setiap hari selama 10 jam (300-500 Lux). Jasad buah* G. mbrekobenum *adalah keras dan mempunyai penghasilan biasa, berbentuk seragam dan kestabilan yang tinggi. Uji kaji secara* in vitro *menunjukkan bahawa ekstrak daripada jasad buah* G. mbrekobenum *merencat pembahagian beberapa sel barah seperti HepG2, MDA-MB-231 dan U87. Ekstrak air jasad buah telah diuji dan didapati bahawa kandungan polisakarida mentah adalah sebanyak 1.12%. Data menunjukkan bahawa kulat ini merupakan varieti bernilai yang berpotensi. Ini merupakan kajian yang pertama melaporkan kaedah penanaman dan aktiviti anti-barah* G. mbrekobenum.

Kata kunci: Ganoderma; *kulat perubatan; polisakarida*

Introduction

Ganoderma is a well-known genus of medicinal fungus; the most famous species are *Ganoderma lucidum* (Curtis) P. Karst. and *Ganoderma sinense* (Zhao & Zhang 2000). Based on the data from more than 1000 specimens from Asia, Europe, North America, South America, Africa, and Oceania, 137 *Ganoderma* species were identified, and a total of 24 *Ganoderma* species were found in China (Li et al. 2015a; Xing & Cui 2018; Xing et al. 2018). The new species *Ganoderma lingzhi* named in 2012 has replaced *G. lucidum*, which was commercially cultivated in East Asia according to the molecular studies (Cao et al. 2012). *G. lingzhi* (*G. lucidum*), a popular traditional medicinal fungus in China, is used for the prevention and treatment of various human diseases, especially cancer (Lin 2001; Mehmet et al. 2017; Sliva et al. 2012; Wu et al. 2012).

Previous studies have shown that several species in this genus also possess various bioactivities (Hsu & Cheng 2018), such as the anti-tumor activity of *Ganoderma leucocontextum* (Li et al. 2019), antibacterial

activity of *Ganoderma australe* (Luangharn et al. 2017), hypouricemic effects of *Ganoderma applanatum* (Yong et al. 2018), antioxidant and antidiabetic activity of *Ganoderma pfeifferi* and *Ganoderma resinaceum* (Raseta et al. 2020). On the other hand, some *Ganoderma* species are the pathogens of plants, such as *Ganoderma boninenseis* and *Ganoderma zonatum* have exerted a severe impact on the palm oil industry (Elliott et al. 2018; Isaac et al. 2018). Prior studies have established many varieties of *Ganoderma* genus were functional. A tentative inference is that other varieties of *Ganoderma* genus maybe has similar functions.

In the present study, *Ganoderma* specimens from Tanzania in East Africa were collected and assessed by morphological and molecular analyses. *Ganoderma mbrekobenum* was identified, which was found only in Ghana and was not studied extensively (Crous et al. 2016). Thus, the cultivation, *in vitro* anti-tumor activity and active constituent determination of the fruiting body were investigated to show the characteristics of the fungus.

Materials and Methods

Materials

The wild-type sample (I160003) was collected in Dar-es-Salaam, Tanzania. For comparison, two samples of *G. lingzhi* (GL) were used. GL-46 (Hanzhi no. 2) was collected from Zhejiang Province, and GL-54 (Hunong no. 1) from Shanxi Province. The strains were isolated and incubated on potato dextrose agar slants (PDA, 20% potato extract, 2% glucose, 2% agar, pH 7.0) at 25 ℃ without light and preserved at 4 ℃. All specimens were dried for identification and conservation. The specimens were deposited in the Herbarium of Guangdong Institute of Microbiology (HMGIM), Guangzhou, China.

Morphological studies

All specimens were examined under the microscope (LEICA DM2500, Solms, German) to assess their spores, basidia, and cystidia in 5% KOH or 1% Congo Red. The pileipellis was examined in 5% KOH (Liu et al. 2016). The samples were also examined by an electron microscope (Hitachi S-3000N, Tokyo, Japan).

Phylogenetic analysis

Ezup pillar of the fungal genomic DNA extraction kit (Sangon Biotech, China) was used to extract total genomic DNA from dried specimens, including the original and the artificially domesticated specimens. The DNA obtained by precipitation was dried at room temperature and resuspended in 20 μL of TE (Tris-EDTA) buffer and stored at -20 ℃ until further use (White et al. 1990).

PCR was conducted using the following primer pair: *ITS1* (5'-TCC GTA GGT GAA CCT GCG G-3') and *ITS4* (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990). The PCR amplification for ITS was as described by Xing et al. (2018). The amplification products were sequenced by Beijing Genomics Institute (Shenzhen, China). All the newly generated sequences were deposited in GenBank. In addition to the sequences generated from this study, the reference sequences were selected from GenBank for phylogenetic analyses (GenBank accession numbers showed on phylogenetic tree). The ITS data included sequences from 34 *Ganoderma* samples and two *Amauroderma* samples, representing 17 taxa (Table 1). MEGA 7.0 software and neighbor-joining method (NJ) were used to construct the phylogenetic tree, and the bootstrap value was 1000.

TABLE 1. Species, specimens, geographic origin and GenBank accession numbers of sequences used in this study

Species name	Voucher no.	Geographic origin	GenBank accession numbers(ITS)	References
Ganoderma australe	HMAS86596	United Kingdom: England	AY884180	Wang & Yao (2006 Unpublished)
G. australe	K(M)120828	United Kingdom: England	AY884183	Wang & Yao (2006 Unpublished)
G. australe	HMAS86595	United Kingdom: England	AY884184	Wang & Yao (2006 Unpublished)
*G. boninens*e	WD 2028	Japan	KJ143905	Zhou et al. (2014)
G. boninense	WD 2085	Japan	KJ143906	Zhou et al. (2014)
*G. curtis*ii	CBS 100131	NC, USA	JQ781848	Zhou et al. (2014)
G. curtisii	CBS 100132	NC, USA	JQ781849	Zhou et al. (2014)
*G. destruct*ans	CMW 43670	Pretoria, South Africa	KR183856	Coetzee et al. (2015)
G. destructans	CBS 139793 (type)	Pretoria, South Africa	NR132919	Coetzee et al. (2015)

*G. flexipe*s	Wei5494	China	JN383979	Cao & Yuan (2013)
G. flexipes	Wei5491	China	JQ781850	Cao et al. (2012)
*G. leucocontext*um	Dai 15601	Xizang, China	KU572485	Xing et al. (2016)
G. leucocontextum	G86	Xizang, China	KX055558	Hu et al. (2016 Unpublished)
*G.ling*zhi	Cui 9166	Shangdong, China	KJ143907	Zhou et al. (2014)
G.lingzhi	Dai12449	Jiangsu, China	JQ781873	Cao et al. (2012)
G.lingzhi	GL-46	Zhejiang, China	--	This study
G.lingzhi	GL-54	Shanxi, China	--	This study
*G. lucid*um	Dai3937	Jilin, China	JQ781853	Cao et al. (2012)
G. lucidum	MT 26/10 (BRNM)	Czech Republic	KJ143912	Zhou et al. (2014)
G. mbrekobenum	SSP:10	India: Badi, Raisen, Madhya Pradesh	KY865253	Parihar et al. (2018 Unpublished)
G. mbrekobenum	UMN7-4 GHA	Ghana	KX000898	Crous et al. (2016)
G. mbrekobenum	I160003	Dar-es-Salaam, Tanzania	MK453307*	This study
G. mbrekobenum	AC-I160003	Dar-es-Salaam, Tanzania	MN097540*	This study
G. multipileum	CWN 04670	Taiwan, China	KJ143913	Zhou et al. (2014)
*G. resinace*um	Rivoire 4150	France, Europe	KJ 143915	Zhou et al. (2014)
G. resinaceum	CBS 194.76	Netherlands, Europe	KJ 143916	Zhou et al. (2014)
G. sichuanense	HMAS 42798 (Holotype)	Sichuan, China	JQ781877	Cao et al. (2012)
G. sichuanense	Cui 7691 (BJFC)	Guangdong, China	JQ781878	Cao et al. (2012)
*G. tropic*um	Yuan 3490	Yunnan, China	JQ781880	Cao et al. (2012)
G. tropicum	BCRC37122	Taiwan: Luku, Nantou	EU021457	Wang et al. (2009)
*G. tsug*ae	Dai 12751b	CT, USA	KJ143919	Zhou et al. (2014)
G. tsugae	Dai 12760 (BJFC)	CT, USA	KJ143920	Zhou et al. (2014)
G. zonatum	FL-02	FL, USA	KJ143921	Zhou et al. (2014)
G. zonatum	FL-03	FL, USA	KJ143922	Zhou et al. (2014)
Outgroup				
Amauroderma rude	CANB 795782	Australia	KU315198	Costa-Rezende et al. (2016)
Amauroderma rugosum	Dai 13716	China	KU219985	Cui & Zhao (2014)

ARTIFICIAL CULTIVATION

The component of mother-culture media, secondary spawn medium and fruiting material were listed in Table 2. Inoculation, cultivation and management were conducted according to the methods described by Hu et al. (2011). The fruiting temperature was 27-30 °C, relative humidity 85-90%, and daily processed lighting for 10 h (300-500 Lux). Fruit bodies were harvested when spores were ejected. Fruit bodies were weighted and then dried at 50 °C. Experiments were repeated three times using 20 bags each time.

TABLE 2. Culture medium of various stages in artificial cultivation

Type of medium	Component
Mother-culture media	20% potatoes, 2% glucose, 1% the potassium dihydrogen phosphate, 0.3% magnesium sulfate, trace vitamin B1, and 2% agar in 1 L ddH_2O, natural pH
Secondary spawn medium	98% sorghum, 2% light calcium carbonate, water content 50±2%, natural pH
Fruiting material	38% cottonseed hull, 50% sawdust, 10% bran, 2% calcium carbonate, water content 63%, natural pH

TUMOR CELL PROLIFERATION ASSAY

The fruit bodies of cultivation were collected, smashed, and soaked in ethyl acetate for 10 h, followed by ultrasonic extraction two times, 40-min each; then, the extract was pooled. The organic phase was collected after filtration, the liquid was evaporated, and the ethyl acetate extract was stored at 4 °C.

Tumor cells, HepG2 (hepatoblastoma cell), U87 (glioma cell), and MDA-MB-231 (breast cancer cell) were obtained from the typical culture preservation committee cell bank of Chinese Academy of Sciences and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% penicillin and streptomycin, and 10% fetal bovine serum (FBS) (Corning Life Sciences, China). Cells (5×10^4 cells/well) were seeded in 96-well plates in medium and incubated at 37 °C in an incubator (Thermo Scientific, USA) containing 5% CO_2 for 24 h.

The ethyl acetate extract was solubilized in dimethyl sulfoxide (DMSO) at a concentration of 200 $mgmL^{-1}$ of mother liquor, diluted with DMEM containing 10% FBS to obtain the final concentration 12.5, 25, 50, 100, and 200 μgmL^{-1}, respectively. A volume of 100 µL/well sample solution (12.5, 25, 50, 100, and 200 μgmL^{-1}) was added to the absorbed cell culture fluid in 96-well plates. Each sample was added in five wells. Also, a blank control (BC) with medium and negative control (NC) with the cells, the highest concentration of drug dissolution medium, and the culture were set; the final concentration of DMSO was 0.2%. Subsequently, the plate was incubated at 37 °C and 5% CO_2 for 30 h.

Cell Counting Kit-8 (CCK-8) (DongRen Chemical Technology, China) was used to evaluate the tumor cell apoptosis. A volume of 10 µL CCK-8 reagent was added to each well and cultured in for 0.5 h. The absorbance values were measured at 450 nm using an ultraviolet spectrophotometer (Multiskan GO, Thermo Scientific, USA).

DETERMINATION OF POLYSACCHARIDES AND GANODERIC ACID A

The crude mushroom polysaccharide was analysed by spectrophotometry according to Xing et al. (2008). To determine ganoderic acid A content, 2 g fruit body was smashed, passed through 40 mesh sieve, mixed with 75 mL of anhydrous ethanol, refluxed for 45 min, cooled, and filtered. The residue was washed with 10 mL of anhydrous ethanol. This step was repeated twice, and the filtrates merged. Ethanol was vaporized in a 60 °C water bath or by rotary vacuum evaporation. The concentrate was diluted with anhydrous ethanol, 3 mL solution was absorbed, filtered by 0.22-µm Millex syringe filters (Thermo Scientific, USA), and stored as described previously (Jia et al. 2017). Ganoderic acid A standard solution (2.5 $mgmL^{-1}$) was prepared by dissolving the compound in methanol, followed by filtration. The resulting filtrate was stored in a 9-mm screw vial.

The ganoderic acid A content was then analyzed by HPLC (Shimadzu LC-20A, Shimadzu Corporation, Kyoto, Japan) under the following conditions: YMC-Pack ODS-A C18 chromatographic column (S-5 microns, 12 nm); Mobile phase of acetonitrile: 0.075% phosphoric acid aqueous solution, isocratic elution (0 - 35 min, 33% acetonitrile); Column temperature: 30°; Flow rate: 1.0 $mLmin^{-1}$; Injection volume: 10 µL; Detection wavelength: 257 nm.

STATISTICAL ANALYSIS

Results were analysed by paired t-test by using SPSS 21 software, where $P < 0.05$ indicated a significant difference.

RESULTS & DISCUSSION

MORPHOLOGICAL IDENTIFICATION

Based on morphological observations, the characteristics of the fungus were as follows: Mature basidiomata pileate, stipitate, dimidiate, applanate, woody to corky when dried, homogenous context structure, pileus maroon to liver brown when dried, surface hard and glabrous, margin rounded, thickened, maroon to liver brown. The most obvious feature was pileus maroon and flabellate (Figure 1). Furthermore, the hyphal system was dimitic, generative hyphae were slightly inconspicuous, branched, thin-walled, and showed hyaline, skeletal hyphae were prevalent in the basidiocarp, occasionally branched, pale to dark brown, 2.5-7-µm-thick, and tapering towards the end. Basidiospores were brown, ovoid to broadly ellipsoid with a truncate base, bitunicate, verruculose, and 8-11.5 × 6-8 µm in size (Figure 2). The morphology of this fungus was similar to that of *G. mbrekobenum* (Crous et al. 2016).

FIGURE 1. Wild appearance of I160003

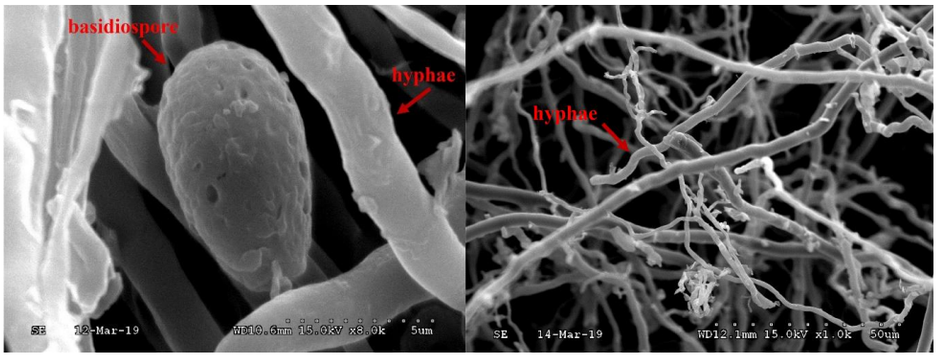

FIGURE 2. The spores (left) and hyphae (right) of I160003 under electron microscopy. The hyphae and spores were magnified at ×1000 and ×8000, respectively

MOLECULAR IDENTIFICATION

The sequences of the wild-type fruit body (I160003) and artificial fruit body (AC-I160003) have been deposited in GenBank. The accession numbers are MK453307 and MN097540, by standard nucleotide BLAST against the NCBI database. The sequence shares 98.97 and 99.14% similarity to that of *G. mbrekobenum* (KX000898), which was submitted by E.C. Otto (Crous et al. 2016). The phylogenetic tree of the *Ganoderma* species based on ITS sequence data showed that *G. lingzhi* (Wu et al. 2012), *G. curtisii* (Berk.) Murrill, *G. tropicum* (Jungh.) Bres., *G. multipileum* (Hou), *G. destructans* Coetzee (Marinc & Wingf.) and *G. flexipes* Pat. formed a clade with a 86 bootstrap value, while GL-46, GL-54, and *G. lingzhi* formed a clade with a 99 bootstrap value. These species were grouped together with *G. resinaceum* Boud. as a clade with a 97 bootstrap value, while *G. lucidum* (Curtis) P. Karst., *G. tsugae* Murrill, and *G. leucocontextum* formed another clade with a 100 bootstrap value, known as the *G. lucidum* complex (Li et al. 2015a). I160003, AC-I160003, *G. mbrekobenum* (KY865253), and *G. mbrekobenum* (KX000898) formed a clade with a 100 bootstrap value; these were related to *G. zonatum* Murrill, *G. boninense* Pat., and *G. australe* (Fr.) Pat., and formed a clade with a 54 bootstrap value. Together with morphological and phylogenetic analysis, this fungus was determined as the *G. mbrekobenum* (Figure 3).

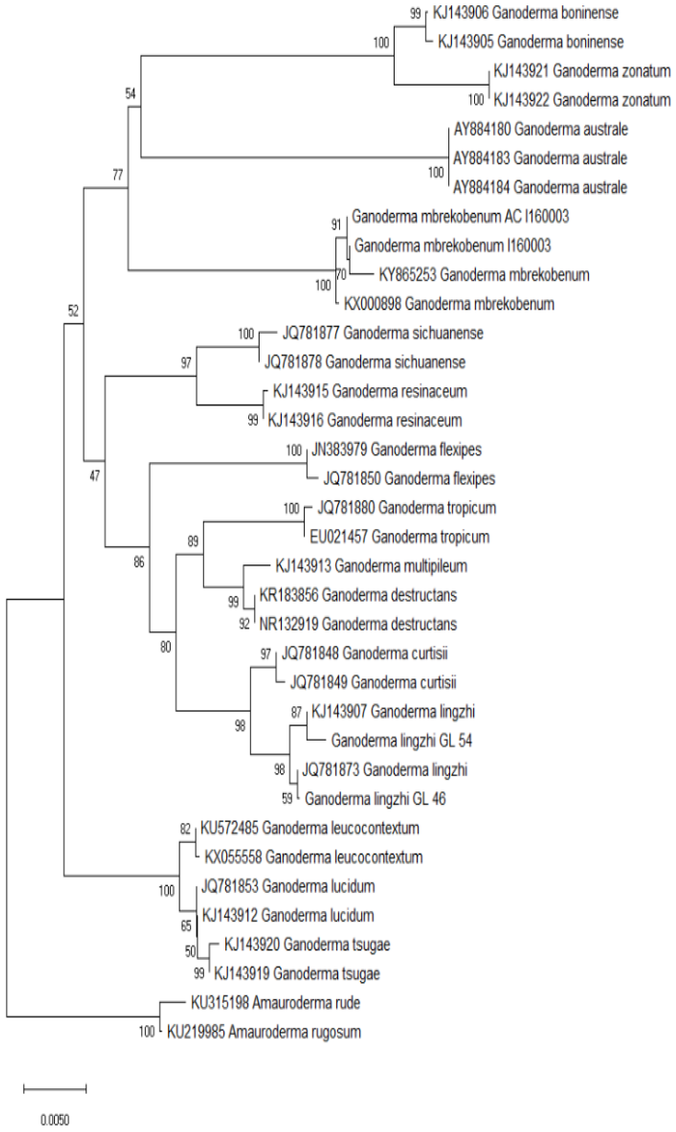

FIGURE 3. Phylogenetic tree of the *Ganoderma* species based on ITS sequence data by NJ method. *Amquroderma rude* species were selected as an outgroup, and 34 *Ganoderma* sequences were used for the phylogenetic tree construction

The phylogenetic tree of the *Ganoderma* species was constructed based on ITS sequence data and it showed that *G. mbrekobenum* is closely related to *G. zonatum* Murrill, *G. boninense* Pat., and *G. australe* (Fr.) Pat, which are subtropical or tropical varieties (Li et al. 2015b; Saccardo & Saccardo 1905; Syahriel et al. 2018). *G. mbrekobenum* was found in Ghana (Crous et al. 2016) and Tanzania, which are in tropical climate. Therefore, we have come to the conclusion that the phylogenetic relationships of *Ganoderma* species are related to climatic types.

CULTIVATION

To evaluate the developing value of *G. mbrekobenum*, we first studied the artificial cultivation of *G. mbrekobenum* as a new *Ganoderma* species. The fruit body of *G. mbrekobenum* was similar to the wild-type state, and the most distinct feature was pileus maroon and flabellate. The growth cycle was 78 days. The tabwet weight of *G. mbrekobenum* was significantly lower than the samples of *G. lingzhi* in the market. However, the appearance, including individual weight, the length of stipe, and the width of pileus, was uniform (Table 3, Figure 4). The fruit body of *G. mbrekobenum* is much harder than the other *G. lingzhi* species. Additionally, *G. mbrekobenum* is a less-spore variety when compared to *G. lingzhi*.

Cultivation of *G. mbrekobenum* was repeated three times from 2017 to 2019. We found that the normal fruit body could be cultivated using the conventional method in subtropical areas. Sawdust served as the main ingredient of the substrate; the fruiting temperature was 27-30 ℃, relative humidity 85-90%, and the light exposure was for 10 h (300-500 Lux). The artificial cultivation was characterized by high stability. Interestingly, there was barely any resistance from *G. mbrekobenum*, and it could grow normally using a mildewed medium. In the artificial cultivation, it could grow in three waves. However, 74.3% of production was concentrated in the first wave, and 92.5% of the production was concentrated in the first and second waves.

FIGURE 4. The cultivating fruit body of G. *mbrekobenum* I160003 (left) compared to *G. lingzhi* (middle: GL-46; right: GL-54)

TABLE 3. First wave wet weight of the *Ganoderma* fruiting body

Sample	I160003 *G. mbrekobenum*	GL-46 *G. lingzhi*	GL-54 *G. lingzhi*
Wet weight (single) (g)	57.09±0.31[b]	69.36±6.36[a]	67.50±5.51[a]
Length of stipe (mm)	22.43±6.1[1]b	34.87±11.68[ab]	49.70±15.62[a]
Width of pileus (mm)	93.87±11.08[a]	109.15±38.74[a]	116.79±22.17[a]
Thickness of pileus (mm)	14.31±0.92[a]	14.05±2.48[a]	11.95±0.24[a]
First wave biological conversion rate (%)	16.3	19.8	19.3

Note: Values were the means (n=3)±SD. Values in the same row of the same item with different lower case letters indicated significant differences at 0.05 level

The first wave biological conversion rate of *G. mbrekobenum* was 16.3%, which was slightly lower than the other two *G. lingzhi* market species (19.8 and 19.3%), but higher than some other *Ganoderma* species, such as *G. australe* (12.8%) (Luangharn et al. 2017) and *G. leucocontextum* (10.1-11.4%) (Hu et al. 2017).

INHIBITION OF TUMOR CELLS *in vitro*

The ethyl acetate extract of *Ganoderma* species was reportedly contains small molecules such as triterpenoids, alkaloids, nucleosides, flavones, organic acids, amino acids, and fatty acids, which possess the biological activity of inhibiting tumors. In this study, the effects of *G. mbrekobenum* ethyl acetate extract were evaluated on human cancer cells, including HepG2, U87, and MDA-MB-231, and the inhibition rate of tumour cell was positively correlated with the concentration (Table 4). The results showed that this extract inhibited the growth of cancer cells in a concentration-dependent manner (Table 5). The half-maximal inhibitory concentration (IC50) test determined that the human cancer cell MDA-MB-231 was sensitive to the ethyl acetate extract treatment (IC50: 114.97±14.1 μgmL^{-1}). Conversely, *G. lingzhi* GL-46 and GL-54 inhibited HepG2 and U87 more obviously than *G. mbrekobenum* I160003, but the effect did not differ significantly on MDA-MB-231.

TABLE 4. Inhibition rate of the ethyl acetate extract of *Ganoderma* fruiting body

Sample	Concentration (μgmL^{-1})	HepG2 inhibition rate (%)	U87 inhibition rate (%)	MDA-MB-231 inhibition rate (%)
I160003	25	3.4±2.33	24.5±5.75	14.6±0.92
	50	16.2±4.59	28.5±8.64	19.6±6.76
	100	34.7±2.12	42.5±6.33	40.9±5.31
GL-46	25	11.9±0.30	29.9±0.60	27.9±5.91
	50	13.3±0.54	38.4±0.06	28.9±6.17
	100	57.5±2.87	58.4±1.42	32.7±9.93
GL-54	25	4.0±0.65	12.8±4.77	19.2±1.62
	50	6.7±0.96	16.4±4.76	22.8±3.43
	100	48.1±0.42	54.0±3.71	38.6±2.09

Note: Values were the means (n=3)±SD

TABLE 5. IC50 value of the ethyl acetate extract of *Ganoderma* fruiting body (μgmL^{-1})

Sample	I160003 *G. mbrekobenum*	GL-46 *G. lingzhi*	GL-54 *G. lingzhi*
HepG2	163.37±18.79^{a}	85.83±3.45^{b}	102.67±10.95^{b}
U87	130.49±8.25^{a}	58.03±2.25^{c}	102.93±1.30^{b}
MDA-MB-231	114.97±14.1^{a}	104.64±9.85^{a}	115.77±1.70^{a}

Note: Values were the means (n=3)±SD. Values in the same row of same item with different lower case letters indicate significant differences at 0.05 level

Some studies assessed the bioactivity of other *Ganoderma* species; for example, exopolysaccharide from *G. applanatum* exhibited selective activity against tumor cells (SiHa) at 22.88 and 228.5 μgmL^{-1} concentrations (Osińska-Jaroszuk et al. 2014). The antibacterial activity of *G. boninense* extracts was found to inhibit *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* and *Escherichia coli* (ATCC 35218) remarkably (Syahriel et al. 2018). The extracts of *G. australe* inhibited *Micrococcus luteus*, *Bacillus subtilis*, *S. aureus*, and *Salmonella typhimurium* bacteria, but were not effective against *E. coli* and *Pseudomonas aeruginosa* (Luangharn et al. 2017). The current study showed that the ethyl acetate extract of *G. mbrekobenum* inhibits proliferation of HepG2, U87, and MDA-MB-231 *in vitro*, especially MDA-MB-231, which was similar to *G. lingzhi* and other *Ganoderma* species (Liu et al. 2018).

DETERMINATION OF POLYSACCHARIDES AND GANODERIC ACID A

The crude polysaccharide content of the *G. mbrekobenum* fruit body was 1.12%, which was significantly higher than the value of *G. lingzhi* samples (0.92 and 0.96%), and 24% higher than the minimum limit prescribed in Chinese Pharmacopoeia (0.9%) (Chinese Pharmacopoeia Committee 2015). Ganoderic acid A content of *G. mbrekobenum* fruit body was significantly lower than that of *G. lingzhi* (Table 6).

TABLE 6. Crude polysaccharide content of *Ganoderma* fruiting body

Sample	I160003	GL-46	GL-54
	G. mbrekobenum	*G. lingzhi*	*G. lingzhi*
Crude polysaccharide content (%)	1.12 ± 0.006^{a}	0.92 ± 0.06^{b}	0.96 ± 0.05^{b}
Ganoderic acid A content (mgg^{-1})	0.07 ± 0.001^{c}	8.65 ± 0.07^{a}	6.51 ± 0.03^{b}

Note: Values were the means (n=3)±SD. Values in the same row of same item with different lower case letters indicate significant differences at 0.05 level

The main bioactive compounds of *Ganoderma* spp. are polysaccharides, triterpenoids, and sterols. At present, over 300 compounds of triterpenoids were reported in *Ganoderma* spp. (Jia et al. 2017). The crude polysaccharide content is a critical quality index of the *Ganoderma* species fruit body. The present results indicated that the content of polysaccharide of *G. mbrekobenum* was significantly higher in *Ganoderma* species than the other varieties of *G. lingzhi* commonly used in China. Thus, we speculated that *G. mbrekobenum* might be a potential variety among *Ganoderma* spp.

Ganoderic acid A had been first reported in *G. lucidum* in 1982 by Kubota et al., therefore, it was tested in our study. However, the content of ganoderic acid A in *G. mbrekobenum* was significantly lower than in *G. lingzhi*. This may caused by differences of effective constituent in *G. lingzhi* and other *Ganoderma* species (Bao & Wang 2014; Li et al. 2013).

CONCLUSION

This is the first study that reports the cultivation method and the anti-tumor activity of *G. mbrekobenum*. This study showed that polysaccharides are the main ingredients in *G. mbrekobenum*. It is known that polysaccharides from medicinal fungi are highly effective in anti-tumor and immuno-enhancing activities, and the treatment of hypertension. Therefore, we proposed that *G. mbrekobenum* is a high quality *Ganoderma*. Moreover, this study provides the scientific basis for the development and utilization of the genus of *Ganoderma*. In future research, the biological characteristics need to be investigated to increase *Ganoderma* production as well as its effective composition.

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REFERENCES

Bao, H. & Wang, X. 2014. Studies on chemical constituents of *Ganoderma sinense*. *Journal of Fungal Research* 12(4): 187-196.

Cao, Y., Wu, S.H. & Dai, Y.C. 2012. Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi". *Fungal Diversity* 56(1): 49-62.

Cao, Y. & Yuan, H. 2013. *Ganoderma mutabile* sp. nov. from southwestern China based on morphological and molecular data. *Mycological Progress* 12(1): 121-126.

Chinese Pharmacopoeia Committee. 2015. *Chinese Pharmacopoeia*. Beijing: People's Press.

Coetzee, M.P.A., Marincowitz, S., Muthelo, V.G. & Wingfield, M.J. 2015. *Ganoderma* species, including new taxa associated with root rot of the iconic *Jacaranda mimosifolia* in Pretoria, South Africa. *IMA Fungus* 6(1): 249-256.

Crous, P.W., Wingfield, M.J., Richardson, D.M., Le Roux, J.J., Strasberg, D., Edwards, J., Roets, F., Hubka, V., Taylor, P.W., Heykoop, M., Martín, M.P., Moreno, G., Sutton, D.A., Wiederhold, N.P., Barnes, C.W., Carlavilla, J.R., Gené, J., Giraldo, A., Guarnaccia, V., Guarro, J., Hernández-Restrepo, M., Kolařík, M., Manjón, J.L., Pascoe, I.G., Popov, E.S., Sandoval-Denis, M., Woudenberg, J.H., Acharya, K., Alexandrova, A.V., Alvarado, P., Barbosa, R.N., Baseia, I.G., Blanchette, R.A., Boekhout, T., Burgess, T.I., Cano-Lira, J.F., Čmoková, A., Dimitrov, R.A., Dyakov, M.Y., Dueñas, M., Dutta, A.K., Esteve-Raventós, F., Fedosova, A.G., Fournier, J., Gamboa, P., Gouliamova, D.E., Grebenc, T., Groenewald, M., Hanse, B., Hardy, G.E., Held, B.W., Jurjević, Ž., Kaewgrajang, T., Latha, K.P., Lombard, L., Luangsa-Ard, J.J., Lysková, P., Mallátová, N., Manimohan, P., Miller, A.N., Mirabolfathy, M., Morozova, O.V., Obodai, M., Oliveira, N.T., Ordóñez, M.E., Otto, E.C., Paloi, S., Peterson, S.W., Phosri, C., Roux, J., Salazar, W.A., Sánchez, A., Sarria, G.A., Shin, H.D., Silva, B.D., Silva, G.A., Smith, M.T., Souza-Motta, C.M., Stchigel, A.M., Stoilova-Disheva, M.M., Sulzbacher, M.A., Telleria, M.T., Toapanta, C., Traba, J.M., Valenzuela-Lopez, N., Watling, R. & Groenewald, J.Z. 2016. Fungal planet description sheets: 400-468. *Persoonia-Molecular Phylogeny and Ecolution of Fungi* 36(1): 316-458.

Costa-Rezende, D.H., Gugliotta, A.D., Mello, Goes-Neto, A., Reck, M.A., Robledo, G.L. & Drechsler-Santos, E.R. 2016. *Amauroderma calcitum* sp. nov. and notes on taxonomy and distribution of *Amauroderma* species (Ganodermataceae). *Phytotaxa* 244(2): 101-124.

Elliott, M.L., Des, J., Elizabeth, A., Ortiz, J.V. & Macias, T. 2018. Genetic variability of *Ganoderma zonatum* infecting palms in Florida. *Mycologia* 110(2): 339-346.

Hsu, K. & Cheng, K. 2018. From nutraceutical to clinical trial: Frontiers in *Ganoderma* development. *Applied Microbiology & Biotechnology* 102(21): 9037-9051.

Hu, H.P., Liu, Y.C., Mo, W.P., Huang, L.H., Zhang, Y.F., Li, T.H., Chen, R. & Xie, Y.Z. 2017. Isolation, characterization and anti-cancer activity of two *Ganoderma leucocontextum* srains. *Acta Edulis Fungi* 24(1): 50-54.

Hu, H.P., Zhang, Y.F., Zhang, Z., Shao, M.C., Wu, Q.P., Keto, M. & Yang, X.B. 2011. Bioactive components in cultivated fruit bodies of three wild *Ganoderma* strains collected from Tanzania. *Acta Edulis Fungi* 18(3): 64-66.

Isaac, I.L., Yin, W.A.W.C., Bakar, M.F.A., Idris, A.S., Bakar, F.D.A., Bharudin, I. & Murad, A.M.A. 2018. Transcriptome datasets of oil palm pathogen *Ganoderma boninense. Data in Brief* 17: 1108-1111.

Jia, H.Y., Wang, Y.T., Zhang, Z.H., Feng, N., Liu, Y.F., Zhou, S.Z., Zhang, Z., Zhang, J.S. & Tang, Q.J. 2017. Determination of triterpenoids in *Ganodema lingzhi* from different areas and species by HPLC. *Microbiology China* 44(1): 238-244.

Kubota, T., Yukihiro, A., Iwao, M. & Hideo, M. 1982. Structures of ganoderic acid A and B, two new lanostane type bitter triterpenes from *Ganoderma lucidum* (FR.) KARST *Helvetica Chimica Acta* 65(2): 611-619.

Li, P., Deng, Y., Wei, X. & Xu, J. 2013. Triterpenoids from *Ganoderma lucidum* and their cytotoxic activities. *Natural Product Research* 27(1): 17-22.

Li, T., Hu, H., Deng, W., Wu, S., Wang, D. & Tamdrin, T. 2015a. *Ganoderma leucocontextum*, a new member of the *G. lucidum* complex from southwestern China. *Mycoscience* 56(1): 81-85.

Li, Y., Li, T.H., Yang, Z.L., Bau, T. & Dai, Y.C. 2015b. *Atlas of Chinese Macrofungal Resources.* Zhengzhou: Zhongyuan People's Publishing House. pp. 401-412.

Li, X.M., Xie, Y.Z., Peng, J.J., Hu, H.P., Wu, Q.P. & Yang, B.B. 2019. Ganoderiol F purified from *Ganoderma leucocontextum* retards cell cycle progression by inhibiting CDK4/CDK6. *Cell Cycle* 18(21): 1-14.

Lin, Z.B. 2001. The integrated studies on *Ganoderma lucidum* Kayst. guided by traditional Chinese medical theories. *Chinese Journal of Integrated Traditional Western Medicine* 21(12): 883-884.

Liu, W., Tang, Q.J., Zhang, G.Y., Feng, N. & Han, W. 2018. *In vitro* anti-tumor and immunological activity of *Ganoderma lobatum*. *Microbiol China*. 45(4): 819-824.

Liu, Y.C., Liu, P., Hu, H.P., Li, D. & Li, Y. 2016. *Tricholosporum*, a newly recorded genus of Agaricomycetes in China. *Phytotaxa*. 289(3): 263-270.

Luangharn, T., Karunarathna, S.C., Khan, S., Xu, J., Mortimer, P. & Hyde, K. 2017. Antibacterial activity, optimal culture conditions and cultivation of the medicinal *Ganoderma australe*, new to Thailand. *Mycosphere* 8(8): 1108-1123.

Mehmet, S.D., Mehmet, C.Y., Ayşe, G., İzzet, Y. & Akdag, M.Z. 2017. The protective effect of melatonin and *Ganoderma lucidum* against the negative effects of extremely low frequency electric and magnetic fields on pulp structure in rat teeth. *Biotechnology & Biotechnological Equipment* 31(5): 979-988.

Osińska-Jaroszuk, M., Jaszek, M., Mizerska-Dudka, M., Błachowicz, A., Rejczak, T.P., Janusz, G., Wydrych, J., Polak, J., Jarosz-Wilkołazka, A. & Kandefer-Szerszeń, M. 2014. Exopolysaccharide from *Ganoderma applanatum* as a promising bioactive compound with cytostatic and antibacterial properties. *BioMed Research International* 2014(1): 743812-743812.

Raseta, M., Popovic, M., Capo, I., Stilinovic, N., Vukmirovic, S., Milosevic, B. & Karaman, M. 2020. Antidiabetic effect of two different *Ganoderma* species tested in alloxan diabetic rats. *RSC Advances* 10(17): 10382-10393.

Saccardo, P.A. & Saccardo, D. 1905. Supplementum universale. Pars VI. Hymenomycetae-Laboulbeniomycetae. *Sylloge Fungorum* 17: 860.

Sliva, D., Loganthan, J., Jiang, J., Jedinak, A., Lamb, J.G., Terry, C., Baldridge, L.A., Adamec, J., Sandusly, G.E. & Dudhgaonkar, S. 2012. Mushroom *Ganoderma lucidum* prevents colitis-associated carcinogenesis in mice. *PLoS ONE* 7(10): 1-13.

Syahriel, A., Yee, S.L., Sylvia, J.D., Arnnyitte, A. & Khim, P.C. 2018. *Ganoderma boninense* isolated from Sabah, Malaysia exhibits potent antibacterial activity against clinically important bacterial pathogens. *Bangladesh Journal of Pharmacology* 13(1): 10-12.

Wang, D.M., Wu, S.H., Su, C.H., Peng, J.T., Shi, Y.H. & Chen, L.Z. 2009. *Ganoderma multipileum*, the correct name for '*G. lucidum*' in tropical Asia. *Botanical Studies* 50(4): 451-458.

White, T.J., Bruns, T.D., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols A Guide to Methods & Applications,* edited by Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. New York: Academic Press, Inc. pp. 315-322.

Wu, Q.P., Xie, Y.Z., Deng, Z.Q., Li, X.M., Yang, W.N., Jiao, C.W., Ling, F., Li, S.Z., Pan, H.H. & Yee, A.J. 2012. Ergosterol peroxide isolated from *Ganoderma lucidum* abolishes microRNA miR-378-mediated tumor cells on chemoresistance. *PLoS ONE* 7(8): 1-14.

Xing, J.H. & Cui, B.K. 2018. *Ganoderma* species diversity, classification and phylogenetic studies. *Chinese Society of Fungus Academic Conference* in Shandong Taian, China. p. 295.

Xing, J.H., Sun, Y.F., Han, Y.L., Cui, B.K. & Dai, Y.C. 2018. Morphological and molecular identification of two new *Ganoderma* species on *Casuarina equisetifolia* from China. *Mycokeys* 34: 93-108.

Xing, J.H., Song, J., Decock, C. & Cui, B.K. 2016. Morphological characters and phylogenetic analysis reveal a new species within the *Ganoderma lucidum* complex from South Africa. *Phytotaxa* 266(2): 115-124.

Xing, Z.T., Men, D.Y., Tang, Q.J., Wang, N., Li, M.R. & Guan, S.M. 2008. *NY/T 1676-2008 Determination of Crude Mushroom Polysaccharides*. The Ministry of Agriculture of the People's Republic of China. Beijing: China Agriculture Press.

Yong, T.Q., Chen, S.D., Xie, Y.Z., Chen, D.L., Su, J.Y., Shuai, O., Jiao, C.W. & Zuo, D. 2018. Hypouricemic effects of *Ganoderma applanatum* in hyperuricemia mice through OAT1 and GLUT9. *Frontiers in Pharmacology* 8(996): 1-11.

Zhao, J.D. & Zhang, X.Q. 2000. *Flora Fungorum Sinicorum Vol. 18 Ganodermataceae*. Beijing: Science Press.

Zhou, L.W., Cao, Y., Wu, S.H., Josef, V., Li, D.W., Li, M.J. & Dai, Y.C. 2014. Global diversity of the *Ganoderma lucidum* complex (Ganodermataceae, Polyporales) inferred from morphology and multilocus phylogeny. *Phytochemistry* 114: 7-15.

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