

# Screening of nutritive values, *in vitro* antioxidant, anticancer and antimicrobial activities from *Boletus griseipurpureus* Corner

Yuttana Sudjaroen, Kanittada Thongkao

Department of Applied Sciences, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Dusit, Bangkok 10300, Thailand

## Abstract

**Background:** *Boletus griseipurpureus* Corner is a popular edible mushroom in the South of Thailand, which were rich in protein and very low in fat and possessed antibacterial. **Aims:** The aims of the study are to evaluate nutritive values and biological activities of *B. griseipurpureus* including antioxidant, anticancer, cytotoxicity and antimicrobial activities. **Materials and Methods:** Nutritive values of fresh mushrooms were analyzed mainly according to AOAC. Total phenolic content (TPC) and antioxidant activity of dichloromethane (BD) and methanol (BM) extracts were performed by of Folin–Ciocalteu reagent; DPPH radical, 2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) cation radical scavenging assays and oxygen radical absorbance capacity. Anticancer activities of BD and BM were performed by resazurin microplate assay and tested with five cancer cell lines. Cytotoxicity of BD and BM were performed by same method and used normal cells. Antimicrobial activities of BD and BM against herpes simplex virus type 1 (HSV-1) and *Mycobacterium tuberculosis* were tested according to green fluorescent protein-based assay; *Plasmodium falciparum* was tested according to microculture radioisotope techniques; antibacterial and anti-*Candida albicans* tests were performed according to CLSI. **Results:** The relatively high content of copper, zinc, and selenium was contained in *B. griseipurpureus* when compared with Thai RDI values. Preferable TPC and antioxidant activities of BD and BM were also revealed. No anticancer activity (to KB, MCF7, NCI-H187, HepG2 and CaCO<sub>2</sub> cell lines) and cytotoxicity of (to Vero and human dermal fibroblasts, neonatal cells) from both extracts. Only, BM was inhibited HSV-1 (IC<sub>50</sub> = 41.67 µg/ml). **Conclusions:** The basidiomes of *B. griseipurpureus* were the relatively high content of antioxidant trace elements; and possessed antioxidant and HSV-1 inhibitory activities.

**Key words:** Anticancer, anti-herpes simplex virus type I, antimicrobial, antioxidant, *Boletus griseipurpureus* Corner, cytotoxicity

## INTRODUCTION

The edible mushrooms are both popular delicacies and important nutraceuticals and have shown that mushrooms are low in fat and rich in polysaccharides, proteins, and minerals, making them a good food for preventing diabetes and cardiovascular disease.<sup>[1-3]</sup> Evaluation of the chemical composition and biological activities of edible mushrooms has recently been an active area of research. Studies of this type enable the discovery of fungal species with high nutritional value and important biological activities. In particular, the mineral content of mushrooms has recently become a universal concern. On the one hand, numerous reports have pointed out

that mushrooms are excellent accumulators of minerals, so they can be a good source of minerals for human nutrition.<sup>[4-6]</sup> Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health. They produce a wide range of

### Address for correspondence:

Dr. Yuttana Sudjaroen, Faculty of Science and Technology, Suan Sunandha Rajabhat University, 1 U-Thong-Nok Rd., Dusit, Bangkok 10300, Thailand, Phone: (66)-2-160-1143-5, Fax: (66)-2-160-1143-6. E-mail: yuttana.su@ssru.ac.th

**Received:** 20-02-2017

**Revised:** 03-03-2017

**Accepted:** 08-03-2017

secondary metabolites with high therapeutic value. Health promoting properties - e.g., antioxidant, antimicrobial, anticancer, cholesterol lowering, antihyperglycemic and immunostimulatory effects - have been reported for some species of mushrooms. Both fruiting bodies and the mycelium contain compounds with wide ranging antioxidant and antimicrobial activities.<sup>[4,7-11]</sup> *Boletus* mushrooms are wild edible mushrooms that are consumed worldwide. They are popular because their flesh is tender and rich in nutrients.<sup>[4]</sup>

In Thailand and nearby countries, e.g., Malaysia wild edible mushrooms are important sources of food for local people.<sup>[12-15]</sup> Wild and cultivated mushrooms are considered to be good for human consumption because they are generally low in energy and fat but high in protein, fiber and carbohydrate.<sup>[16]</sup> They may also have strong antioxidant activity.<sup>[3,4,11,17,18]</sup> *Boletus griseipurpureus* Corner 1972 (*Boletaceae*) is a wild edible ectomycorrhizal mushroom,<sup>[19-21]</sup> rich in protein and low in fat. Thai local name is called “Hed Sa med,” characterized by (1) Macroscopic features included pileus 3.0-6.0 cm across, convex to plane, gray to purple or light purple, surface covered with gray black short soft hairs when young; fresh white and firm when young, later softer, unchanging when injured; tube small and firm, pallid white when young, later pinkish to pale brown; and stipe 3.0-6.0 cm long, 1.5-3.0 cm across, purple, cylindrical to clavate, increasing in thickness to clavate toward the base, sometime with pale brown net at the apex. (2) Microscopic features: Basidia 24.0-40.0 × 8.0-10.5 μ; clavate; basidiospores 3.1-4.9 × 7.8-10.7 μ, cylindric, pinkish pale brown and smooth.<sup>[15]</sup> Many of these are ectomycorrhizal fungi, often associated with specific host trees. *B. griseipurpureus* is most frequently associated with *Melaleuca leucadendron* (Sa Med Khao or cajeput tree)<sup>[19-21]</sup> and *Acacia mangium*. This mushroom has been reported as being ectomycorrhizal with *M. leucadendron*, *Acacia auriculiformis*, *Gustavia gracillima*, *Eucalyptus camaldulensis*, and *Casuarina equisetifolia*<sup>[15]</sup> but this needs confirmation. *B. griseipurpureus* can command a high price and a popular edible mushroom in the South of Thailand,<sup>[19-21]</sup> which has a bitter taste but is quite tasty in local cuisine. In the previous study, the proximal analysis of *B. griseipurpureus* basidiomes showed that basidiomes were rich in protein and very low in fat.<sup>[20]</sup> The proximate composition of *B. griseipurpureus* was compared with three other Thai edible fungi, including *Phlebopus portentosus*, another sought after ectomycorrhizal bolete, protein concentration in *B. griseipurpureus* was higher than in many ectomycorrhizal fungi and fat concentration in *B. griseipurpureus* was lower than in *P. portentosus* but similar to *C. bibarius* (0.7%). Most edible mushrooms have high carbohydrate contents; however, *B. griseipurpureus* (33%) was lower than other wild edible Thai mushrooms which ranged from 42% to 65%. Thus, the food value of *B. griseipurpureus* was high in terms of the relatively high protein and low-fat contents.<sup>[20]</sup> Recently, study was reported antibacterial activities of *B. griseipurpureus* (basidiocarp) extracts were evaluated with *Escherichia coli* ATCC 25922,

*Staphylococcus aureus* ATCC 29523 and methicillin-resistant *S. aureus* (MRSA) 189 by using the agar-well diffusion method and found that crude methanol extract (200 mg/ml) of basidiomes inhibited the growth of all bacteria *in vitro* more than the crude ethyl acetate extract. We were interested to conduct the research study, which aimed to evaluate more nutritive values/composition of *B. griseipurpureus*, such as some of antioxidant trace elements and vitamins, and also biological activities for determine health promoting benefits including antioxidant, anticancer, cytotoxicity, and antimicrobial activities of *B. griseipurpureus* extracts. This finding can promote its beneficial values for consumers in local area and more widen cultivate as agricultural product in the Southern part of Thailand.

## MATERIALS AND METHODS

### Sample Extraction

Basidiomes of *B. griseipurpureus* were collected from under *M. leucadendron* in Ranong province, which nearby Ranong campus, Suan Sunadha Rajabhat University. Additional basidiomes were opportunistically purchased from city and roadside markets. Identification of *B. griseipurpureus* was clarified by local experts. The characteristic of *B. griseipurpureus* was recorded such as size, color, spore print and morphology of pileus and stripe. The microscopic characteristic was done as described by Seehanan and Petcharat.<sup>[15]</sup> Fresh basidiomes (1 kg) were dried in constant weigh before evaluation of nutritive values. Another, fresh basidiomes (450 g) were selected, cut in small pieces and then air dried. The air dried basidiomes (60 g) was grinded in powder and kept for screening biological test. Bring ground basidiomes for continuous extraction, then, extract with dichloromethane and methanol using Soxhlet apparatus. Finally, get the solvent evaporated through rotary evaporation apparatus under vacuum. The extracts were evaluated for total phenolic content (TPC) and antioxidant assays. The extracts could also be dissolved in dimethyl sulfoxide (DMSO) and be test to the anticancer and antimicrobial activities onward. All assays were carried out in triplicate, and the results were described as mean values and standard deviation.

### Evaluation of the Nutritive Values

#### Proximate analysis

The proximate analysis was performed according to the methods to be described, or based on the Official Methods of Analysis of AOAC International, 16<sup>th</sup> ed.<sup>[22]</sup> The fresh samples were used for the water, crud protein, crude fat, ash, carbohydrate content determination.<sup>[22]</sup>

#### Determination of β-carotene and vitamin E

- Measure β-carotene was applied from the extract method of Munzuroglu *et al.*<sup>[23]</sup> High performance liquid

chromatography (HPLC) separations were accomplished at room temperature with a Perkin-Elmer liquid chromatograph system (Series 1100), consisting of a sample injection valve (Cotati 7125) with a 20 ml sample loop, an ultraviolet (UV) spectrophotometric detector (Cecil 68174), integrator (HP 3395) and a Techsphere ODS-2 packed (5 mm particle and 80 Å pore size) column (250 × 4.6 i.d.) with a methanol: acetonitrile:chloroform (47:42:11, v/v) mobile phase at 1 ml/min flow rate.

- b. Measure vitamin E was applied from the extract method of Qian *et al.*<sup>[24]</sup> Chromatographic separations were performed on a 150 × 3.9 mm Novapak C column (Waters). Methanol was used as mobile phase at a flow-rate of 1.5 ml/min and a pressure of 1000 p.s.i. (1 p.s.i. = 6894.76 Pa) All injections were 50-ml loop injections on an M710B autosampler (waters). A Model M510 waters pump and a Model M490 waters variable Wavelength UV–visible detector set at 290 nm were used. All quantitation was by peak area using a Waters M740 integrator. Based on the established chromatographic conditions, repeated injections of 0.1, 0.5, 1, 5 and 10 mg of the standard vitamin E was made duplicated onto the HPLC system. The retention time for vitamins E was 4.1 min. A Shimadzu MPS-2000 universal spectrophotometric scanner was used to determine the spectrograms of vitamin E in *n*-butanol.

### Determination of minerals

The microwave-assisted treatment was adapted from that employed by us for the determination of the mineral profile of diets.<sup>[25]</sup> Calcium, iron, copper, zinc, and selenium determinations were done by inductively coupled plasma optical emission spectrometry (ICP-OES) techniques. ICP-OES Optima 5300 DV inductively coupled plasma optical emission spectrometer Perkin Elmer (Norwalk, CT, USA) equipped with an autosampler AS 93-plus, and an ultrasonic nebulizer U6000AT+ Cetac (Nebraska EEUU) was used for all mineral determination. The calibration standards were prepared in 0.5% nitric acid. The calibration range for all elements was evaluated from 0.05 to 2 mg/L except calcium for which calibration curves was prepared from 2 to 10 mg/L. Ruthenium (1 mg/L) was used as internal standard and added to all samples, reagent blanks and standards.

### TPC

In this step, 0.1 ml of 1 mg sample extract was input into the test tube, mixing with 4.6 ml distilled water and 1 ml of Folin–Ciocalteu reagent. After that, the extract was left inside the room in room temperature for 3 min. Next, 3 ml of 2% Na<sub>2</sub>CO<sub>3</sub> (w/v) was filled into the tube and shaken with the speed of 150 RpM for 2 h. Then, the extract was measured to find out the light absorbance at 760 nm by comparing with the gallic acid at the intensity of 1, 0.875, 0.75, 0.625, 0.5, 0.375, 0.25, and 0.125 mg/ml. The TPC was calculated into mg of gallic acid per g of the extract.<sup>[26]</sup>

### Assays for Antioxidant Activity

- a. DPPH radical scavenging assay to measure the decreasing light absorbance of DPPH radical<sup>[27]</sup> using negative control by DPPH radical ( $6 \times 10^{-5}$  M), promptly measure at nm and positive control using vitamin C.
- b. 2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) cation radical scavenging assay similar to the 1<sup>st</sup> method but using ABTS radical instead<sup>[28]</sup> and also using Trolox (soluble vitamin E) as standard substance to create standard graph (0.5-5.0 mg/ml concentration). The antioxidant activity of the dried seablite would be shown in Trolox equivalent antioxidant capacity (TEAC)/g of the dried seablite extracts.
- c. Oxygen radical absorbance capacity (ORAC) to measure the ability of extract to scavenge oxygen radical<sup>[29]</sup> and the florescent signal generated by fluoresceine sodium salt (Sigma-aldrich, Inc.) was measured by FLUOstar OPTIMA microplate reader (BMG) on 1 h. The antioxidant activity of dried seablite would be also shown in TEAC/g of the dried seablite extracts.

### Anticancer Activity and Cytotoxicity Tests

Five cell lines including epidermoid carcinoma of the oral cavity (KB) ATCC CCL-17, breast adenocarcinoma (MCF-7) ATCC HTB-22, small cell lung carcinoma (NCI-H187) ATCC CRL-5804, human hepatocarcinoma (HepG2) ATCC HB-8065, and human colon adenocarcinoma (CaCO<sub>2</sub>) ATCC HTB-37 were used in this study. The resazurin microplate assay developed by O'Brien *et al.*<sup>[30]</sup> was performed for anticancer test. In brief, the cells were cultured in proper condition and diluted by culture medium at  $2.2\text{--}3.3 \times 10^4$  cells/ml. The next step was to add the 5% DMSO 50 µl into cell suspension 45 µl in the 384-well plates. Then, the extract was incubated at 37°C in the incubator which contained 5% of CO<sub>2</sub>. After incubation (3-5 days), 12.5 µl of resazurin (62.5 µg/ml) was added. The incubation was continued for 4 h, then measured fluorescence signal by SpectraMax M5 multidetection microplate reader (Molecular Devices, USA) at excitation and emission wavelength of 530 and 590 nm, respectively. Dose-response curve could be done in the 6<sup>th</sup> test. 3-fold serial intensity dilution and the intensity of the cell-restraint extract 50% (IC<sub>50</sub>) could be calculated by SOFTMax Pro software (Molecular Devices, USA). Ellipticine, doxorubicin, and tamoxifen were used as positive control. 0.5% DMSO and water were used as negative control. For cytotoxicity test, Vero cells (African green monkey) and human dermal fibroblasts, neonatal (HDFn) C-004-5C were used for evaluated cytotoxicity of karaunda fruit by the same method.



## Antimicrobial Activity Test

### *Ant herpes simplex virus type 1 (HSV-1) test*

Before the test of antiviral activity, there should be the cytotoxicity test conducted first to make sure that the extract is non-cytotoxic. The antiviral test was conducted by green fluorescent protein (GFP)-based assay.<sup>[31]</sup> The extracts diluted by 10% DMSO at 10 µl/well were added into 96-well plate. Next, added GFP-expressing Vero cell suspension  $1 \times 10^5$  cells/ml mixed with HSV-1 (ATCC VR260)  $5 \times 10^5$  PFU/ml for 190 µl/well. Then, the sample was incubated at 37°C by incubator which has 5% of CO<sub>2</sub> for 4 days. After that, the fluorescence signal was measured by SpectraMax M5 multidetection microplate reader (Molecular Devices, USA) at excitation and emission wavelength 485 and 535 nm, respectively (bottom-reading mode). Fluorescence signal from the 4<sup>th</sup> day of incubation will be deducted on the 1<sup>st</sup> day (day = 0) of incubation. The IC<sub>50</sub> was calculated by SoftMax Pro software (Molecular Devices, USA) from testing 6 levels of 2-fold serial dilution extracts. Acyclovir was used as positive control and 0.5% DMSO was used as negative control.

### *Antibacterial and anti-Candida albicans tests*

Six bacteria, *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (ATCC 17978), and *Enterococcus faecalis* (ATCC 29212); and one yeast, *Candida albicans* (ATCC 90028) were used as tested organisms. The bacterial inoculum was prepared in tryptic soy broth, in case of *C. albicans*, Sabouraud dextrose broth was used. The number of cfu/ml in the inoculum was determined and standardized. A 0.5 McFarland standard is comparable to a bacterial suspension of 10<sup>8</sup> cfu/ml. The minimum inhibition concentration (MIC) was determined by the broth microdilution method using 96-well microtiter plates.<sup>[32]</sup> Concentrations range from 1.0 to 50.0 mg/mL for extracts was used in experiment against every microorganism tested. The starting solutions of extracts were obtained by measuring off a certain quantity of extract and dissolving it in DMSO. The dilutions of extracts were prepared in Müller-Hinton broth for bacterial cultures and potato dextrose agar broth for *C. albicans* cultures. The MIC was determined by establishing visible growth of microorganisms. The boundary dilution without any visible growth was defined as the MIC for the tested microorganism at the given concentration. As a positive control of growth inhibition, streptomycin was used in case of bacteria; amphotericin B in case of yeast was used as positive control and 0.5% DMSO was used as negative control.

### *Antimalarial activity test*

*Plasmodium falciparum* (K1, multi-drug resistant strain) was cultured in the test tube (*in vitro*) developed by Trager and Jensen method.<sup>[33]</sup> It was cultured by RPMI 1640 medium

which had 20 mM of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 32 mM of NaHCO<sub>3</sub> and 10% of heat inactivated human serum with 3% of erythrocyte mixed together. Then, it was incubated at 37°C by 3% CO<sub>2</sub> in CO<sub>2</sub> incubator. The culture medium and erythrocyte were changed every day during the test. The evaluation of *in vitro* antimalarial test was performed by microculture radioisotope techniques.<sup>[34]</sup> This experiment used 1 and 10 µg/ml extract to prevent *P. falciparum* to calculate the IC<sub>50</sub>. Dihydroartemisinin and 0.1% DMSO was used as positive control and negative control, respectively.

### *Antimycobacterium test*

GFP expressing *Mycobacterium tuberculosis* H<sub>37</sub>Ra strain (H<sub>37</sub>Ra *gfp*) culture was developed by Changsen *et al.*<sup>[35]</sup> and Collins *et al.*<sup>[36]</sup> H<sub>37</sub>Ra *gfp* was cultured on plate 7H10 agar consisting of kanamycin 30 µg/ml. The incubation at 37°C was 4 weeks long. After that, the single colony of the cell was taken to culture on 7H9 broth which had 0.2% of glycerol v/v, 0.1% of casitone w/v, 0.05% of Tween 80 v/v, 10% of Middlebrook OADC enrichment solution (BD Biosciences) v/v and 30 µg/ml of kanamycin. All substances were incubated at 37°C in 200 rpm shaker incubator until the 550 nm optical density was around 0.5-1. For batch cultivation, 1/10 of the ingredient above was taken to incubate at the same condition. Then, the cells were cleansed and suspended by phosphate-buffered saline buffer and then were sonicated 8 times (15 s per time). The cultures were divided into tubes and kept at -80°C for 2-3 months before experiment session. During the test session, the cells were tested their density in 384-well plate at around  $1 \times 10^5$  CFU/ml/well. The tests took 4 times (quadruplicate), or within 4 wells/test. Each testing plate was contained 5 µl of 0.5% DMSO (diluted by serial dilution) and 45 µl cell suspension. The plate was incubated at 37°C for 10 days. To find out the fluorescence signal, I used SpectraMax M5 multidetection microplate reader (Molecular Devices, USA) with excitation and emission wavelength 485 and 535 nm respectively (bottom-reading mode). The fluorescence signal on 10<sup>th</sup> incubation day was deducted on = 0 of incubation. It could be calculated in MIC by rifampicin, ofloxacin, isoniazid, and ethambutol as positive control and 0.5% DMSO as negative control.

## Statistical Analyses

TPC and antioxidant values are expressed as mean ± standard deviation. Statistical analyses were performed with the EXCEL (version 11) and SPSS (version 13) software packages, to determine independent *t*-test was test for different among mean of antioxidant values between *B. griseipurpureus* extracted with dichloromethane (BD) and *B. griseipurpureus* extracted methanol (BM); and Pearson's bivariate correlation test was carried out to calculate correlation coefficients (R<sub>2</sub>) between the content of TPC and the DPPH, ABTS radical scavenging and ORAC activities.

## RESULTS

The nutrition values of *B. griseipurpureus* were presented in Table 1. It was found that basidiomes of BD contained

**Table 1: Nutritive values of *B. griseipurpureus***

Basidiomes	Results	Units	LOD	Thai RDI (unit)
Ash	0.22	g/100 g	-	-
Carbohydrate	5.44	g/100 g	-	300 g
Total fat	0.14	g/100 g	-	65 g
Moisture	10.1	g/100 g	-	-
Protein (g) (%N×6.25)	4.28	g/100 g	-	50 g
Vitamin A	Not detected	µg/100 g	7.00	800 µg
Vitamin E	Not detected	µg/100 g	0.05	10 mg
Sodium	228.08	mg/kg	-	2400 mg
Magnesium	32.31	mg/kg	-	350 mg
Calcium	33.98	mg/kg	-	800 mg
Iron	10.41	mg/kg	-	15 mg
Copper	1.832	mg/kg	-	2 mg
Zinc	12.71	mg/kg	-	15 mg
Selenium	1.66	mg/kg	-	70 µg

LOD: Limit of detection, Thai RDI: Thai recommended dietary intake, *B. griseipurpureus*: *Boletus griseipurpureus*

crude extract lower than basidiomes BM by concentration =  $5.1 \pm 0.5$  and  $19.4 \pm 0.9$  mg of gallic acid equivalent/g of extract, respectively, which was related to preferable potential of antioxidant activities, which were 50-80 and 120-200 µmol TEAC for BD and BM, respectively [Table 2]. BD and BM extracts were not possessed anticancer activity to KB-oral cavity cancer, MCF7-breast cancer, NCI-H187-small lung cancer, HepG2-hepatocarcinoma, and CaCO<sub>2</sub>-colon adenocarcinoma cell lines at maximum concentration = 100 µg/ml [Table 3]. In the same way to anticancer activities, no any cytotoxic effect of both extracts to Vero cells and HDFn-neonatal dermal fibroblast [Table 3], which implied that both extract were not harm human normal cells and also human cancer cell lines. Only, BM was significantly inhibited HSV-1 at IC<sub>50</sub> = 41.67 µg/ml (BD was lack of activity), was shown in Table 4. However, both of extracts were not inhibited *M. tuberculosis* H<sub>37</sub>Ra strain, *P. falciparum*, K1 strain, all tested bacteria and *C. albicans* [Table 5].

## DISCUSSION

The nutritive screening of *B. griseipurpureus* was revealed high protein and low fat, which correspond to the previous study.<sup>[20]</sup> The relatively high content of copper, zinc, and selenium was antioxidant trace elements, which were contained in basidiomes of *B. griseipurpureus* when compared with Thai RDI values. Preferable TPC and antioxidant activities of BD and BM were also similar to other *Boletus* species as previous studies.<sup>[3,4,11,16-18]</sup> This study

**Table 2: Total phenolic content and antioxidant activities of BD and BM**

Extract**	TPC (mg GAE)	DPPH (µmol TEAC)	ABTS* (µmol TEAC)	ORAC (µmol TEAC)	R <sup>2</sup>
BD	5.1±0.5	52.0±1.3	56.4±8.2	78.6±3.4	0.965*
BM	19.4±0.9	136.5±9.1	153.2±12.6	184.3±13.8	1.000*

TPC: Total phenolic content, GAE: Gallic acid equivalent/g of extract, TEAC: Trolox equivalent antioxidant capacity/g of extract, ORAC: Oxygen radical absorbance capacity, ABTS: 2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), BD: *B. griseipurpureus* extract with dichloromethane, BM: *B. griseipurpureus* extract with methanol, *B. griseipurpureus*: *Boletus griseipurpureus*. \*Correlation is significant at  $P < 0.01$  level. \*\*The significant different of antioxidant values (from three methods) between BD and BM at  $P < 0.05$  level

**Table 3: Anticancer activity of BD and BM against KB, MCF7, NCI-H187, HepG2 and CaCO<sub>2</sub> cell lines; and cytotoxicity of BD and BM with HDFn and Vero cells<sup>a</sup>**

Extract (100 µg/ml)	%cytotoxicity of KB <sup>a</sup>	%cytotoxicity of MCF7 <sup>b</sup>	%cytotoxicity of NCI-H187 <sup>c</sup>	%cytotoxicity of HepG2 <sup>d</sup>	%cytotoxicity of CaCO <sub>2</sub> <sup>e</sup>	%cytotoxicity of HDFn <sup>f</sup>	%cytotoxicity of Vero <sup>g</sup>
BD	-5.94	-12.70	0.253	-5.11	7.74	8.77	-19.25
BM	1.05	-16.01	-3.92	-8.42	2.62	-2.45	-12.16

### Interpretation

% cytotoxicity	Activity
<50	Non-cytotoxic
≥50	Cytotoxic

<sup>a</sup>IC<sub>50</sub> of positive control: Ellipticine=1.81 µg/ml, doxorubicin=0.655 µg/ml; <sup>b</sup>IC<sub>50</sub> of positive control: Tamoxifen=6.96 µg/ml, doxorubicin=8.07 µg/ml; <sup>c</sup>IC<sub>50</sub> of positive control: Ellipticine=1.65 µg/ml, doxorubicin=0.088 µg/ml; <sup>d</sup>IC<sub>50</sub> of positive control: Ellipticine=1.82 µg/ml; <sup>e</sup>IC<sub>50</sub> of positive control: Ellipticine=14.17 µg/ml; <sup>f</sup>IC<sub>50</sub> of positive control: Ellipticine=2.89 µg/ml; <sup>g</sup>IC<sub>50</sub> of positive control: Ellipticine=1.43 µg/ml;

<sup>h</sup>Negative control: 0.5% DMSO. DMSO: Dimethyl sulfoxide, BD: *B. griseipurpureus* extract with dichloromethane, BM: *B. griseipurpureus* extract with methanol, HDFn: Human dermal fibroblasts, neonatal, *B. griseipurpureus*: *Boletus griseipurpureus*

**Table 4:** Antiviral activity of BD and BM against HSV-1

Sample	Final conc. (µg/ml)	Fluorescence unit (0 day)	Fluorescence unit (4 days)	% inhibition	Activity	IC <sub>50</sub> (µg/ml)
BD	50.00	2.304±166	3.183±165	5.18	Inactive	-
BM	50.00	2.215±21	4.624±508	60.08	Active	41.67
Acyclovir (positive control)	20.00	1.808±153	5.088±327	91.27	Active	2.17
	10.00	1.760±98	4.733±337	80.27	Active	
	5.00	1.845±172	4.833±452	80.82	Active	
	2.50	1.812±163	4.089±402	55.32	Active	
	1.25	1.806±146	3.243±329	25.19	Active	
	0.63	1.715±140	2.871±182	15.09	Active	
0.5% DMSO (negative control)	-	2.105±179	5.628±327	-	-	-

DMSO: Dimethyl sulfoxide, BD: *B. griseipurpureus* extract with dichloromethane, BM: *B. griseipurpureus* extract with methanol, HSV: Herpes simplex virus type 1, *B. griseipurpureus*: *Boletus griseipurpureus*

**Table 5:** The summary of antimicrobial activity of BD and BM against HSV-1, *M. tuberculosis*, *C. albicans* and *P. falciparum*<sup>a</sup>

Antimicrobial activity	BD		BM	
	% Inhibition	Activity	% Inhibition	Activity
<i>M. tuberculosis</i> <sup>a</sup>	-47.07	Inactive	-18.30	Inactive
<i>P. falciparum</i> <sup>d</sup>	-42.10	Inactive	9.74	Inactive
<i>S. aureus</i> <sup>c</sup>	22.35	Inactive	25.46	Inactive
<i>E. coli</i> <sup>c</sup>	15.28	Inactive	21.17	Inactive
<i>K. pneumoniae</i> <sup>c</sup>	14.20	Inactive	19.46	Inactive
<i>P. aeruginosa</i> <sup>c</sup>	12.18	Inactive	15.16	Inactive
<i>A. baumannii</i> <sup>b</sup>	9.45	Inactive	14.48	Inactive
<i>E. faecalis</i> <sup>c</sup>	10.25	Inactive	15.87	Inactive
<i>C. albicans</i> <sup>d</sup>	8.26	Inactive	11.15	Inactive

<sup>a</sup>IC<sub>50</sub> of positive control: Rifampicin=0.0125 µg/ml, isoniazid=0.0469 µg/ml, ofloxacin=0.391 µg/ml, ethambutol=0.938 µg/ml; <sup>b</sup>IC<sub>50</sub> of positive control: Dihydroartemisinin=2.47 nM, mefloquine=64.12 nM; <sup>c</sup>IC<sub>50</sub> of positive control: Streptomycin=31.25, 31.25, 1.95, 15.62, 15.62, 15.62 µg/ml for *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and *E. faecalis*, respectively; <sup>d</sup>IC<sub>50</sub> of positive control: Amphotericin B=0.108 µg/ml; <sup>e</sup>Negative control: 0.5% DMSO. DMSO: Dimethyl sulfoxide, BD: *B. griseipurpureus* dichloromethane extract, BM: *B. griseipurpureus* methanol extract, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *A. baumannii*: *Acinetobacter baumannii*, and *E. faecalis*: *Enterococcus faecalis*, *M. tuberculosis*: *Mycobacterium tuberculosis*, *P. falciparum*: *Plasmodium falciparum*, *C. albicans*: *Candida albicans*, HSV: Herpes simplex virus type 1, *B. griseipurpureus*: *Boletus griseipurpureus*

was revealed a lack of anticancer activities of BD and BM (maximum concentration = 100 µg/ml) against KB, MCF7, NCI-H187, HepG2 and CaCO<sub>2</sub> cell lines, and no cytotoxicity to normal cell also (Vero and HDFn cells). Anticancer activity of *Boletus* species was reported in literature review, however, its activity via immunomodulation and cell cycle arrest.<sup>[37,38]</sup> Thus, there may conclude that BD and BM had no cytotoxic effect with cancer cell lines and normal cells, which are safe when concerning for consumers. In case of antiviral activity, only BM was inhibited HSV-1 (IC<sub>50</sub> = 41.67 µg/ml). Rare report for antiviral activity of *Boletus* species was described by Sun *et al.*,<sup>[39]</sup> hallucinogenic mushroom *Boletus speciosus*, which was exhibited HIV-1 reverse transcriptase inhibitory activity with an IC<sub>50</sub> of 7.1 µM.

However, BD and BM were a lack of antimicrobial activities along with various pathogens including, Six bacteria (*S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii* and *E. faecalis*); yeast, *C. albicans*; malaria, *P. falciparum* and *M. tuberculosis*. Previous study was reported antibacterial activities of *B. griseipurpureus* extracted by methanol and ethyl acetate, which revealed antimicrobial activities against *E. coli*, *S. aureus* and MRSA by using the agar-well diffusion method and found that crude methanol extract (200 mg/ml) of basidiomes inhibited the growth of all bacteria *in vitro* more than the crude ethyl acetate extract.<sup>[21]</sup> Our negative results were contrasted with the previous study, which may due to the low concentration of extracts, different of antibacterial assays and solubility



of extracts in mixture of assays, however, our suggestion to “cutoff” of biological assays are actually to be lower than 200 mg/ml. Time variation of mushroom collection is also important to concern that fresh basidiomes were changed from white to brown color until black color after keep during 24 h (4°C). This study was concluded prior reported for nutritional composition, antioxidant, anticancer, cytotoxicity and antimicrobial activities of two *B. griseipurpureus* extracts and characterization of active constituents are need to conduct in further study.

## CONCLUSION

The basidiomes of *B. griseipurpureus* were the relatively high content of antioxidant trace elements. TPC and antioxidant activities of BD and BM were preferable as other *Boletus* species. Only BM had HSV-1 inhibitory activity ( $IC_{50} = 41.67 \mu\text{g/ml}$ ). BD and BM had no cytotoxic effect with cancer cell lines and normal cells and also other pathogens in this study.

## ACKNOWLEDGMENT

We would like to express their gratitude to the Office of Higher Education, Ministry of Education, Thailand, and Research and Development Institute of Suan Sunandha Rajabhat University, Bangkok, Thailand, for the funding support. Authors are grateful to Faculty of Science and Technology, Saun Sunandha Rajabhat University, National Center for Genetic Engineering and Biotechnology for research facility support. Authors would like to sincerely thank Dr. Kowit Suwannahong, Department of Occupational Health, Safety and Environment, Faculty of Public Health, Western University, Kanchanaburi, for coordinating laboratory service and data interpretation. Authors would like to also sincerely thank Dr. Amornrat Aung-aud-chariya, Srivijaya Trang Campus, Rajamangala University of technology for provide useful *Boletus griseipurpureus* information as expertise.

## REFERENCES

- Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira IC. Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chem Toxicol* 2008;46:2742-7.
- Lau BF, Abdullah N, Aminudin N. Chemical composition of the tiger's milk mushroom, *Lignosus rhinocerotis* (Cooke) Ryvarden, from different developmental stages. *J Agric Food Chem* 2013;61:4890-7.
- Jaworska G, Pogon K, Skrzypczak A, Bernas E. Composition and antioxidant properties of wild mushrooms *Boletus edulis* and *Xerocomus badius* prepared for consumption. *J Food Sci Technol* 2015;52:7944-53.
- Liu Y, Chen D, You Y, Zeng S, Li Y, Tang Q, *et al.* Nutritional composition of boletus mushrooms from Southwest China and their antihyperglycemic and antioxidant activities. *Food Chem* 2016;211:83-91.
- Kojta AK, Falandysz J. Metallic elements (Ca, Hg, Fe, K, Mg, Mn, Na, Zn) in the fruiting bodies of *Boletus badius*. *Food Chem* 2016;200:206-14.
- Kalac P, Svoboda L. A review of trace element concentrations in edible mushrooms. *Food Chem* 2001;69:273-81.
- Oyetayo VO, Dong CH, Yao YJ. Antioxidant and antimicrobial properties of aqueous extract from *Dictyophora indusiata*. *Open Mycol J* 2009;3:20-6.
- Mau JL, Chang CN, Huang SJ, Chen CC. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chem* 2004;87:111-8.
- Barros L, Calhella RC, Vaz JA, Ferreira IC, Baptista P, Estevinho LM. Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *Eur Food Res Technol* 2007;225:151-6.
- Ferreira IC, Baptista P, Vilas-Boas M, Barros L. Free-radical scavenging capacity and reducing power of wild edible mushrooms from Northeast Portugal: Individual cap and stipe activity. *Food Chem* 2007;100:1511-6.
- Kosanic M, Rankovic B, Dašić M. Mushrooms as possible antioxidant and antimicrobial agents. *Iran J Pharm Res* 2012;11:1095-102.
- Lee SS, Chang YS, Noraswali MN. Utilization of macro fungi by some indigenous communities for food and medicine in Peninsular Malaysia. *For Ecol Manage* 2009;257:2062-5.
- Phosri C, Watling R, Martin MP, Whalley AJ. The genus *Astraeus* in Thailand. *Mycotaxon* 2004;89:453-63.
- Sanmee R, Dell B, Lumyong P, Izumori K, Lumyong S. Nutritive value of popular wild edible mushrooms from Northern Thailand. *Food Chem* 2003;82:527-32.
- Seehanan S, Petcharat V. Some species of wild boletes in Thailand. *J Agric Technol* 2008;4:109-18.
- Cheung PC. The nutritional and health benefits of mushrooms. *Nutr Bull* 2010;35:292-9.
- Wong JY, Chye FY. Antioxidant properties of selected tropical wild edible mushrooms. *J Food Compost Anal* 2009;22:269-77.
- Kumari D, Reddy MS, Upadhyay RC. Nutritional composition and antioxidant activities of 18 different wild *Cantharellus* mushrooms of Northwestern Himalayas. *Food Sci Technol Int* 2011;17:557-67.
- Aung-aud-chariya A, Bangrak P, Dell B, Kamlangdee N. Genetic study of *Boletus griseipurpureus* from Melaleuca forest. In: International Conference on Biotechnology and Healthy Living October 20-22. The 22<sup>nd</sup> Annual Meeting of the Thai Society for Biotechnology. Thailand: Prince of Songkla University, Trang Campus; 2010.
- Aung-Aud-Chariya A, Bangrak P, Dell B, Lumyong S, Kamlangdee N. Some species of wild boletes in Thailand. *J Agric Technol* 2012;8:1991-8.

21. Aung-Aud-Chariya A, Bangrak P, Lumyong S, Phupong W, Aggangan NS, Kamlangdee N. RNA polymerase II second largest subunit molecular identification of *Boletus griseipurpureus* corner from Thailand and antibacterial activity of basidiocarp extracts. *Jundishapur J Microbiol* 2015;8:e15552.
22. AOAC. Official Methods of Analysis of AOAC International. 16<sup>th</sup> ed., Vol. II. Alington: AOAC International; 1995.
23. Munzuroglu O, Karatas F, Geckil H. The vitamin and selenium contents of apricot fruit of different varieties cultivated in different geographical regions. *Food Chem* 2003;83:205-12.
24. Qian H, Sheng M. Simultaneous determination of fat-soluble vitamins A, D and E and pro-vitamin D2 in animal feeds by one-step extraction and high-performance liquid chromatography analysis. *J Chromatogr A* 1998;825:127-33.
25. Mir-Marqués A, Domingo A, Cervera ML, de la Guardia M. Mineral profile of kaki fruits (*Diospyros kaki* L.). *Food Chem* 2015;172:291-7.
26. Singlenton VL, Orthoffor R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 1999;299:152-78.
27. Yen CG, Duh DP. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *J Agric Food Chem* 1994;42:629-32.
28. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999;26:1231-7.
29. Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, *et al.* Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. *J Agric Food Chem* 2003;51:3273-9.
30. O'Brien J, Wilson I, Orton T, Pognan F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur J Biochem* 2000;267:5421-6.
31. Hunt L, Jordan M, De Jesus M, Wurm FM. GFP-expressing mammalian cells for fast, sensitive, noninvasive cell growth assessment in a kinetic mode. *Biotechnol Bioeng* 1999;65:201-5.
32. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard, CLSI Document M7-A6. 6<sup>th</sup> ed. Wayne, PA: CLSI; 2003.
33. Trager W, Jensen JB. Human malaria parasites in continuous culture. *Science* 1976;193:673-5.
34. Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob Agents Chemother* 1979;16:710-8.
35. Changsen C, Franzblau SG, Palittapongarnpim P. Improved green fluorescent protein reporter gene-based microplate screening for antituberculosis compounds by utilizing an acetamidase promoter. *Antimicrob Agents Chemother* 2003;47:3682-7.
36. Collins LA, Torrero MN, Franzblau SG. Green fluorescent protein reporter microplate assay for high-throughput screening of compounds against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 1998;42:344-7.
37. Hou Y, Ding X, Hou W, Song B, Wang T, Wang F, *et al.* Pharmacological evaluation for anticancer and immune activities of a novel polysaccharide isolated from *Boletus speciosus* frost. *Mol Med Rep* 2014;9:1337-44.
38. Lemieszek MK, Cardoso C, Ferreira Milheiro Nunes FH, Ramos Novo Amorim de Barros AI, Marques G, Pozarowski P, *et al.* *Boletus edulis* biologically active biopolymers induce cell cycle arrest in human colon adenocarcinoma cells. *Food Funct* 2013;4:575-85.
39. Sun J, Ng TB, Wang H, Zhang G. A novel hemagglutinin with antiproliferative activity against tumor cells from the hallucinogenic mushroom *Boletus speciosus*. *Biomed Res Int* 2014;2014:340467.

**Source of Support:** Nil. **Conflict of Interest:** None declared.