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Original Article

Boletus griseipurpureus Corner: Antibacterial, Antioxidant Properties and Phytochemical Compositions

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ABSTRACT

This study aims to investigate the properties of *B. griseipurpureus* Corner concerning its efficacy against various bacterial strains and its antioxidant capacity, along with its phytochemical composition. The antibacterial activity was evaluated using the agar well diffusion method, revealing pronounced efficacy against gram-negative bacteria, particularly noteworthy against *E. coli* ESBL182, known for its antibiotic resistance. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *B. griseipurpureus* Corner extract against *E. coli* ESBL182 were determined to be 75 and 300 mg/ml, respectively. The extract exhibited bacteriostatic activity by inhibiting cell growth, leading to a reduction in optical density at 600 nm. Additionally, examination of the supernatant from untreated and treated *E. coli* ESBL182 cells with *B. griseipurpureus* Corner extract indicated absorbance values of 0.088 and 0.248 at 260 nm, and 0.045 and 0.286 at 280 nm, respectively. Further investigation utilizing a scanning electron microscope revealed alterations in the morphology of treated cells, which displayed elongation and fragmentation, in contrast to untreated cells. The DPPH assay indicated that the IC_{50} of *B. griseipurpureus* Corner extract was 31.22 mg/ml. The IC_{50} value obtained from the ABTS assay was 47.31 mg/ml. Additionally, the FRAP assay revealed that the concentration of ascorbic acid equivalent in *B. griseipurpureus* Corner extract was 1.06 mg/g crude extract. Phytochemical analysis, conducted using a spectrophotometer at wavelengths of 750 nm and 510 nm, respectively, indicated phenolic and flavonoid contents of 0.22 mg gallic acid/g fresh weight and 3.23 mg quercetin/g fresh weight in the extract.

Key words: *Boletus griseipurpureus* Corner, Antibacterial activity, Antioxidant property, Phytochemical compositions, Phenolic compound, Flavonoid compound.

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INTRODUCTION

Currently, edible mushrooms are widely utilized for both culinary and medicinal purposes across the globe. These mushrooms harbor beneficial compounds, such as phenolic chemicals, which are secondary metabolites present not only in mushrooms but also in other plants. Notably, these compounds possess medicinal properties and exhibit biological capabilities as antioxidants, as evidenced by previous studies.[1-3]. Mushrooms are notable for their carbohydrate and protein content, with fat being the exception.[4,5] Among edible species utilized in cooking, *Boletus regius* stands out for its significant levels of phenolic compounds, known for their antioxidant effects.[6] Moreover, research indicates that *B. fragrans* exhibits antioxidant characteristics, owing to the presence of phenolic chemicals and sucrose in its extract.[7] Notably, mushrooms like *B. edulis* demonstrate remarkable antioxidant properties and possess antihyperglycemic effects.[8] Additionally, polysaccharide extracts derived from mushrooms showcase significant antioxidant properties[9].

B. griseipurpureus Corner belongs to the Boletaceae family and is an edible mushroom cultivated in southern Thailand, including Narathiwat province. Locally known as Hed-Sa-Med, it typically grows in association with host plants such as melaleuca, eucalyptus, and sea pine due to its ectomycorrhizal nature.[10,11] The methanolic extract of *B. griseipurpureus* Corner has shown activity against *Pseudomonas aeruginosa* TISTR2370 and *Enterococcus faecalis* TISTR379.[12] However, recent findings[11] reported toxicity in the liver and kidney associated with high doses of *B. griseipurpureus* Corner extract at 2000 mg/mL.

Utilizing insights from similar species such as *B. regius* and *B. fragrans*, known for their antioxidant properties, this study aims to explore the efficacy of *B. griseipurpureus* Corner against a range of bacterial strains including *Escherichia coli* ESBL182, *Salmonella typhimurium* TISTR292, *Pseudomonas aeruginosa* TISTR1467, *Staphylococcus aureus* TISTR517 & MRSA, *Bacillus cereus* ATCC11778, and *Micrococcus luteus* TISTR884. Additionally, the study seeks to assess the antioxidant capacity and phytochemical composition of *B. griseipurpureus* Corner.

MATERIAL AND METHODS

Preparation of *Boletus griseipurpureus* Corner. Extract

Boletus griseipurpureus Corner specimens were acquired from the Faculty of Natural Resources at Prince of Songkla University (Collection No. BGC-2566-01), where they had already been morphologically identified. The specimens were rinsed with tap water, drained, crushed thoroughly, then macerated with 95% ethanol in a 4:1 ratio for three days. The process was repeated two times. The extract was filtered using cotton cloth, followed by filter paper no. 1, and then evaporated using an evaporation machine (Heidolph, Hei-VAP Precision, Germany). The crude extract was dried

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at room temperature for three days and stored in a desiccator until needed. The *B. griseipurpureus* Corner. extract was diluted using a two-fold dilution procedure using DMSO (Dimethyl sulfoxide) in the experiment.

The yield percentage was determined using the following formula:

Yield (%) = (weight of dried extract x 100) / weight of fresh specimen

Antibacterial activity

This experiment followed the methodology outlined in previous study.[13] Initially, the agar-well diffusion method was utilized, as illustrated in Figure 1. All indicator strains, including *Escherichia coli* ESBL182, *Salmonella thyphimurium* TISTR292, *Pseudomonas aeruginosa* TISTR1467, *Staphylococcus aureus* TISTR517 and MRSA which is the Methicillin-resistant strain of *S. aureus*, *Bacillus cereus* ATCC11778, and *Micrococcus luteus* TISTR884, were cultured in the Mueller-Hinton agar (MHA) and then incubated at 35 °C for 18 hours. Subsequently, a single pure colony of each strain was transferred to the Mueller-Hinton Broth (MHB) and incubated at 35 °C and 150 rpm for 18 hours. The cell culture broth of each strain underwent two-fold serial dilution, adjusted to McFarland No. 0.5, and was swabbed onto MHA plates. Wells, 6 mm in diameter, were then cut using a sterile tip and filled with 100 ml of various concentrations of *B. griseipurpureus* Corner extract (9.38, 18.75, 37.5, 75, 150, and 300 mg/ml). After incubating the MHA plates at room temperature for 5 hours, they were further incubated at 35 °C for 18 hours. The inhibition zones were measured using a Vernier caliper, The Minimum Inhibitory Concentration (MIC) was evaluated. The Minimum Bactericidal Concentration (MBC) was determined by streaking the clear zone of the lowest MIC concentration onto an MHA agar plate, followed by incubation at 35 °C for 18 hours. Bacterial growth corresponding to *B. griseipurpureus* Corner extract was then investigated. This experimental procedure was repeated three times in triplicate.

Mode of action

The assay was carried out as described previously.[13] A concentration of 300 mg/ml of the *B. griseipurpureus* Corner extract was given to actively growing *E. coli* ESBL182 cells in MHB at mid-log phase. The cells in the medium MHB without *B. griseipurpureus* Corner extract were used as the control group for comparison. The culture broth's absorbance was quantified at 600 nm with a spectrophotometer (EON, Bio-Tex, USA).

Scanning Electron scanning microscope (SEM)

This assay was performed following the previously described method.[14] *E. coli* ESBL182, a drug-resistant bacterium producing spectrum beta-lactamases that affect enzymes in extended-spectrum cephalosporins, was chosen due to its sensitivity to *B. griseipurpureus* Corner extract. Cells of *E. coli* ESBL182 were collected from the edge of the clear zone on the MIC plate displaying the MBC value. They were then washed twice with 0.15M phosphate buffer pH 7.2, followed by fixation with 2.5% Glutaraldehyde (C5H8O2) for 2 hours. Subsequently, the cells were washed twice with phosphate buffer and distilled water, respectively. Dehydration was achieved using a graded acetone series (5—100%). The cells were dried using the Critical Point Drying method, affixed to a stub with carbon tape and carbon paint, coated with gold using a Sputter Coater, and observed under a scanning electron microscope.

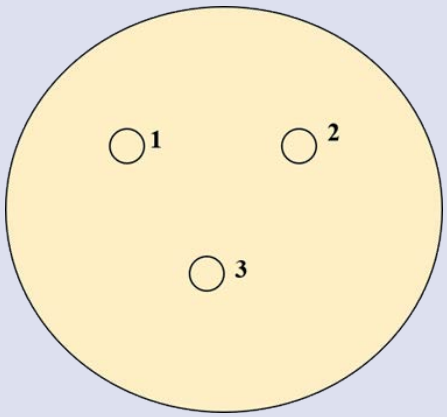

Figure 1: Agar well diffusion method of *B. griseipurpureus* Corner. extract against *E. coli* ESBL 182, well 1 is the extract; well 2 is DMSO; well 3 is gentamicin (10 µg).

Release of intracellular UV-absorbing material test

The investigation into the leakage of ultraviolet light-absorbing material followed the procedure outlined in a previous study.[15] A cell suspension of *E. coli* ESBL182 was adjusted to McFarland No. 0.5 and combined with a concentration of 300 mg/ml of *B. griseipurpureus* Corner extract, with a ratio of 1:1 (v/v), while a cell suspension of *E. coli* ESBL182 without *B. griseipurpureus* Corner extract served as the control. The crude extract mixture was then incubated at 35 °C for 24 hours before being centrifuged at 4 °C at 10,000 rpm for 20 mins. The UV absorbance of the cell-free supernatant was measured at 260 and 280 nm using a spectrophotometer (EON, Bio-Tex, USA).

Antioxidant assay

DPPH radical-scavenging activity

The DPPH assay was slightly adapted from a previously described method.[16] The oxidant utilized in this protocol was 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich). The extract of *B. griseipurpureus* Corner was diluted to concentrations of 25, 12.5, 6.25, and 3.125 mg/ml using a two-fold dilution technique. Each crude extract concentration (1 mL) was combined with 1 mL of DPPH solution and then incubated in darkness for 30 mins at room temperature. Absorbance at 517 nm was measured using a spectrophotometer (Thermo Fisher Scientific, SPN-1840-21800). Methanol was used as a substitute for the crude extract in the control, while only methanol was tested in the blank. Ascorbic acid served as a standard at concentrations ranging from 1 to 10 g/mL.

Antioxidant activity was determined as mg/ml of ascorbic acid equivalent using the following formula:

$$\%\text{Radical scavenging} = [(A_0 - A_{30})/A_0] \times 100$$

The IC_{50} was calculated using a graph of percentage inhibition against *B. griseipurpureus* Corner extract concentration.

ABTS radical-scavenging activity

The ABTS assay was slightly altered from the method employed in a previous research.[16] Solutions of ABTS and potassium persulfate were prepared at concentrations of 7 mM and 2.45 mM, respectively, and combined in a 1:2 ratio before being incubated for 12 hours. The extract of *B. griseipurpureus* Corner was diluted to concentrations of 25, 12.5, 6.25, and 3.125 mg/ml using a two-fold dilution method. The analysis commenced with 150 ml of *B. griseipurpureus* Corner extract mixed with 2850 ml of the working solution and incubated in darkness for 2 hours. Absorbance was measured at 734 nm using a spectrophotometer (Thermo Fisher Scientific, SPN-1840-21800). Ascorbic acid served as a standard at specific concentrations. The antioxidant activity of the *B. griseipurpureus* Corner extract was expressed as mole ascorbic acid per gram of fresh weight.

Ferric reducing activity (FRAP assay)

The FRAP assay was slightly altered from the method employed in a previous research.[16] The FRAP solution was prepared by combining 25

ml of 300.0 mM acetate buffer, 2.5 ml of 10 mM TPTZ solution, and 2.5 ml of 20 mM FeCl3 solution in a ratio of 10:1:1. The extract of *B. griseipurpureus* Corner was diluted to concentrations of 25, 12.5, 6.25, and 3.125 mg/ml using a two-fold dilution method. Then, 1 ml of each concentration of *B. griseipurpureus* Corner extract was mixed with 1 ml of water and incubated in darkness for 30 mins at room temperature. Absorbance was measured at 593 nm using a spectrophotometer (Thermo Fisher Scientific, SPN-1840-21800). Distilled water served as the blank, while ascorbic acid was used as the standard. The antioxidant activity of *B. griseipurpureus* Corner extract was expressed as mg ascorbic acid per gram of fresh weight.

Preliminary phytochemical screening test

Minor modifications were made to the procedure described in a previous study regarding the dilution of the *B. griseipurpureus* Corner extract with sterile distilled water (at a 1:100, w/v) for phytochemical screening.[17]

Flavonoids screening

A 1 ml aliquot of *B. griseipurpureus* Corner crude extract was mixed with 2 ml of a 2% sodium hydroxide solution. Upon addition of 3 drops of acid solution, a yellow color appeared, indicating a positive outcome. Subsequent observation revealed a colorless solution.

Saponin screening

In a 100 ml beaker, 1 ml of *B. griseipurpureus* Corner crude extract was diluted with 20 ml of sterile distilled water and stirred with a magnetic bar for 15 mins. A positive result was indicated by the presence of a consistent 1 cm foam layer.

Phenols and tannin screening

A 10-min heating process was applied to 1 ml of *B. griseipurpureus* Corner crude extract. The extract was subsequently supplemented with 2 ml of a 3% $FeCl_3$ solution. A favorable outcome was signified by the manifestation of a green or blue hue.

Determination of phenolic content

The Folin-Ciocalteu method, which was modified slightly from an earlier investigation[16], was employed to determine the total phenolic content. To begin, a solution was prepared by combining 10µl of *B. griseipurpureus* Corner extract with 1 ml of sterile distilled water. Following this, 300 µl of 20% Na_2CO_3 was added, which was followed by the addition of 100 µl of Folin-Ciocalteu's phenol reagent. A one-hour incubation at ambient temperature in the absence of light followed after the mixture was meticulously combined. Using a UV-Vis spectrophotometer, the optical density was quantified at a

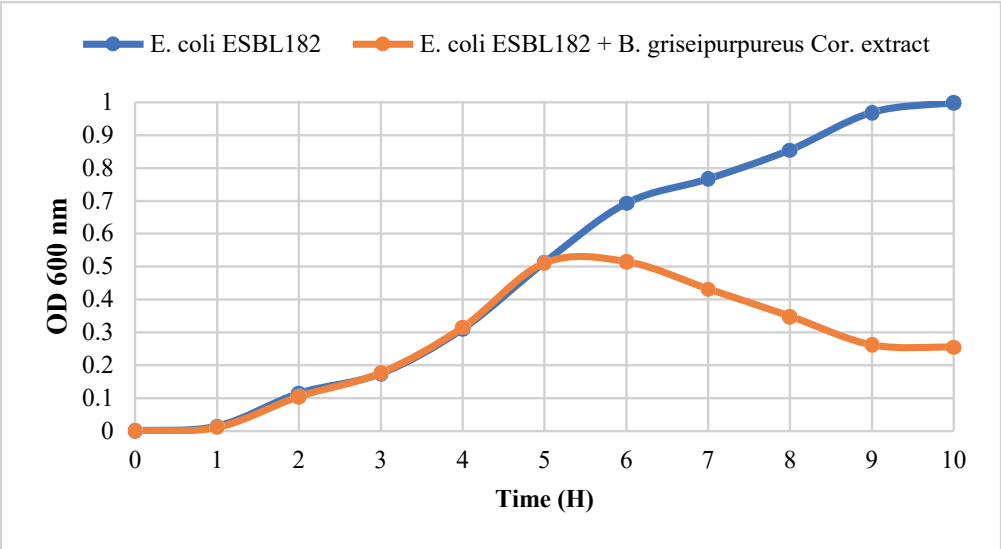

Figure 2: Growth curve (Mode of action) of *E. coli* ESBL182.

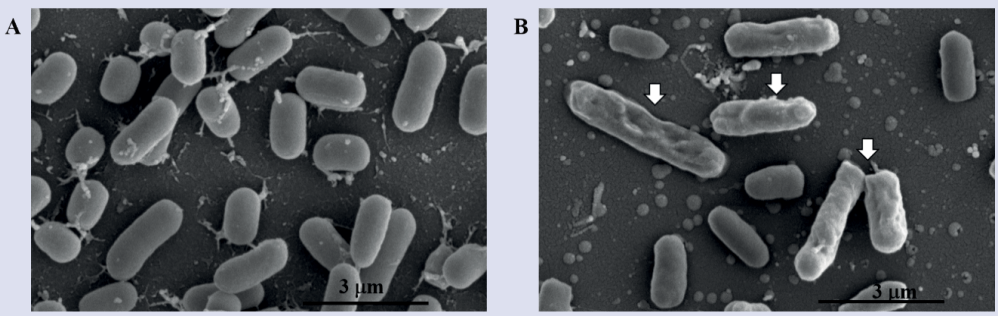

Figure 3: Scanning electron micrographs (SEM) of (A) untreated and (B) treated *E. coli* ESBL182 with *B. griseipurpureus* Corner. extract. Magnification of x 25000, bar = 3 µm.

concentration of 735 nm. With a range of concentrations from 0.02 to 0.4 mg/mL, a calibration curve was constructed using gallic acid as the standard. The phenolic content in its entirety was quantified in milligrams of gallic acid equivalent (GAE) per gram of fresh weight.

Determination of flavonoid content

The quantification of total flavonoid content was performed utilizing the Folin-Ciocalteu method, which was modified slightly from a prior investigation.[16] A mixture was prepared by combining 40 µl of *B. griseipurpureus* Corner extract with 1 ml of sterile distilled water and 60 µl of a 5% $NaNO_2$ solution. The components were combined meticulously and left to incubate for a duration of 10 mins at room temperature. Following this, 120 µl of 10% AlCl3 were added to the reaction mixture, which was then supplemented with 700 µl of 1M NaOH and 80 µl of sterile distilled water. The resulting mixture was incubated at room temperature for an additional 15 mins after being thoroughly mixed once more. Utilizing a UV-Vis spectrophotometer, subsequent optical density measurements were taken at a specific wavelength of 510 nm.

RESULTS AND DISCUSSION

Antibacterial activity

The agar-well diffusion method effectively suppressed bacterial growth when using *B. griseipurpureus* Corner extract, as illustrated in Table 1. Notably, gram-negative bacteria exhibited greater susceptibility to the extract compared to gram-positive bacteria. Particularly noteworthy is the sensitivity of *E. coli* ESBL182, an antibiotic-resistant strain prevalent in hospitals, due to its production of extended-spectrum beta-lactamases targeting cephalosporins. This phenomenon aligns with findings from various studies, wherein plant extracts predominantly inhibited gram-positive bacteria over gram-negative strains. For instance, *Hamamelis virginiana* L. leaf extract demonstrated efficacy against *E. coli* ESBL.[18] Antibiotic-resistant bacteria have also been shown to be susceptible to the antimicrobial properties of essential oils including niaouli oil, cinnamon leaf oil, and tea tree oil.[19] In contrast, the efficacy of French mushroom extracts was found to be more pronounced against gram-positive bacteria as opposed to their gram-negative counterparts.[20] In a similar fashion, mushroom extracts that were accessible in Malaysia exhibited efficacy against a range of bacteria, such as *S. aureus*, *B. cereus*, *S. typhimurium*, and *E. coli*, as determined by minimum inhibitory concentrations (MIC values) varying from 500 mg/mL to 1000 mg/mL.[21] Multidrug-resistant ESKAPE pathogens that were isolated from clinical wound infections exhibited comparable efficacy when treated with methanolic and aqueous extracts of *Neoboletus luridiformis* and *Boletus edulis*.[22] Additionally, a water extract of *Triwanofungus camphoratus* exhibited efficacy against *Listeria monocytogenes*[23], while the water extract of *Pleurotus pulmonarius* demonstrated effectiveness against both gram-positive and gram-negative bacteria.[24]

Mode of action

This study observed a decrease in the optical density at 600 nm of treated *E. coli* ESBL182 cells when *B. griseipurpureus* Corner extract was added to the mid-log phase of their growth, compared to untreated cells, as illustrated in Figure 2. Similarly, exposure of *Salmonella enteritidis* to 20 mg/ml of methanolic mushroom extract extended the lag phase to 7 hours, with bactericidal activity observed at 40 mg/ml. Untreated *S. aureus* exhibited a normal growth curve, while those treated with 2.5 and 5 mg/ml of mushroom extract closely resembled the negative control culture medium.[25] These findings suggest the potential bacteriostatic activity of *B. griseipurpureus* Corner extract.

Scanning electron microscope (SEM)

In this research, the optical density at 600 nm of *E. coli* ESBL182 treated with *B. griseipurpureus* Corner extract during the mid-log phase exhibited a reduction compared to untreated cells, as depicted in Figure 2. Similarly, exposure of *Salmonella enteritidis* to 20 mg/ml of methanolic mushroom extract extended the lag phase to 7 hours, with bactericidal effects observed at 40 mg/ml. Notably, untreated *S. aureus* followed a normal growth curve, whereas those treated with 2.5 and 5 mg/ml of mushroom extract paralleled the negative control culture medium.[25] These findings suggest the potential bacteriostatic activity of *B. griseipurpureus* Corner extract.

Release of intracellular UV-absorbing material E. coli ESBL182

After 24 hr of incubation, the cell-free supernatant from both treated and untreated cells exhibited absorbances at 260 nm of 0.088 and 0.248, respectively, while the absorbance at 280 nm was 0.045 and 0.286, as detailed in Table 2. This finding confirms the release of cellular contents. Furthermore, SEM experiments corroborated cell fracture, as depicted in Figure 3. Consequently, it can be inferred that the extract induces bacterial cell destruction by causing cell rupture or expansion, leading to cell death. Similarly, the methanol extract of *Coriolus versicolor* disrupted the cell membranes of *S. aureus* and *S.*

Table 1: Antibacterial activity of *B. griseipurpureus* Corner extract.

Indicator strains	Inhibition zone (mm) (Mean ± SE)	MIC (mg/ml)	MBC (mg/ml)
E. coli ESBL182	16.00 ± 0.333	75	300
S. thyphimurium TISTR292	21.67 ± 0.192	9.38	150
P.aeruginosa TISTR1467	19.00 ± 0.000	75	150
S. aureus TISTR517	12.67 ± 0.192	75	75
S. aureus MRSA	11.67 ± 0.192	150	300
B.cereus ATCC11778	15.67 ± 0.192	75	75
M.luteus TISTR884	15.67 ± 0.192	75	75

Table 2: Release of intracellular UV-absorbing material *E. coli* ESBL182.

	Untreated cells (Mean ± SE)	Treated cells (Mean ± SE)
Nucleic acid (A_{260nm})	0.088±0.001	0.248±0.002
Protein (A_{280nm})	0.045±0.003	0.286±0.001

*Values are mean (n=3) ± SE, Data were analyzed using the pair sample T-test (P<0.05).

Table 3: Phytochemical screening of *B. griseipurpureus* Corner extract.

Phytochemicals	Presence of phytochemicals
Flavonoid	+
Phenols	+
Tannin	+
Saponin	-
Anthraquinones	-

Table 4: Antioxidant activity and Phytochemical content of *B. griseipurpureus* Corner extract.

Method	*B. griseipurpureus* Corner
% Yield	5.05
DPPH (IC_{50}) mg/ml	31.22 ± 0.01
ABTS (IC_{50}) mg/ml	47.31 ± 0.01
FRAP (mg ascorbic acid/g crude extract)	1.06 ± 0.02
Phenolic compound (mg Gallic acid equivalent/g fresh weight)	0.22 ± 0.01
Flavonoid compound (mg Quercetin/g fresh weight)	3.23 ± 0.01

*Values are mean (n=3) ± SE, Data were analyzed using the pair sample T-test (P<0.05).

enteritidis, resulting in the release of material absorbing at 260 nm from within the cell.[25] Previous studies have reported on the mechanisms of action of antimicrobial agents, including disruption of cell wall synthesis, alteration of cell membrane permeability, interference with chromosome replication, and inhibition of protein synthesis. Additionally, metabolic dysfunction and cell death occur when the synthesis of cell wall components such as peptidoglycan or inner cell wall lipids is disrupted.[26,27]

Preliminary phytochemical screening

B. griseipurpureus Corner extract contains various phytochemical constituents, as illustrated in Table 3. Similarly, *B. regius* is known for its high phenolic content.[28] Furthermore, phytochemical screening of *Heliotropium indica* extract revealed the presence of alkaloids, cardiac glycosides, flavonoids, terpenoids, saponins, tannins, and phenols.[17]

Antioxidant activity test and phytochemical content

The yield of *B. griseipurpureus* Corner extract was 5.05%. In the DPPH assay, the extract demonstrated an IC_{50} value of 31.22 mg/mL, while in the ABTS assay, it exhibited an IC_{50} value of 47.31 mg/mL. Furthermore, the FRAP assay indicated a FRAP value of 1.06±0.02 mg ascorbic acid/g crude extract. The extract also displayed a total phenolic content of 0.22 mg gallic acid equivalent (GAE)/g fresh weight and a total flavonoid content of 3.23 mg quercetin/g fresh weight, as presented in Table 4. Additionally, *B. griseipurpureus* Corner extract collected from Maha Sarakham Province, Northeast Thailand, showed IC_{50} values of 0.09±0.04 mg/ml and 1.03±0.03 mg/ml, as analyzed by the DPPH and ABTS methods, respectively. Moreover, the FRAP assay revealed a value of 3608.89±120.68 mg $FeSO_4$/g extract.[16] Other mushroom extracts, including *Pleurotus pulmonaris*, *Ganoderma lucidum*, and *Flammulina velutipes*, displayed IC_{50} values ranging from 2.81±0.02 mg/ml to 10.57±0.27 mg/ml.[24] Similarly, the ethanol extract of *B. griseipurpureus* Corner contained phenolic and flavonoid compounds at concentrations of 26.12±0.36 and 273.84±4.56, respectively.[16] Additionally, the ethyl acetate fraction of the leaf extract of Indonesian *Anacardium occidentale* L. exhibited high contents of phenolic (508.89 mg GAE/g dw) and flavonoid (184 mg QE/g dw) compounds.[29]

CONCLUSION

The research findings underscore the promising potential of *B. griseipurpureus* Corner extract in inhibiting gram-negative bacteria and drug-resistant strains, positioning it as a prospective candidate for future medical interventions. Additionally, its substantial antioxidant properties enhance its therapeutic value, highlighting its multifaceted benefits. The consumption of *B. griseipurpureus* Corner as a dietary supplement not only offers potential health advantages but also serves as a natural approach to overall well-being. This emphasizes the significant role of natural remedies such as *B. griseipurpureus* Corner in promoting health and combating bacterial infections. The study's outcomes pave the way for further exploration and development in the realm of medicinal mycology, opening new avenues for innovative research and potential pharmaceutical applications. As such, the investigation underscores the importance of harnessing the potential of natural products like *B. griseipurpureus* Corner in addressing contemporary health challenges and advancing medical science.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this manuscript.

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