JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2018. Vol. **12**(2), p. 567-576

Antioxidant and Antimicrobial Activities of *Polycephalomyces nipponicus*

Pimchanok Somsila[1], Uthai Sakee[2], Akeapot Srifa[3] and Watchara Kanchanarach[3]*

[1]Department of Biology, Faculty of Science, Mahasarakham University, Kantarawichai District, Maha Sarakham Province, 44150, Thailand.
[2]Department of Chemistry, Faculty of Science, Mahasarakham University, Kantarawichai District, Maha Sarakham Province, 44150, Thailand.
[3]Microbiology and Applied Microbiology Research Unit, Faculty of Science, Mahasarakham University, Kantarawichai District, Maha Sarakham Province, 44150, Thailand.

http://dx.doi.org/10.22207/JPAM.12.2.15

(Received: 12 March 2018; accepted: 17 April 2018)

The aim of this work was to investigate total phenolic and flavonoid content, antioxidant properties and to assess antimicrobial activities of three different extracts from *Polycephalomyces nipponicus* TBRC 6537 mycelia . The 50% (v/v) aqueous methanol extract was found to have the highest total phenolic content, while methanol extract exhibited the highest total flavonoid content. Water and methanol extracts showed the strongest DPPH radical scavenging activity. In addition, water and 50% (v/v) aqueous methanol extracts had stronger ABTS radical scavenging activity than that of methanol extract. The antimicrobial activity of each extract was quantified by determining minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFC) against five human pathogenic bacteria and one human pathogenic fungus. The water extract was found to have the strongest activity against *Escherichia coli*, *Staphylococcus epidermidis*, *Bacillus cereus* and *Candida albicans*. Furthermore, aqueous methanol extract exhibited strongest activity against *Staphylococcus aureus* with the lowest MIC and MBC. The methanol extract had moderate antimicrobial activity against *Pseudomonas aeruginosa*, whereas the aqueous methanol and water extracts did not inhibit growth. This study has revealed that *P. nipponicus* TBRC 6537 mycelia extracts might be a useful source of natural antioxidant and antimicrobial compounds for the pharmaceutical industry.

Keywords: Antioxidant activity ; Antimicrobial activity ; Insect pathogenic fungus; *Polycephalomyces nipponicus.*

Insect pathogenic fungi, especially *Cordyceps* and *Ophiocordyceps* spp. belonging to the Ascomycota phylum and Hypocreales order are famous traditional Chinese medicinal mushrooms [1]. They have been used as food and herbal medicines in China, Japan, Korea and Taiwan for many centuries. These species, including *Ophiocordyceps sinensis*, *Cordyceps militaris* , *Cordyceps cicadae*, *Ophiocordyceps sobolifera* and *Cordyceps pruinosa* contain valuable active compounds such as cordycepin, cordycepic acid, ergosterol, polysaccharides, nucleosides, manitol and peptides [2,3] Many are pharmacologically active exhibiting immunomodulatory, anti-inflammatory, anti-tumour, antioxidant, antimicrobial, antihyperglycaemic, antiapoptosis and hepatoprotective activities [4]. In addition, *Polycephalomyces nipponicus* (former name:

* To whom all correspondence should be addressed.
Tel.: +66981047211;
Email: watchara.k@msu.ac.th

Cordyceps nipponica) [5] has been shown to have anti bacterial [6] and antimalarial activities [7].

Antioxidants are compounds that can eliminate free radicals and protect cells from damage caused by oxidative stress [8]. Nowadays, natural antioxidants from mushrooms have attracted much interest because they are safer than synthetic antioxidants in the pharmaceutical and food industries [9]. Furthermore, pathogenic microorganisms are increasingly resistant to commercial drugs so medicinal agents from natural sources with efficacy against pathogenic microorganisms are being sought as alternative medicines. There have many benefits for human health because they are non-toxic and there are no side effects [10]. Natural antioxidant substances found in fruiting bodies, mycelia and broth of mushrooms include phenolics, flavonoids, and polysaccharides which have been shown to have the capacity to scavenge free radicals and inhibit pathogenic bacterial and fungal growth [11,12,13]. Several studies have demonstrated that various extracts of insect pathogenic fungi had potent activity as scavengers of free radicals *in-vitro* [2,14, 15,16].

The antimicrobial activities of insect pathogenic fungal species have been reported [15,17, 18,19]. Methanolic extract from the fruiting body of *C. militaris* contains antibacterial and antifungal compounds [20]. An aqueous extract from *O. sinensis* possed antibacterial activity [21]. Zhang *et al*.[22] demonstrated that a polysaccharide from *C. cicadae* showed strong antibacterial activity against gram positive and gram negative bacteria. However, there have been few studies of biological activities from *P. nipponicus*. In this study, we investigated the antioxidant and antimicrobial activities in methanol, 50% methanol and water extracts of *P. nipponicus* TBRC 6537 mycelia.

MATERIALS AND METHODS

All chemicals and reagents were commercially available and used without purification.

Fungal strain

Polycephalomyces nipponicus TBRC 6537 was purchased from Thailand Bioresource Research Center (TBRC). The stock culture was grown on potato dextrose agar (PDA) slant at 28 ^{o}C for 7 days and then kept at 4 ^{o}C.

Submerged culture

The mycelia of *P. nipponicus* TBRC 6537 was cultured on PDA agar plate at 28 °C for 14 days and punched out with sterile cork borer (6 mm in diameter). This 20 mycelia plugs were grown on 400 ml of synthetic medium (dextrose 20 g/L, peptone 10 g/L, yeast extract 5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, K_2HPO_4 1 g/L, thiamin 0.002 g/L and glycine 4 g/L, pH 6.8) in a 1,000 mL Erlenmeyer flasks . The culture was incubated at 28 °C for 30 days under static culture and filtered through Watman No.4 filter paper. Collected mycelia were freeze dried.

Extraction

Samples of dried mycelium of *P. nipponicus* TBRC 6537 were ground before extraction. Approximately 2 g of dried and powdered samples was sequentially extracted using methanol, 50 % methanol and water, at 60 °C for 30 min using an ultrasonic cleaning bath (Bandelin Electronic, Ultrasonic Bath DT 255 H). The extract was filtered through Whatman No.1 filter paper and the filtrate evaporated to dryness under reduced pressure in a rotatory vacuum evaporator. The dried sample of mycelial extract was weighed and the yield was determined. The dried mycelial extracts were kept in darkness at 4 °C until further analyses.

Total phenolic content

The total phenolic compound (TPC) of mycelial extracts was determined by the Folin-Ciocalteu method [23] with some modifications. 500 µl of mycelia extract was mixed with 1,500 µl of Folin-Ciocalteu reagen (diluted 10 times in water) and held in the dark for 3 min before 1,500 µl of 7.5% Na_2CO_3 was added. The absorbance of the mixed reaction was measured at 765 nm after incubation in the dark for 30 min. TPC was expressed as mg gallic acid equivalents (GAE) per gram extract. Values presented are the average of three measurements.

Total flavonoid content

The total flavonoid content of mycelia extracts was determined by the method described by Chang *et al*.[24] with some modifications. 200 µl of mycelia extract was mixed with 75 µl of 5% $NaNO_2$. After incubation in the dark at room temperature for 5 min, 150 µl of 10%$AlCl_3$ was added. The mixed reaction was incubated for 6 min and 500 µl of 1M NaOH added, followed by 1,075 µl of distilled water to a final volume of 2 ml. The

absorbance of the mixed reaction was measured at 415 nm after incubation at room temperature for 30 min. Flavonoid content was expressed as mg quercetin equivalent (QE) per gram dried extract. Values presented are the average of three measurements.

Antioxidant property

DPPH assay

The DPPH radical-scavenging activity of mycelial extracts was determined using the method based on Reis *et al*.[20] using ascorbic acid as a standard. 100 µl of mycelia extract at various concentrations was mixed with 900 µl of 0.1 mM radical DPPH solution. The absorbance of the mixed reaction was measured at 517 nm after incubation in the dark for 30 min. The DPPH scavenging percentage was calculated as follows: Scavenging DPPH radical (%) = $[(A_0-A_s) / A_0] \times 100$

Where A_0 is the absorbance of a negative control and A_s is the absorbance of the reaction mixture. The antioxidant activity is expressed as IC_{50}. IC_{50} was calculated by interpolation from a graph plotting concentration against scavenging activity. Tests were conducted in triplicate.

ABTS assay

The 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid free radical (ABTS•+) neutralization was determined using a spectrophotometric method described by Re *et al* .[25] with some modifications. 100 µl of mycelia extract at various concentrations was mixed with 900 µl of ABTS cation radical solution. The absorbance of the mixed reaction was measured at 734 nm after incubation in the dark for 20 min. The ABTS scavenging percentage was calculated as follows:

Scavenging ABTS radical (%) = $[(A_0-A_s) / A_0] \times 100$

Where A_0 is the absorbance of a negative control and A_s is the absorbance of the reaction mixture. ABTS radical cation scavenging activity was express as equivalents of ascorbic acid. Tests were conducted in triplicate.

Antimicrobial activity

Test microorganism

P. aeruginosa DMST 4739, *E. coli* DMST 4212, *S. aureus* DMST 2933 and *B. cereus* DMST 5040 were obtained from the Public Health Ministry, Bangkok. *S. epidermidis* TISTR 518 *and C. albicans* TISTR 5957 were obtained from a culture collection at Thailand Institute of Scientific and Technological Research. These bacterial strains were used for assessing antibacterial activity of the extracts. The yeast strain was used for assessing the antifungal activity of the extracts.

Agar well diffusion method

Antimicrobial activities of the different extracts were determined by the agar well diffusion method with some modifications [26]. The five tested bacteria were cultured at 37°C for 16–18 hours on nutrient agar and *C. albicans* were cultured at 28°C for 48 hours on Sabouraud dextrose agar. The cell suspensions were adjusted to 1.5×10^8 CFU/ml for bacteria and 1.5×10^6 CFU/ml for yeast in 0.85% NaCl which compared to 0.5 McFarland standard. Bacterial and yeast suspensions were then swabbed with sterilized cotton swab on to Mueller Hinton agar and Sabouraud dextrose agar, respectively. Agar wells were punched out with a sterile cork borer (6 mm in diameter). 100 µl (200 mg/mL) of extracts was added to each well and then incubated at 37°C for 18-24 hours (bacteria) and at 28°C for 24 hours (yeast). Gentamycin (antibiotic) and ketoconazole (antifungal) were used as positive control. 5% DMSO was used as negative control.

Determination of the minimal inhibitory concentration (MICs), minimum bactericidal concentration (MBC) and Determination of minimum fungicidal concentration (MFC)

The antibacterial activity of each mycelia extract was quantified by determining minimum inhibitory concentrations (MICs) using the broth microdilution method [27,28] with some modification against the five bacterial strains. 90 µl of various concentrations of the mycelia extract (2-fold dilutions of mycelia extract in Mueller Hinton broth starting from 400 mg/ml) were added to 96-well microtiter plates . Ten microliters of bacterial suspension (ca. 1.5×10^6 CFU/ml) was applied to each well. After incubation at 37°C for 24 hours, 30 µl of resazurin (0.01%) indicator solution was added in to each well. The plates were further incubated for 2-4 hours. Color change of the indicator from blue to pink indicated viable cell growth. The lowest concentration at which no color change occurred was defined as the MIC value. Samples of the highest dilution with remaining blue color from the MIC were inoculated (10 µl)

onto nutrient agar plates to determine the minimal bactericidal concentration (MBC) with no bacterial growth.

For antifungal activity, the MIC was determined by using the macrodilution method [29] against *C. albicans*. The mycelia extracts were diluted two fold in Sabouraud dextrose broth at concentrations ranging from 0.39 mg/ml to 200 mg/ml. Aliquots of 0.9 ml of yeast suspensions (ca. 2.5×10^3 CFU/ml) were transferred to each tube containing 0.1 mL mycelia extract. MICs were determined after incubation at 28°C for 24-48 hours as the lowest concentration of mycelial extract which could inhibit growth. In order to obtain the MFC, turbidity free media from the MIC assay was streaked onto Sabouraud dextrose agar plates and incubated at 28°C for 48 hours. The MFC was defined as the lowest concentration of mycelia extract which could kill yeast cells.

Statistical Analyses

Neither log-transformed nor original size of clear zone conformed to normal distribution at 95% confidence level, according to Shapiro-Wilk tests ($p<0.05$). Independent-Samples Kruskal-Wallis Tests were therefore performed as non-parametric one-way analyses of variance for these two variables under the null hypothesis that the distributions of these variables are the same across categories of either extraction methods or test microbes. Pairwise comparisons were used as a post hoc analyses should the null hypothesis be rejected.

RESULTS

Extraction yield, total phenolic and flavonoid contents

Extraction yield, total phenolic and flavonoid contents of different extracts from *P. nipponicus* TBRC 6537 mycelia are reported in Table 1

Antioxidant activity

Free radical scavenging activity was investigated with the DPPH and ABTS assays.

Table 1. Extraction yield, total phenolic content and total flavonoid content of different extracts of *P. nipponicus* TBRC 6537 mycelia

Extracts	Extraction yield (%)	Total phenolic content (mg GAE/g dw. extract)	total flavonoid content (mg QE/g dw. extract)
Methanol	12.00	21.624±0.217	67.139±0.648
50% aq.Methanol	8.08	28.249±0.213	28.350±0.182
Water	7.39	16.774±0.130	39.478±0.194

Remark: Values are mean inhibition zone (mm) ± S.D of three replicates

Table 2. IC_{50} values of different extracts of *P. nipponicus* TBRC 6537 mycelia by free radical scavenging method

Extracts	Scavenging activities IC_{50} (mg/ml) DPPH	ABTS
Methanol	0.697±0.000	0.321±0.001[b]
50% aq.Methanol	0.907±0.006[b]	0.157±0.002[a]
Water	0.228±0.001[a]	0.049±0.000[a]
Ascorbic acid	0.029±0.001[a]	0.012±0.001[a]

Remarks: Samples with the same superscript letter indicate that values in the same column are not statistically significantly different at 95 % confidence level.
Values are mean inhibition zone (mm) ± S.D of three replicates

Antioxidant activity is calculated as half inhibitory concentration (IC_{50}); lower values of IC_{50} indicate higher antioxidant activity [30] (Table 2). The results revealed that water extract (IC_{50} 0.228±0.001) and methanol extract (IC_{50} 0.697±0.000) have more effective scavenging ability for DPPH radical than that of 50% aqueous methanol extract (IC_{50} 0.905±0.006). This effectiveness is significantly different ($p = 0.015$), and the pairwise comparison revealed that the means of the 50% aqueous methanol extract and ascorbic acid samples was the only different pair ($p = 0.013$).

In addition, the water extract (IC_{50} 0.049±0.000) and 50% aqueous methanol extract (IC_{50} 0.157±0.002) have more effective scavenging

ability for ABTS radical than of the methanol extract (IC_{50} 0.321±0.001). This effectiveness is significantly different (p = 0.015), and the only different mean pair was between methanol extract and ascorbic acid (p = 0.012).

Antimicrobial Activity

Assays of antimicrobial activity of methanol, 50% aqueous methanol and water extracts from *P. nipponicus* TBRC 6537 mycelia were performed using the agar well diffusion method against five bacterial and one yeast species. The results showed that the different extracts from *P. nipponicus* TBRC 6537 mycelia showed selective antimicrobial activity against the microorganisms tested. The methanol extract displayed potential activity against five bacteria tested with the inhibition zones from 13.67±0.58 to 19.33±0.58 mm. although these size of inhibition zones were not statistically different across methods of extraction (p = 0.293), but statistically different across test microbes (p = 0.008) when using Kruskal-Wallis Test. Pairwise comparisons revealed that the means of clear zone diameter between *S. aureus* DMST 2933 and *S. epidermidis* TISTR 518 (p=0.033) and between *S. aureus* DMST 2933 and *E. coli* DMST 4212 (p=0.021) were significantly different at 95% confidence level. The methanol extract exhibited the highest antibacterial activity against *P. aeruginosa* DMST 4739 (19.33±0.58 mm).

Table 3. Antimicrobial activity of different extracts from *P. nipponicus* TBRC 6537 mycelia against microorganisms tested by the agar well diffusion method

Microorganism	Zone of inhibition (mm)			
	Methanol	50% aq.Methanol	Water	Positive control
P. aeruginosa DMST 4739	19.33±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	18.33±0.58^{a}
E. coli DMST 4212	13.67±0.58^{a}	20.33±0.58^{b}	25.00±±0.00^{d}	17.68±0.58^{a}
S. aureus DMST 2933	14.33±0.58^{a}	15.83±0.29^{b}	0.00±0.00^{a}	17.33±0.29^{a}
S. epidermidis TISTR 518	17.83±0.76^{a}	18.17±0.58^{b}	19.17±2.02^{c}	17.83±0.76^{a}
B. cereus DMST 5040	16.33±0.58^{a}	20.67±0.58^{b}	13.17±0.29^{b}	17.50±0.50^{a}
C. albicans TISTR 5957	0.00±0.00	13.33±1.15	22.33±1.53	18.67±1.15

Remarks: Same letter in inhibition zone diameters indicate that values in same column are not statistically significantly different at 95 % confidence level. Values are mean inhibition zone (mm) ± S.D of three replicates.

Table 4. Minimum inhibitory concentration (MICs) and Minimum microbicidal concentrations (MBCs/MFC) of different extracts from *P. nipponicus* TBRC 6537 mycelia against microorganism tested (mg/ml)

Microorganism		Methanol	50% aq. Methanol	Water
P. aeruginosa DMST 4739	MIC	100	ND	ND
	MBC	200	ND	ND
E. coli DMST 4212	MIC	100	1.56	0.05
	MBC	>200	3.13	0.16
S. aureus DMST 2933	MIC	25	12.5	ND
	MBC	50	50	ND
S. epidermidis TISTR 518	MIC	25	12.5	0.78
	MBC	50	25	1.56
B. cereus DMST 5040	MIC	25	6.25	3.13
	MBC	25	12.5	6.25
C. albicans TISTR 5957	MIC	-	2.5	0.156
	MFC	-	10	0.313

ND = not determined

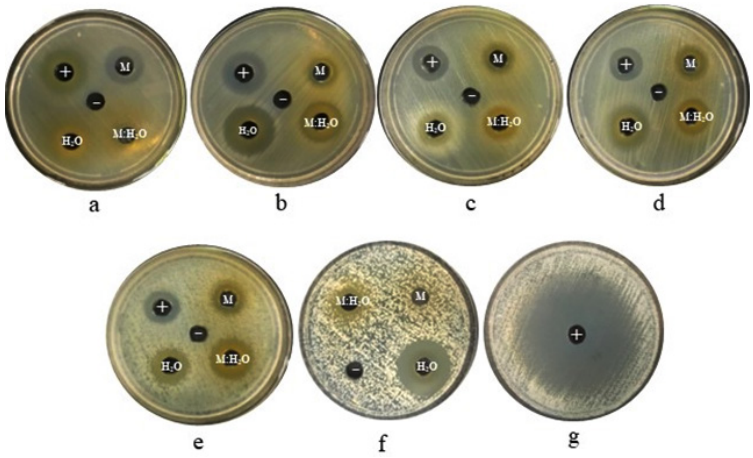

Fig. 1. Antimicrobial activity of methanol (M), 50% aqueous methanol (M:H_2O) and water (H_2O) extracts from *P. nipponicus* TBRC 6537 mycelia against (a) *P. aeruginosa* DMST 4739, (b) *E. coli* DMST 4212, (c) *S. aureus* DMST 2933, (d) *S. epidermidis* TISTR 518, (e) *B. cereus* DMST 5040, (f) *C. albicans* TISTR 5957 and (g) Ketoconazole against *C. albicans* TISTR 5957 (positive control, +). Gentamicin was used as a positive control (+) for bacteria. 5% DMSO was used as a negative control (-)

The 50% aqueous methanol extract had antibacterial activity against four bacteria tested (*E. coli* DMST 4212, *S. aureus* DMST 2933, *S. epidermidis* TISTR 518 and *B. cereus* DMST 5040) with the inhibition zones from 15.83±0.29 to 20.67±0.58 mm, and it did not inhibit *P. aeruginosa* DMST 4739. The 50% aqueous methanol extract exhibited the highest antibacterial activity against *E. coli* DMST 4212 and *B. cereus* DMST 5040 (20.33±0 and 20.67±0.58 58 mm).

The water extract showed antibacterial activity against three bacteria tested (*E. coli* DMST 4212, *S. epidermidis* TISTR 518 and *B. cereus* DMST 5040) with the inhibition zones from 13.17±0.29 to 25.00±00 mm, and it did not inhibit *P. aeruginosa* DMST 4739 or *S. aureus* DMST 2933. The water extract exhibited the highest antibacterial activity against *E. coli* DMST 4212 (25.00±00) and *S. epidermidis* TISTR 518 (19.17±2.02). The 50% aqueous methanol and water extracts had efficient antifungal activities against *C. albicans* TISTR 5957 with the inhibition zones of 13.33±1.15 and 22.33±1.53 mm, while methanol extract had no antifungal activity. (Table 3 and Figure 1.)

The MICs assay and MBCs/MFC of methanol, 50% aqueous methanol and water extracts from *P. nipponicus* TBRC 6537 mycelia against five bacterial and one yeast species are shown in Table 4 . The MIC values of methanol extract for five bacteria tested was ranged from 25 to 100 mg/ml and MBC values rang was 25 to >200 mg/ml. The MIC values of 50% aqueous methanol extract for four bacteria tested was ranged from 1.56–12.5 mg/ml and MBC values was ranged from 3.13 to 50 mg/ml. The MIC values of water extract for three bacteria tested ranged from 0.05 to 3.13 mg/ml and MBC values ranged from 0.16 to 6.25 mg/ml. The MIC values of 50% aqueous methanol and water extracts for *C. albicans* TISTR 5957 were 2.5 and 0.156 mg/ml, respectively. The MFC values of 50% aqueous methanol and water extracts were 10 and 0.313 mg/ml, respectively. The result of these study indicated that the water extract was found to be more effective for microorganisms tested than 50% aqueous methanol and methanol extracts, respectively.

DISCUSSION

The yield of extraction depends on the solvent and its varying polarity, temperature, extraction time, and composition of the sample. Under constant extraction time and temperature, solvent and composition of sample are known as the most important parameters. In this work, *P. nipponicus* TBRC 6537 mycelia extracts were obtained by sequential extraction in an ultrasonic cleaning bath using methanol, 50 % aqueous methanol and water, respectively. Extraction yields

ranged from 7.39% for water extract to 12.00% for methanol extract (Table 1). The yields of extraction by various solvents decreased in the following order: methanol > 50% aqueous methanol > water. It can be seen that the extraction yield of pure methanol (12.00%) is higher than that of 50% aqueous methanol (8.08%) and water (7.39%). This shows that the extraction yield decreases with increasing polarity of the solvent used in extraction. The total phenolic content values of the extracts range from 16.774 mg GAE/g for water extract to 28.249 mg GAE/g for 50% aqueous methanol extract (Table 1) and they decrease in the following order: 50% aqueous methanol > methanol > water. It was found that the total phenolic content of the extracts decreased with increasing water content in the aqueous methanol. The total phenolic contents of the 50% aqueous methanol extract (28.249 mg GAE/) is higher than that of the methanol extract (21.624 mg GAE/g). This may be attributable to the content of more nonphenolic compounds such as carbohydrates and terpene in methanol extracts than in 50% aqueous methanol extract. It may also be caused by the possible complex formation of some phenolic compounds in the extract that are soluble in 50% aqueous methanol.

The flavonoid contents of the extracts are reported in Table 1. It was observed that the extraction efficiency of different solvents on the flavonoid contents was different to that on the phenolic contents. The total flavonoid contents was highest in methanol extract (67.139 mg QE/g), followed by water extract (39.478 mg QE/g) and 50% aqueous methanol extract (28.350 mg QE/g). This indicates that flavonoids are not the dominating phenolic group in *P. nipponicus* TBRC 6537.

The antioxidant activity assay is based on the reduction of DPPH and ABTS. The ability of the extracts and standard ascorbic acid to scavenge free radicals and pair of the odd electron was shown in this assay (Table 2). It was observed that water extract (IC_{50} 0.228±0.001) and methanol extract (IC_{50} 0.697±0.000) were good as (p=0.015) ascorbic acid (IC_{50} 0.029±0.001) in DPPH radical scavenging activity. The water and methanol extracts exhibited the highest DPPH scavenging activities because of the polar compounds that they contains [31]. Additionally, water extract (IC_{50} 0.049±0.000) and 50% aqueous methanol extract (IC_{50} 0.157±0.002) were as good as (p=0.015) ascorbic acid (IC_{50} 0.012±0.001) in ABTS radical scavenging activity. Reis *etal* .[20] reported that methanol extract from the fruiting body of *C. militaris* showed the highest DPPH radical scavenging activity with the lowest EC_{50} value of 12.17 mg/ml. Furthermore, a hot water extract from cultured mycelia of *O. sinensis* had stronger DPPH radical scavenging effect than that of natural mycelium [32]. In addition, exo-polysaccharide (EPS) and intracellular polysaccharide (IPS) extracted from mycelium of *C. cicadae* showed high DPPH scavenging activity [15] which were similar to *C. gracilis* [33]. The study demonstrates that the extracts of *Cordyceps sinensis* (CSE) and *Cordyceps militaris* (CME) in aqueous systems showed strong ABTS radical scavenging activities [34]. The water extract contains polar compounds like polysaccharide that there is correlation to their antioxidant [32,35].

Antimicrobial Activity

The results of antimicrobial activity of the different extracts tested by the agar well diffusion method, MIC and MBC/MFC against *P. aeruginosa* DMST 4739, *E. coli* DMST 4212, *S. aureus* DMST 2933, *S. epidermidis* TISTR 518, *B. cereus* DMST 5040 and *C. albicans* TISTR 5957 are shown in Table 3 and 4. The methanol extract exhibited antibacterial effects against all tested microorganisms except *C. albicans* TISTR 5957. The methanol extract was found to have antibacterial activity against *P. aeruginosa* DMST 4739 while 50% aqueous methanol and water extracts showed no antibacterial activities. Moreover, it exhibited the highest antibacterial activity against *B. cereus* DMST 5040 (MIC and MBC: 25 mg/ml), followed by *S. aureus* DMST 2933 and *S. epidermidis* TISTR 518 (MIC and MBC: 25 and 50 mg/ml), *P. aeruginosa* DMST 4739 (MIC and MBC: 100 and 200 mg/ml) and *E. coli* DMST 4212 (MIC and MBC: 100 and >200 mg/ml). In contrast, the methanol extract of *P. nipponicus* isolate Cod-MK1201 mycelia did not inhibit the growth of any of the gram positive and gram negative bacteria tested [6]. Reis *et al*. [20] who reported that a methanol extract of fruiting *C. militaris* revealed the highest antibacterial activity against *B. cereus* and *P. aeruginosa* with MIC values of 0.015 mg/ml and MBC values of 0.03 mg/ml. Dong *et al*. [14] reported that the methanol

extract from the fruiting body of *C. militaris* was more effective than the fermented mycelial extract against all microorganisms tested. While the fermented mycelial extract showed selective activity and did not inhibit *P. aeruginosa*, *B. subtilis*, *Aspergillus. flavus* and *C. albicans*.

The 50% aqueous methanol showed antibacterial activity against four bacteria tested. It showed the highest antibacterial activity against *E. coli* DMST 4212 (MIC and MBC: 1.56 and 3.13 mg/ml), followed by *B. cereus* DMST 5040 (MIC and MBC: 6.25 and 12.5 mg/ml), *S. epidermidis* TISTR 518 (MIC and MBC: 12.5 and 25 mg/ml) and *S. aureus* DMST 2933 (MIC and MBC: 12.5 and 50 mg/ml). The water extract showed antibacterial activity against three bacteria. It exhibited the highest antibacterial activity against *E. coli* DMST 4212 (MIC and MBC: 0.05 and 0.16 mg/ml), followed by *S. epidermidis* TISTR 518 (MIC and MBC: 0.78 and 1.56 mg/ml) and *B. cereus* DMST 5040 (MIC and MBC: 3.13 and 6.25 mg/ml). Water extract exhibited the highest antifungal activity against *Candida albicans* TISTR 5957 (MIC and MFC: 0.156 and 0.313 mg/ml). This is consistent with Ren *et al*.[21] whose found that the aqueous extract from *O. sinensis* exhibited antibacterial activity on *B. subtilis* and *S. epidermidis* with MIC values of 938 and 469 µg/ml, respectively, Sharma *et al*.[15] reported that polysaccharide from *C. cicadae* showed broad spectrum for all pathogenic microorganisms tested.

Based on the results above, *E. coli* DMST 4212, *S. epidermidis* TISTR 518, *B. cereus* DMST 5040 and *C. albicans* TISTR 5957 were the most sensitive to water extract with the lowest MIC and MBC/MFC. Also *S. aureus* DMST 2933 was the most sensitive to 50% aqueous methanol extract. Howerver, *P. aeruginosa* DMST 4739 showed moderate to methanol extract. Therefore, the antimicrobial activity of this different extracts from *P. nipponicus* TBRC 6537 mycelia would be related to its total phenolic and flavonoid contents.

The results of this research have shown that *P. nipponicus* TBRC 6537 mycelia showed strong DPPH and ABTS radicals scavenging activities. In addition, it was found to possess a potent antimicrobial activity. These could be used as a source of natural antioxidants and antimicrobial agents.

ACKNOWLEDGEMENTS

This work was financially supported by Mahasarakham University grant year 2017. We would like to thank Dr.Janet Jennifer Luangsa-ard for her suggestion. We thank Dr.Adrian Roderick PLANT for proofreading.

REFERENCES

1. Sung, G.H., Hywel-Jones, N.L., Sung, J.M., Luangsa-ard, J.J., Shrestha, B., Spatafora, J.W. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology.*, 2007; **57**(1): 5-59.
2. Chiua, C.P., Hwang, T.L., Chan, Y., El-Shazly, M., Wu, T.Y., Lo, I.W., Hsu, Y.M., Lai, K.H., Hou, M.F., Yuan, S.S., Changa, F.R., Wua, Y.C. Research and development of *Cordyceps* in Taiwan. *Food Science and Human Wellness.*, 2016; **5**: 177-185.
3. Wong, J. H., Ng, T. B., Wang, H., Wing Sze, S. C., Zhang, K. Y., Li, Q., Lu, X. Cordymin, an antifungal peptide from the medicinal fungus *Cordyceps militaris*. *Phytomedicine*, 2011; **18**: 387-392.
4. Ng, T. B., Wang, H. X. Pharmacological actions of *Cordyceps*, a prized folk medicine. *J. Pharm. Phramacol*., 2005; **57**: 1509-1519.
5. Kepler, R., Ban, S., Nakagiri, A., Bischoff, J., Hywel-Jones, N., Owensby, C.A., Spatafora, J.W. The phylogenetic placement of hypocrealean insect pathogens in the genus Polycephalomyces: an application of one fungus one name. *Fungal Biol*., 2013; **117**(9): 611-622.
6. Sangdee, K., Seephonkai, P., Buranrat, B., Surapong, N., Sangdee, A. Effects of ethyl acetate extracts from the *Polycephalomyces nipponicus* isolate cod-MK1201 (Ascomycetes) against human pathogenic bacteria and a breast cancer cell line. *Int. J. Med Mushrooms.*, 2016; **18**(8):733-743.
7. Isaka, M., Tanticharoen, M., Kongsaeree, P., Thebtaranonth, Y. Structures of cordypyridones A D, antimalarial N hydroxy and N methoxy 2 pyridones from the insect pathogenic fungus *Cordyceps nipponica*. *J. Org. Chem*., 2001; **66**: 4803-4808.
8. Stajic ,M., Vukojevic, J., Knezevic, A., Lauševic, S.D., Milovanovic, I. . Antioxidant protective effects of mushroom metabolites. *Curr. Top Med. Chem*., 2013; **13**:2660-2676.
9. Shebis, Y., Iluz, D., Kinel-Tahan, Y., Dubinsky, Z., Yehoshua, Y. Natural antioxidants function and sources. *Food. Nutr. Sci*., 2013; **4**: 643-649.

10. Patel, S.H., Suthar, J.V., Patel, R.K., Zankharia, U.S., Jani, V.R., Gajjar, K.N. Antimicrobial activity investigation of *Aegle marmelos*, *Couroupita guianesis*, *Manilkara hexandra*, Cow Urine and Dung. *Res. J. Pharm. Biol. Chem. Sci*., 2015; **6**(4): 1014-1022.
11. Prasad, R., Varshney, V.K., Harsh, N. S. K., Kumar, M. Antioxidant capacity and total phenolics content of the fruiting bodies and submerged cultured mycelia of sixteen higher Basidiomycetes Mushrooms from India. *Int. J. Med Mushrooms*., 2015; **17**(10): 933-941.
12. Rao, J.R., Millar, B.C., Moore, J.E. Antimicrobial properties of shiitake mushrooms (*Lentinula edodes*). *Int. J. Antimicrob Agents*., 2009; **33**: 591-592.
13. Kozarski, M., Klaus, A., Jakovljevic, D., Todorovic, N., Vunduk, J., Petroviæ, P., Niksic, M., Vrvic, M.M., Griensven, L.v. Antioxidants of edible mushrooms. *Molecules*., 2015; **20**: 19489-19525.
14. Dong, C., Yang, T., Lian, T. A Comparative study of the antimicrobial, antioxidant, and cytotoxic activities of methanol extracts from fruit bodies and fermented mycelia of caterpillar medicinal mushroom *Cordyceps militaris* (Ascomycetes). *Int. J. Med Mushrooms*., 2014; **16**(5): 485-495.
15. Sharma, S.K., Gautam, N., Atri, N.S. Optimized extraction, composition, antioxidant and antimicrobial activities of exo and intracellular polysaccharides from submerged culture of *Cordyceps cicadae*. *BMC. Complement. Altern. Med*., 2015; **15**: 446.
16. Lu, J., Gu, G., Hao, L., Jin, Z., Wang, X. Characterization and in vitro antioxidant activity of a polysaccharide from *Cordyceps sobolifera*. *J. Food. Process. Preserv*., 2016; **40**(3): 447-452.
17. Ahn, Y.J., Park, S.J., Lee, S.G., Shin, S.C., Choi, D.H. Cordycepin: selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against *Clostridium* spp. *J. Agric. Food. Chem*., 2000; **48**: 2744-48.
18. Imtaij, A., Lee, T.S. Screening of antibacterial and antifungal activities from Korean wild mushrooms. *World. J. Agric. Sci*., 2007; **3**(3): 316-321.
19. Xiao, J.H., Xiao, D.M., Sun, Z.H., Xiong , Q., Liang, Z.Q., Zhong, J.J. Chemical compositions and antimicrobial property of three edible and medicinal *Cordyceps* species. *J. Food. Agric .Environ*., 2009; **7**(3-4): 91-100.
20. Reis, F.S., Barros, L., Calhelha, R.C., Ciric, A., Griensven, L.J.V., Sokovic, M., Ferreira, I.C. The methanolic extract of *Cordyceps militaris* (L.) Link fruiting body shows antioxidant, antibacterial, antifungal and antihuman tumor cell lines properties. *Food. Chem. Toxicol.*, 2013; **62**: 91-98.
21. Ren, L., Hemar, Y., Perera, C.O., Lewis, G., Krissansen, G.W., Buchanan, P.K. Antibacterial and antioxidant activities of aqueous extracts of eight edible mushrooms. *Bioact. Carbohydr. Diet. Fibre*., 2014; **3**: 41-51.
22. Zhang, Y., Wua, Y.T., Zheng, W., Han, X.X., Jiang, Y.H., Hu, P.L., Tang, Z.X., Shi, L.E. The antibacterial activity and antibacterial mechanism of a polysaccharide from *Cordyceps cicadae*. *J. Funct. Foods*., 2017; **38**: 273-279.
23. Liu, X.L., Dong, M.S., Chen, X.H., Jiang, M., L.v, X., Yan, G. Antioxidant activity and phenolics of an endophytic *Xylaria* sp. From *Ginkgo biloba*. *Food. Chem.*, 2007; **105**: 548-554.
24. Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C., Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Food. Drug. Anal*., 2002; **10**(3): 178-182.
25. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Even, C.R. Antioxidant activity apply an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.*, 1999; **26**: 1231-1237.
26. Rahman, S.F.S.A., Sijam, K., Omar, D. Identification and antibacterial activity of phenolic compounds in crude extracts of *Piper sarmentosum* (Kadok). *J. Pure. APPL. Microbio*., 2014; **2**(8): 483-490.
27. Dung, N. T., Kim, J. M., Kang, S. C. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. *Food. Chem. Toxicol*., 2008; **46**: 3632-3639.
28. Sarker, S.D., Nahar, L., Kumarasamy, Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *J. Methods*., 2007; **42**: 321-324.
29. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard, 2nd ed. Wayne, Pennsylvania, NCCLS Document M27-A2 (ISBN 1-56238-469-4)., 2002.
30. Sánchez, C. Reactive oxygen species and antioxidant properties from mushrooms. *Synth Syst. Biotechnol*., 2017; **2**:13-22.
31. Shashidhar, M.G., Giridhar, P., Sankar, K.U., Manohar, B. Bioactive principles *Cordyceps sinensis*: a potent food supplement - a review. *J.*

Funct. Foods., 2013; **5**: 1013-1030.

32. Dong, C.H., Yao, Y.J. In vitro evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of *Cordyceps sinensis*. *LWT: Food Sci. Technol*., 2008; **41**: 669-677.
33. Sharma, S.K., Gautam, N., Atri, N.S. Optimization, composition, and antioxidant activities of exo- and intracellular polysaccharides in submerged culture of *cordyceps gracilis* (Grev.) Durieu & Mont. *J. Evid. Based Complementary. Altern Med*., 2015; 2015: 1-8.
34. Yu, H.M., Wang, B.S., Huang, S.C., Duh, P.D. Comparison of protective effects between cultured *Cordyceps militaris* and natural *Cordyceps sinensis* against oxidative damage. *J. Agric. Food Chem.*, 2006; **54**(8): 3132-3138.
35. Russell, R., Paterson, M. *Cordyceps* - A traditional Chinese medicine and another fungal therapeutic biofactory ?. *Phytochemistry*., 2008; **69**: 1469-1495.