Antioxidant and Antimicrobial Properties of Aqueous Extract from *Dictyophora indusiata*

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Abstract: The antioxidant and antimicrobial properties of hot water extract (WE) obtained from *Dictyophora indusiata* were investigated. The free radical scavenging ability of WE on DPPH• was 97.35% at 2 mg/ml concentration. The reducing power of WE was moderate (1.22 at 2 mg/ml). Similarly, the WE displayed average scavenging effect on hydroxyl radical (52.28% at 2 mg/ml) and superoxide anion scavenging effect (48.64% at 2 mg/ml). However, the WE exhibited a very weak ferrous ion chelating effect of 18.56% at 2mg/ml concentration. Antimicrobial assay revealed that WE from *D. indusiata* can inhibit both bacteria and fungi used as indicators for antimicrobial effect at concentration of 200 mg/ml. The results suggest that WE possess good antioxidant and antimicrobial properties.

Keywords: Antioxidant, Antimicrobial, Hot water, *Dictyophora indusiata*, Extract.

INTRODUCTION

Mushrooms possess high contents of qualitative protein, crude fibre, minerals and vitamins [1, 2]. Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health [3, 4]. They produce a wide range of secondary metabolites with high therapeutic value [5]. Health promoting properties, e.g. antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory effects, have been reported for some species of mushrooms [6-8]. Both fruiting bodies and the mycelium contain compounds with wide-ranging antioxidant and antimicrobial activities [7, 9, 10].

As sources of antioxidants, edible mushrooms are desirable since they are safe to eat and known not to place additional stress on the body [4]. Hence, edible mushrooms serve as a good alternative to synthetic antioxidant such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) which are known to be carcinogenic [11, 12].

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment [13]. Hence, they are rich sources of natural antibiotics. The activity of the exudates from mushroom mycelia against protozoa such as the parasite that causes malaria, *Plasmodium falciparum* [14, 15] and other microorganisms [13] had been reported. Chinese Shiitake mushroom (*Lentinus edodes*) has also been reported to possess both anti-tumor and antimicrobial properties [16]. Presently, most antimicrobial that are available are sourced from microscopic fungi [13]. Based on the reports above, edible mushrooms may be the source of new antimicrobial that can combat the emergence of resistant microbial strains that is now rampant.

Dictyophora indusiata (Vent.) Desv. (≡*Phallus indusiatus* Vent.) is an edible mushroom that is considered a good delicacy by the Chinese. The species is also known as "Veiled Lady Mushroom" and belongs to the family of *Phallaceae* Corda. The fruiting body begins as an "egg" stage, from which the phallic-looking basidioma emerges over the course of just a few hours. The antioxidant property of methanolic extract from *D. indusiata* had been reported [17]. Moreover, the antimicrobial property of ethyl acetate, ethanol, acetone and volatile oil extracts from *Dictyophora* species have also been reported [18, 19].

In the present study, *in vitro* experiments, such as DPPH• scavenging, reducing activity, hydroxyl ion and superoxide anion radicals scavenging ability and iron chelating activity, were performed to assess the antioxidant potential of aqueous extract from *D. idusiata*. Moreover, the antimicrobial potential of the extract was assessed against some selected pathogenic and food spoilage organisms. The present study will reveal the biopharmaceutical potential of aqueous extract obtained from *D. indusiata*.

MATERIALS AND METHODOLOGY

Fungal Materials

The fungal materials used in this study were dried fruit bodies of *D. indusiata* from a mushroom farm and bought in Gutian County, Fujian Province, People's Republic of China. The fruit bodies were further dried at 45 ^{o}C in the laboratory to a constant weight and ground into powder before the extraction process.

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Chemicals

2-deoxy-o-ribose and 1,1-diphenyl-2-picrylhydrazyl (DPPH•), Folin-Ciocalteau-reagent, Gallic acid were purchased from Sigma-Aldrich (Steinheim, Germany); 2-thiobarbituric acid (TBA) from Acros Organics (Geel, Belgium); ethylenediaminetetraacetic acid (EDTA), nystatin and tetracylcline from Amresco (Ohio, USA); sodium acetate trihydrate, acetic acid, hydrogen peroxide (H_2O_2), trichloroacetic acid (TCA) and ascorbic acid from Beijing Chemical Reagents Company (Beijing, China); Ampicillin from Huabei Pharm., China; butylated hydroxytoluene (BHT) from China National Pharmaceutical Group Shanghai Chemical Reagents Company (Shanghai, China); detection Kit of superoxide anion radical scavenging activity from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All reagents were analytical grade.

Hot Water Extraction Process

The powdered *D. indusiata* was soaked in 10 part volume of distilled water at 95 to 100 °C for 2 h. The mixture of water and the residue was centrifuged for 10 min at 3214 g to obtain the water extract. This procedure was repeated twice. The water extract was filtered through a filter-paper under pressure using a Millipore machine and the filtrate concentrated to one third of the total volume by using a rotary evaporator under reduced pressure at 50 °C, and the resultant extract was lyophilized to dryness in vacuum. The lyophilized water extract (WE) powder was stored in dark at 4 °C before use.

Antimicrobial Assay

Indicator Organisms Used for Antimicrobial Assay

A total of 12 microbial strains made up of 8 bacteria, 2 yeasts and two moulds were used. The microbial strains were *Bacillus subtilis* ATCC 6633, *Bacillus cereus* CMCC1.1846, *Alcaligenes faecalis* CMCC1.1837, *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC 6358P, *Pseudomonas aeruginosa* ATCC10145, *Shigella dysenteriae* CMCC 51252, *Salmonella typhimurium* CMCC 1.1174, *Candida albicans* ATCC10231, *Cryptococcus neoformans* 1038, *Aspergillus niger* 1349 and *Aspergillus flavus* 1348. Microbial strains were obtained from China General Microbial Culture Collection Centre (CGMCC).

Antimicrobial Activity

Antimicrobial activity of WE from *D. indusiata* was determined by the agar well diffusion method [8]. The bacterial strains used as indicator organism were cultivated on Nutrient Agar Medium at 30 ± 1 °C for 24 h while the fungal strains were cultivated on Yeast Malt Extract Agar at 26 ± 1 °C for 48 to 72 h. Aliquot of culture (100 µl) was evenly spread on the surface of the solidified agar. Wells of 7 mm were bored in the agar with sterile cork borers. Extract dissolved in 10% dimethyl sulfoxide (DMSO) to the concentration of 10 to 200 mg/ml and filtered through 0.22 µm membrane filter was introduced into the wells. A 100 µl volume was placed in each well. The plates were incubated at 30 ± 1 °C for 24 h for bacteria while the fungi were incubated at 26 ± 1 °C for 48 to 72 h. Tetracycline and ampicillin were used as standard antibacterial while nystatin was used as antifungal standard under the conditions specified for bacteria and fungi respectively. The diameter of the inhibition zones were measured in milliliters. Inhibition zones were measured in triplicates (three plates per indicator organism). Agar well in which 10% DMSO was added served as negative control. The inhibitory action of negative control was not visible.

Assessment of Antioxidant Activities of Water Extrate

Multimechanistic antioxidative assays as listed below were employed in assessing the antioxidant property of the WE from *D. indusiata*.

Scavenging Effect of DPPH• Radicals

Radical scavenging potential of WE was assessed using an ethanolic solution of the "stable" free radical, DPPH•. The method of Blois [20] was used in studying the effect of WE from *D. indusiata* on DPPH• radicals with some modifications. A solution of DPPH• (0.5 mmol/L) in ethanol and 0.05 mol/L acetate buffer (pH 5.5) was prepared. Extract in solution (0.1 ml) at different concentrations was mixed with 2 ml of acetate buffer, 1.9 ml of absolute ethanol and 1 ml DPPH• solution. The mixture was shaken immediately after adding DPPH• and allowed to stand at room temperature in dark for 30 min. The decrease in absorbance at 517 nm was measured using a UNICO 2100 spectrophotometer. BHT was used as positive control and the sample solution without DPPH• was used as blank. The radical scavenging activity was measured as a decrease in absorbance of DPPH• and calculated as:

$$\text{Scavenging activity (\%)} = \frac{Ab - (As - Asb)}{Ab} \times 100$$

where Ab, As and Asb are absorbances at 517 nm of DPPH• of the blank, the extract or the control and the sample blank respectively.

Measurement of Reducing Power

The reducing power of WE from *D. indusiata* was determined by the method of Yen and Chen [21] with some modification. Briefly, extracts (0.0625–2 mg/ml) in PBS (2.5 ml, 0.2 mol/L, pH 6.6) were added to potassium ferricyanide (2.5 ml, 10 mg/ml) and the mixture was incubated at 50 °C for 20 min. 2.5 ml of 10 mg/ml trichloroacetic acid (TCA) was added and centrifuged at 1160 g for 10 min. De-ionised water (2.5 ml) was added to 2.5 ml of the supernatant and 0.5 ml of 1.0 mg/ml ferric chloride. The absorbance was measured at 700 nm against a blank in a spectrophotometer (UNICO 2100). Ascorbic acid was used as control. A higher absorbance of the reaction indicates a higher reducing power.

Scavenging Effect of Hydroxyl Radical

The determination of scavenging effect on hydroxyl radicals was carried out as described in Halliwell *et al.* [22]. The reaction mixture in a final volume of 1.0 ml, containing 0.4 ml of 20 mmol/ml sodium phosphate buffer (pH 7.4), 0.1 ml of 0.125–2 mg/ml extracts, 0.1 ml of 60 nmol/L deoxyribose, 0.1 ml of 10 mmol/L hydrogen peroxide, 0.1 ml of 1 mmol/L ferric chloride, 0.1 ml of 1.04 mmol/L EDTA and 0.1 ml of 2 mmol/L ascorbic acid was incubated at 37 °C for 1 h. Solutions of $FeCl_2$ and ascorbic acid were made up immediately before use in de-ionised water. The reaction was stopped by adding 1 ml of 17 mmol/L thiobarbituric acid (TBA) and 1 ml of 17 mmol/L trichloroacetic acid (TCA). The mixture

was boiled for 15 min, cooled in ice and then the absorbance measured at 532 nm using a UNICO 2100 spectrophotometer. BHT was used as positive control while distilled water in place of extracts or BHT was used as blank and the sample solution without adding deoxyribose as sample blank.

$$\text{Scavenging activity (\%)} = \frac{Ab - (As - Asb)}{Ab} \times 100$$

where Ab, As and Asb are the absorbance at 532 nm of the blank, the extract or BHT and the sample blank without deoxyribose, respectively.

Scavenging Effect of Superoxide Anion Radicals

A commercial kit was used to assay this. Superoxide anion radicals were generated by xanthine/xanthine oxidase system and reacted with 2,4-iodiphenyl-3,4-nitrophenyl-5-phenyltetrazolium chloride to form formazan, a colored compound which was spectrophotochemically quantified at 550 nm. The production of formazan is inversely related to the superoxide anion radical scavenging activity of tested sample. The final results were expressed as the inhibition degree of formazan production. BHT was used as positive control and distilled water in place of extracts or BHT as blank. The percentage of inhibition of superoxide anion radicals was calculated using the formula below.

$$\text{Scavenging activity (\%)} = \frac{Ab - As}{Ab} \times 100$$

where Ab is the absorbance of the blank without extract or BHT and As is the absorbance in the presence of extract or BHT.

Ferrous ion Chelating Assay

The chelation of ferrous ion by the extracts was ascertained by the methods previously described [23]. One millilitre of extracts (0.25–8 mg/ml) was mixed with 3.7 ml of deionised water and then the mixture was reacted with ferrous chloride (2 mmol/L, 0.1 ml) and ferrozine (5 mmol/L, 0.2 ml) for 20 min. The absorbance at 562 nm was determined spectrophotometrically. EDTA was used as positive control. Chelating activity on ferrous ion was calculated using the equation below:

$$\text{Chelating Activity (\%)} = \frac{Ab - As}{Ab} \times 100$$

where Ab is the absorbance of the blank without extract or EDTA and As is the absorbance in the presence of extract or EDTA.

Statistical Analysis

All experiments were carried out in triplicate. Data obtained were analyzed by one way analysis of variance and means were compared by Duncan's multiple range test (SPSS 11.5 version). Differences were considered significant at $p<0.05$.

RESULTS

Antimicrobial Activity of Water Extrate

Table **1** showed the antimicrobial activity of WE. *Alcaligenes faecalis* was the most susceptible bacterium while the least susceptible was *E. coli*. Among the fungi used as indicators for antimicrobial test, *Candida albicans* was more susceptible to the antimicrobial effect of WE than the others.

DPPH• Scavenging

At 0.0625–2 mg/ml concentrations, WE exhibited scavenging ability of 18.12–97.58%. Scavenging activities of *D. indusiata* were well pronounced at concentrations of 0.5 mg/ml to 2 mg/ml (Fig. **1**). The free radical scavenging effect of WE was slightly higher than BHT at concentrations of 0.5 to 2 mg/ml. WE exhibited concentration dependent free radical scavenging effect.

Reducing Activity

Reducing ability of WE was also concentration dependent (Fig. **2**). However, ascorbic acid (positive control) exhibited a better and significantly different ($P<0.05$) reducing power than WE. The WE exhibited a fair reducing power of 0.63 at 0.125 mg/ml concentration and this increased to 1.22 at 2 mg/ml concentration.

Hydroxyl ion Scavenging Activity

WE from *D. indusiata* exhibited moderate and concentration dependent hydroxyl radical scavenging ability (Fig. **3**). The ability of WE to scavenge hydroxyl radicals was below

Table 1. Diameter of Zone of Inhibition (mm) of WE from *D. indusiata* Against Selected Microorganisms

Microorganism	WE (200 mg/ml)	Ampicillin (10 µg/ml)	Tetracycline (30 µg/ml)	Nystatin (40 µg/ml)
Escherichia coli	4.0 ± 1.0	4.0 ± 0.0	10.7 ± 0.6	NT
Alcaligenes faecalis	14.3 ± 0.6	10.0 ± 0.5	23.7 ± 3.7	NT
Salmonella typhimurium	5.5 ± 0.5	8.7 ± 0.5	4.0 ± 0.0	NT
Shigella dysenteriae	8.0 ± 1.0	9.0 ± 1.0	4.0 ± 0.0	NT
Pseudomonas aeruginosa	7.5 ± 0.5	--	4.3 ± 0.5	NT
Bacillus subtilis	6.0 ± 0.5	25.7 ± 1.2	35.0 ± 1.5	NT
Staphylococcus aureus	8.3 ± 0.5	4.3 ± 0.6	5.0 ± 1.0	NT
Bacillus cereus	4.7 ± 0.6	4.5 ± 0.6	25.6 ± 1.2	NT
Aspergillus niger	--	NT	NT	5.7 ± 0.6
Aspergillus flavus	2.5 ± 0.6	NT	NT	4.3 ± 0.5
Candida albican	11.0 ± 1.0	NT	NT	10.3 ± 0.5
Cryptococcus neoformans	7.3 ± 1.2	NT	NT	9.3 ± 1.2

Each value is expressed as mean ± standard deviation (n=3). NT: Not tested; --: No inhibition.

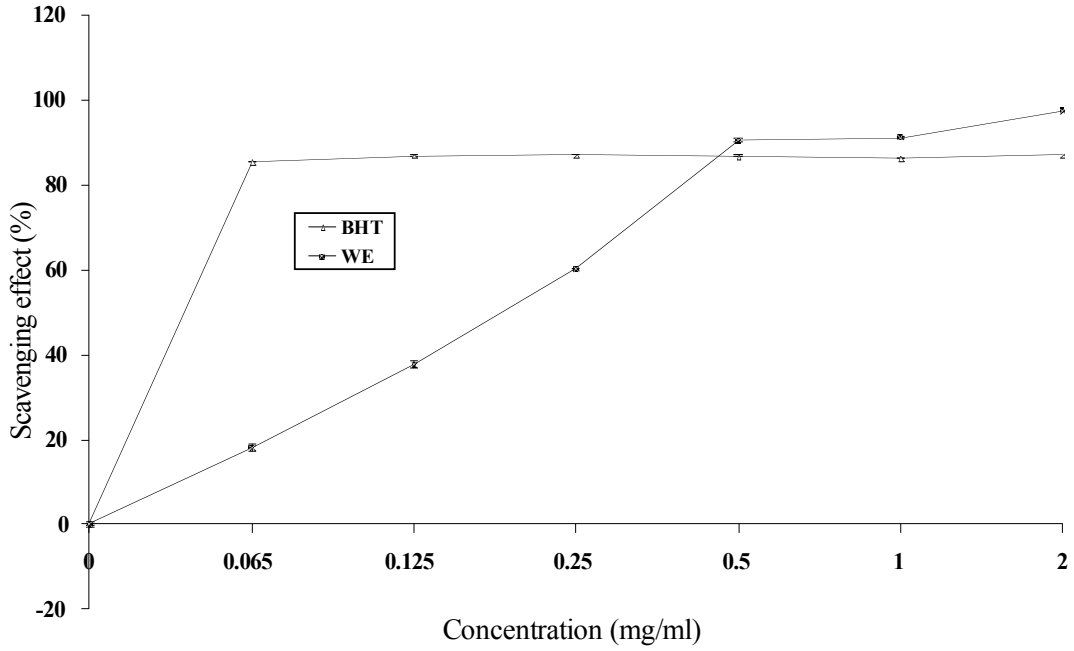

Fig. (1). Free radical scavenging ability of WE from *D. indusiata*. Each value is expressed as mean of three replicates (n=3). BHT: butylated hydroxyl toluene; WE: water extract.

average at concentrations of 0.125–1 mg/ml, however, it was slightly above average (52.28%) at 2 mg/ml. The hydroxyl radical scavenging property of WE was lower and significantly different from what was exhibited by BHT (positive control).

Superoxide Anion Scavenging Ability

The WE exhibited a moderate and concentration dependent superoxide anion activity until 1 mg/ml concentration (Fig. **4**). The ability of WE to scavenge superoxide anion was close to average at 1 mg/ml (45.49%) and 2 mg/ml (47.64%) respectively.

Iron Chelating Activity

Fig. (**5**) shows the ferrous ion chelating ability of WE from *D. indusiata*. The extract displayed a very weak chelating activity of 18.56% at 8 mg/ml concentration. At concentrations of 0.252 mg/ml, iron chelating effect ranges from 2.6–9.88%. However, there was a slight increase to 15.25% and 18.56% in ferrous iron chelating effect at 4 mg/ml and 8

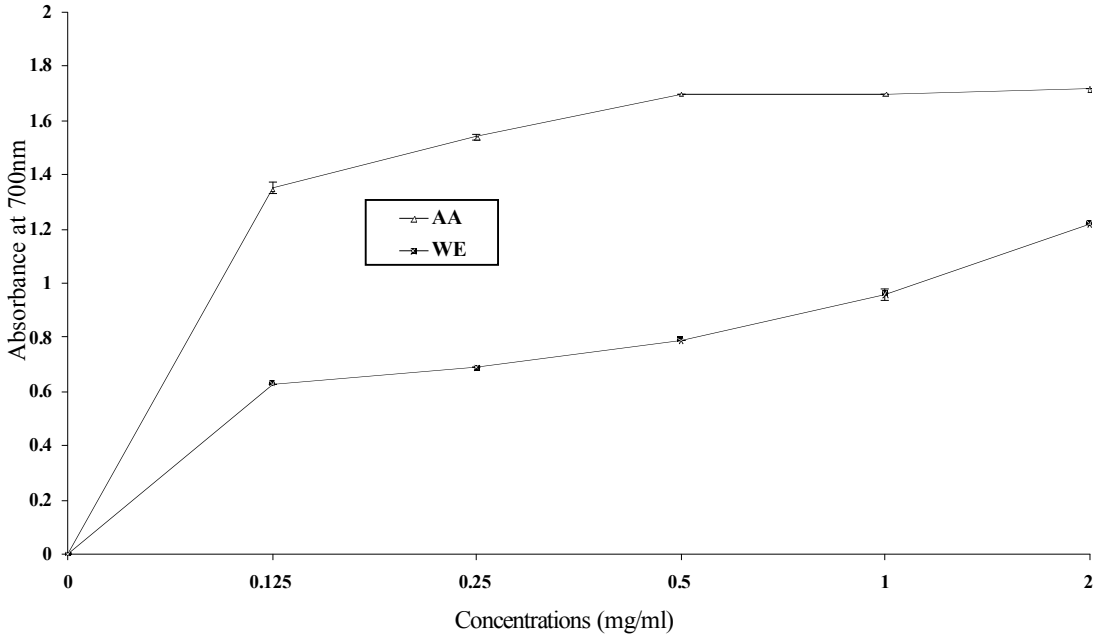

Fig. (2). Reducing power of WE from *D. indusiata*. Each value is expressed as mean of three replicates (n=3). AA: Ascobic acid; WE: Water extract.

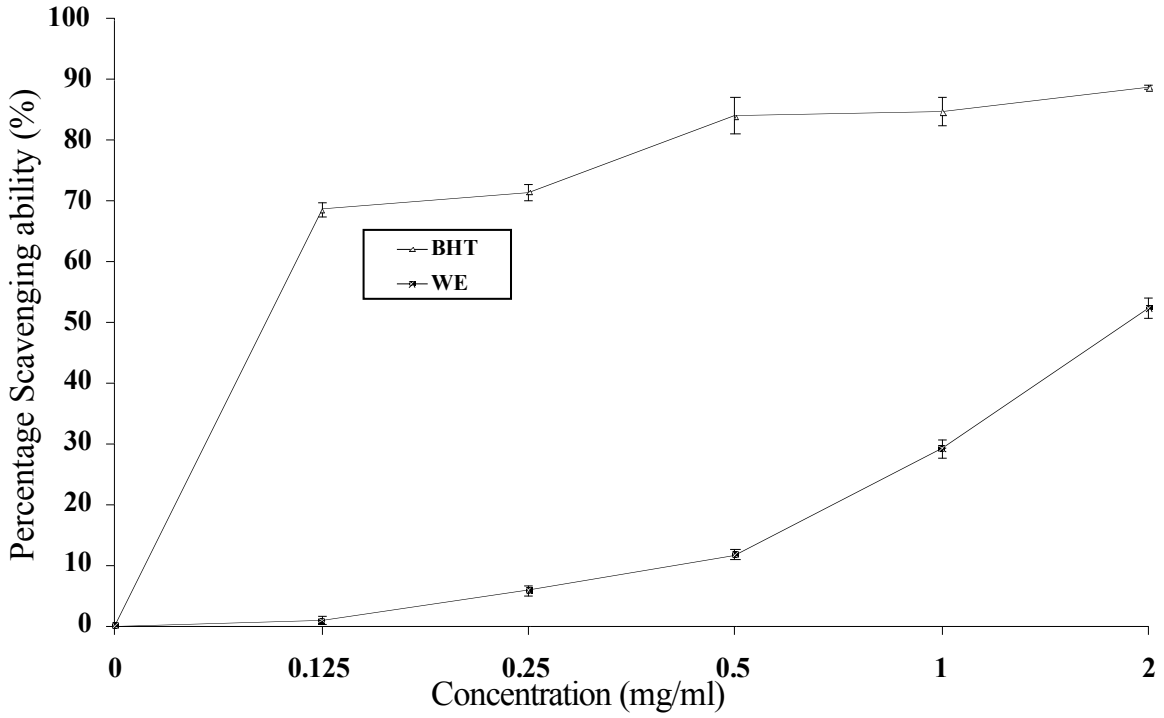

Fig. (3). Hydroxyl radical scavenging ability of WE from *D. indusiat*a. Each value is a mean of three replicates (n=3). BHT: Butylated hydroxyl toluene; WE: Water extract.

mg/ml concentrations respectively. Ferrous iron chelating effect of WE was concentration dependent. The iron chelating activity of positive control (EDTA) was significantly higher than WE at all tested concentrations. EDTA displayed iron chelating activity as high as 97.86% at concentration of 0.25 mg/ml.

DISCUSSION

Mushrooms have been appreciated as sources of food nutrients for centuries, and, especially, used for medicinal purposes in the orients for centuries [13]. In the present study, antioxidant and antimicrobial activities of aqueous extract from *D. indusiata*, an edible mushroom popular

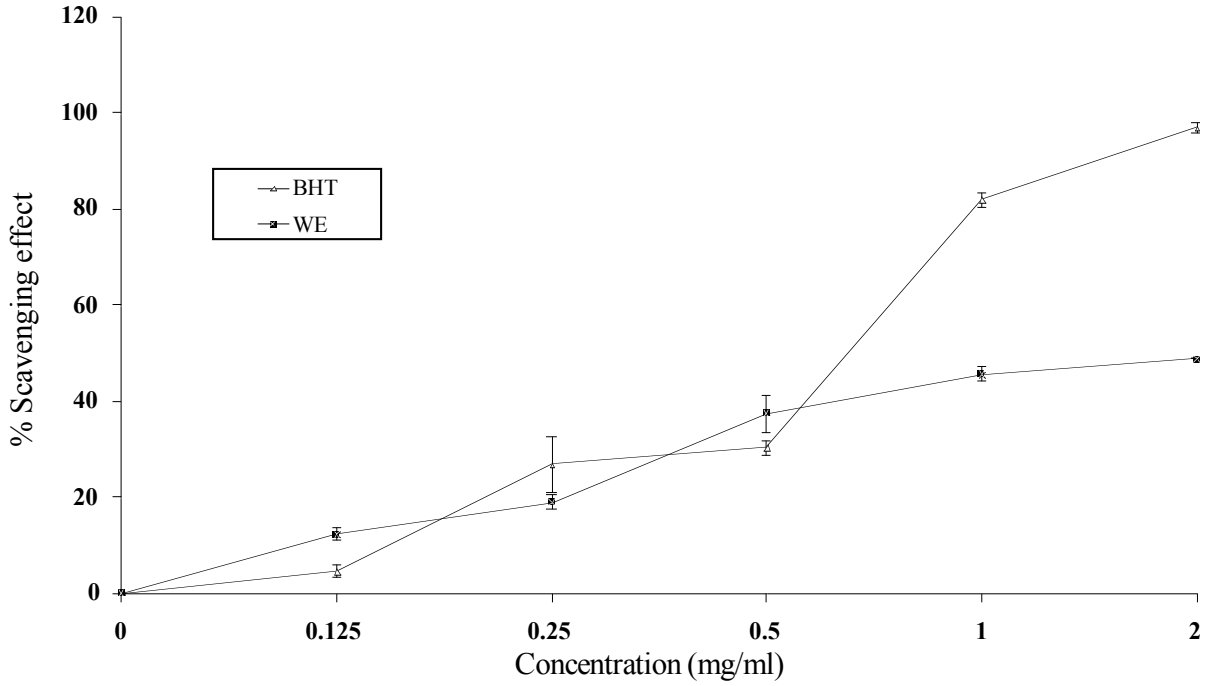

Fig. (4). Superoxide anion scavenging effect of WE from *D. indusiata*. Each value is expressed as mean of three replicates (n=3). BHT: Butylated hydroxytoluene; WE: Water extract.

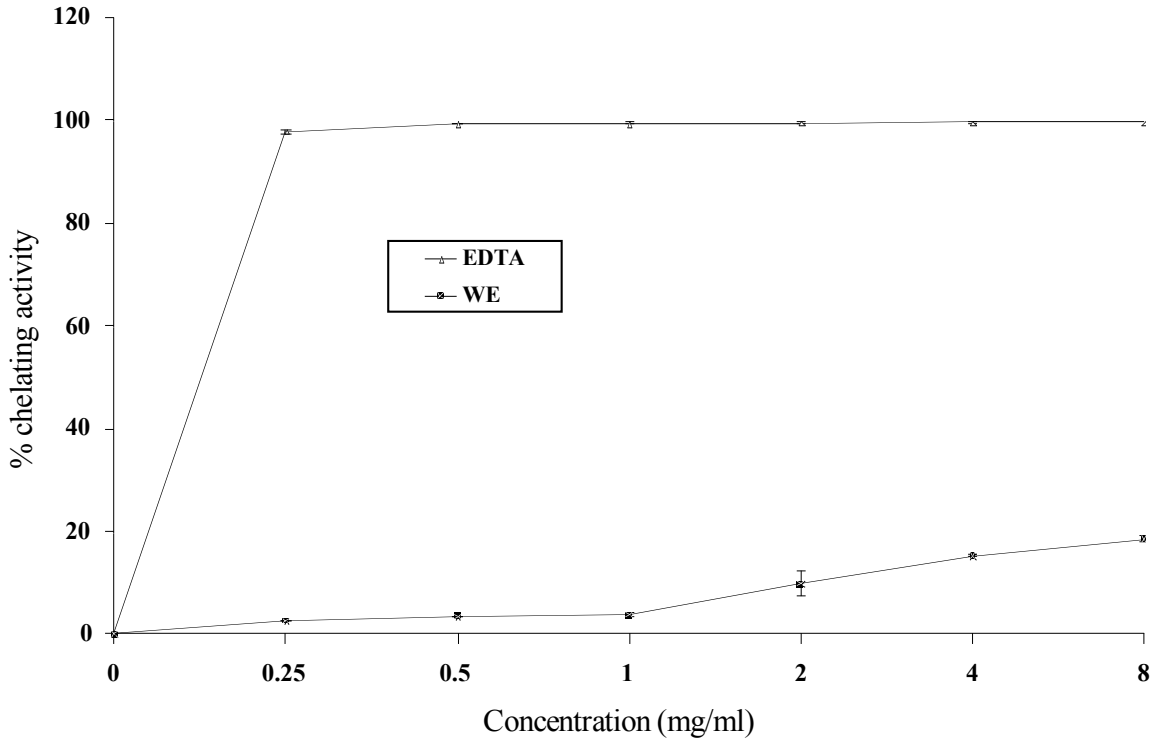

Fig. (5). Ferrous ion chelating activity of WE from *D. indusiata*. Each value is expressed as mean of three replicates (n=3). EDTA: Ethylene diamine tetraacetic acid; WE: Water extract.

among the Chinese, were assessed by using various *in vitro* testing systems.

The hot water extract (WE) from *D. indusiata* was able to inhibit the growth of both bacteria and fungi used as indicator organisms (Table **1**), displaying a wide spectrum antimicrobial activity at concentration of 200 mg/ml whilst Barros *et al*. [8] reported antimicrobial effect of phenolics extracts of Portuguese wild mushrooms to be between 10 to 300 mg/ml. The most susceptible bacteria in the present study was *Alcaligenes faecalis* and the least susceptible was *E. coli*. Among the fungi used as indicators for antimicrobial test, *Candida albicans* was the most susceptible species to the antimicrobial effect of WE.

DPPH• assay is a method used to evaluate antioxidant activities in a relatively short time compared with other methods. Moreover, DPPH• is not affected by certain side reactions such as metal chelation and enzyme inhibition which is a major disadvantage associated with laboratory-generated free radicals [24]. The WE from *D. indusiata* was a good scavenger of free radicals (Fig. **1**). Earlier report on methanolic extract from *D. indusiata* showed an excellent scavenging effect of 92.1% on DPPH• radicals [17] at concentration of 6.4 mg/ml. However, the WE from *D. indusiata* exhibited 97.58% free radical scavenging ability at a lower concentration of 2 mg/ml. The WE also show a good reducing ability when compared to methanolic extract of *D. indusiata*. The reducing power (1.20) of WE at 2 mg/ml concentration was higher than 1.09 reducing power at 3 mg/ml concentration reported for methanolic extract from *D. indusiata* by Mau *et al*. [17].

The WE from *D. indusiata* also exhibited moderate and concentration dependent hydroxyl radical scavenging ability (Fig. **3**). Hydroxyl radicals are highly reactive [25] and can induce severe damage to adjacent molecules [26]. Mau *et al*. [27] had earlier reported a concentration dependent increase in the scavenging abilities of all hot water extracts from *Ganoderma tsugae* on hydroxyl radicals while methanolic extract from *G. tsugae* showed a non concentration dependent hydroxyl radical scavenging ability. Similarly, the WE obtained from *D. indusiata* exhibited a concentration dependent activity (Fig. **3**).

Superoxide anions are biologically important since they are very harmful to cellular components of biological systems because their decomposition can lead to the formation of stronger oxidative species such as singlet oxygen and hydroxyl radicals [25, 28]. Superoxide anion scavenging effect of the WE is also moderate and concentration dependent until 1 mg/ml concentration (Fig. **4**). Dong and Yao [29] had earlier observed that hot water extract from natural and cultured mycelium of *C. sinensis* had a better superoxide scavenging effect than methanolic extract. Hence, the WE from *D. indusiata* hold promise as a source of bioactive that may effectively scavenge superoxides.

The WE displayed a very weak chelating activity of 18.55% at 8 mg/ml concentration. Weak ferrous iron chelating effect of 17.1% at concentration of 3 mg/ml had earlier been reported for methanolic extract from *D. indusiata* [17].

The present report shows that the WE from *D. indusiata* may be effective as antioxidant and antimicrobial agents. The higher antioxidative properties, observed for the WE when compared with antioxidative activity of methanolic extract from *D. indusiata* previously reported by Mau *et al*. [17], may be due to the presence of water soluble phenolics in WE. Water soluble phenolics have been reported to play

important role in reversing oxidative stress [30]. Puttaraju *et al*. [31] have also reported that antioxidant activity of water extract from *Termitomyces* species was better than methanolic extract. Moreover, antimicrobial activity of the WE was well pronounced against both gram positive and gram negative bacteria. Furthermore, *Candida albicans*, a pathogenic yeast, was also susceptible to the antimicrobial action of the extract. The WE from *D. indusiata* may therefore hold promise as a good source of bioactive for biopharmaceutical exploitation. Further work aimed at identification of the specific biological agents responsible for antioxidant and antimicrobial properties in WE is the next focus.

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