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PRELIMINARY STUDY OF THE ANTIOXIDANT PROPERTIES OF OPHIOCORDYCEPS SOBOLIFERA

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Abstract:

The aim of study was to determine preliminary phytochemical analysis and the antioxidant potential of Ophiocordyceps sobolifera. The antioxidant activity of Ophiocordyceps sobolifera was evaluated using 2,2-diphenyl–1–picrylhydrazyl (DPPH) radical scavenging and total antioxidant activity methods. Phytochemical analyses revealed the presence of steroids, triterpenoids, alkaloids, phenolics, flavoinods, protein, carbohydarte and saponins. The antioxidant activity of the ethanol extracts and water extracts showed high antioxidant activity with the lowest half maximal inhibitory concentration (IC_{50}) values to 0.70 from 0.95 mg/mL, realtively. Total antioxidant capacity of the O. sobolifera showed contained from 5.52 ± 0.14 to 12,71 ± 0.23 mg GA/g or from 3.55 ± 0.15 to 7.87 ± .05 µmol AS/g. These data suggest that O. sobolifera is a natural source of antioxidants.

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INTRODUCTION:

The genus *Cordyceps* is wildly found in Asian countries such as Vietnam, Thailan, China, Japan, Korea [1], [2]. Some *Cordyceps* spp. were used as a traditional medicine to immunity modulation, fatigue resistance, longevity elongation, and other functions [3], [4], [5]. *Ophiocordyceps sobolifera* (syn *Cordyceps sobolifera*) belongs to the *Cordyceps* family, is an entomogenous fungi species that is parasitic on wingless cicada nymphs. The previous reports indicated that *Ophiocordyceps sobolifera* had been exhibited significantly HIV-1 reverse transcriptase inhibitory activity [6]. In addition, Chiu et al. reported that polysaccharide extract of *Ophiocordyceps sobolifera* attenuates renal injury in endotoxemic rats [7]. However, through the literature review, the number of studies on chemical composition and antioxidant activity of species is very limited.

The antioxidant activity is one of the most important properties of disease prevention and cure. The methods were determed antioxidant activity which were differ in terms of their assay principle, experimental conditions and mechanism. A certain survey pattern evaluates only one aspect of antioxidant activity. The approach based on the stable free radical DPPH and total antioxidant capacity could be seen as the most effective way for the measurement of the antioxidant activity because of their fast and simple features.

The aim of study was to preliminary phytochemical analysis and elevaluting the antioxidant potential of *Ophiocordyceps sobolifera* by evaluating total antioxidant capacity and DPPH radical scavenging.

EXPERIMENTAL SECTION:

Material samples:

Cordyceps sobolifera is purchased from the United State and grown on biomass in Vietnam, then was taxonomically identified by the Institute of Microbiology and Biotechnology, VietNam national university, HaNoi.

Preparation of ethanol extracts and water extracts:

A sample of *O. sobolifera* (50 g) was packed in separate round bottom flask for sample extraction using different solvents namely ethanol and water. The extraction was conducted with 500 mL of each solvent three times at boiling temperature of solvent for a period of 3 hours. The solutions were combined and evaporated under reduced pressure at 50 ^{0}C, resulting in 3.9038 ± 0.1124 g of the crude ethanol extract and 4.1566 ± 0.1134 g of the crude water extract .

Test of phytochemical of extractions:

The presence of phytochemicals such as alkaloids, saponins, tannins, phenolic, flavonoid, carbohydrate and steroids were carried out different methods [8], [9].

Steroids

Acetic anhydride (1 mL) was added to 2 mL extracts in 2 mL of H_2SO_4. The change in colour from violet to blue or green indicated the presence of steroids.

Terpenoids

The Salkowski test was undertaken to ascertain if terpenoids were present. Five millilitres of extract were mixed in 2 mL of chloroform and layered over 3 mL of concentrated H_2SO_4. A reddish-brown colour of the interface demonstrated the presence of terpenoids.

Alkaloids

Dragendorff's reagent was prepared by mixing 0.4 g of bismuth subnitrate in 10 mL HCl (12 N) with 5 g of potassium iodide in 50 mL distilled water. Then, 0.05 g of the extract were stirred with 1 mL of 1% aqueous HCl on a steam bath. A few drops of Dragendorff's reagent were used to treat 0.5 mL of the filtrate. Orange precipitation indicated the presence of alkaloids.

Phenolics

To 2 mL of sample was added to 2 mL of 0,1% ferric chloride solution and kept in the room temperature. Appearance of violet color indicated the presence of phenolic compounds in the sample.

Flavanoids

One mL of the extract solution was diluted by the mixture of 4 mL of deionized water and 0.3 mL of 5% $NaNO_2$. After 5 minutes, 0.3 mL of 10% $AlCl_3$

solution was added into above solution. Then, 2 mL of 1M NaOH solution was also added prior to be filled to 10 mL by deionized water. The orange colour was indicated the presence of flavanoids.

Saponins

About 0.5 g of sample was shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponin.

Cardiac glycosides

About 0.2 g of sample were dissolved in 1 mL of glacial acetic acid containing 1 drop of ferric chlorde solution. This was then under layered with 1mL of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardic glycosides.

Proteins

To 2 mL of extract, 1 mL of 40% NaOH solution and 1 to 2 drops of 1% CuSO4 solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Carbohydrate

Phenol –sulphuric acid method for the colorimetric determination of carbohydrates in extractions is proposed. To 1 mL of extract, 1 mL of 5% phenol and 5 mL of H_2SO_4 was added. A yellow color indicated the presence of carbohydrate.

Evaluation of the total antioxidant activity using the phosphor-molybdenum method:

The total antioxidant activity of studied samples was determined according to the method described in literature [10] with certain modifications. In brief, a 0.3 mL aliquot of the sample was mixed with 3 mL of a reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), and then the mixture was incubated at 95 ^oC for 90 min. The mixture was then cooled down to 25 ^oC and the absorbance was measured at the wave length of 695 nm against a blank that contained 3 mL of the reagent solution without the sample. The total antioxidant activity was expressed as number of equivalents of gallic acid (GA) [11] and ascorbic acid (AS) [12] (with concentrations of between 0.1 ÷ 0.5 mg/mL) and as the absorbance of the sample (the higher absorbance value indicates the higher antioxidant activity).

Evaluation of DPPH radical scavenging activity:

The DPPH free radical scavenging activity of each sample was determined using the Jasco V-630 Spectrophotometer according to the method described in literature [10]; [13] with certain modifications. The samples were dissolved in 1.5 mL methanol at various concentrations (25, 50, 75 and 100 µg/ mL) and mixed with 1.5 mL of 100 µM DPPH (100 µM DPPH dissolved in methanol before using). The reaction mixture was shaken for 1 minute and incubated at room temperature for 30 minutes. The absorbance was then measured at a wave length of 517 nm. Three milliliters of methanol was used as a blank sample. Radical scavenging activity was evaluated using the IC_{50} value [14].

RESULTS AND DISCUSSION:

Phytochemical analyses

Phytochemical screening of the ethanol extracts and water extracts of *O. sobolifera* showed the presence of steroids, triterpenoids, alkaloids, phenolics, flavoinods, protein, carbohydarte and saponins (Table 1). The presence of compounds having strongly antioxidant activities such as phenolics and flavonoids were showed the potential antioxidant activity of *O. sobolifera*.

Table 1. Phytochemical screening of ethanol extracts and water extracts of *O. sobolifera*

No.	Phytochemicals	Ethanol	Water	Results
1	Steroids	++	+	Yes
2	Triterpenoids	++	+	Yes
3	Alkaloids	+	-	Yes
4	Phenolics	++	++	Yes
5	Flavonoids	++	++	Yes
6	Saponins	-	++	Yes
7	Cardiac glycosides	-	-	No
8	Proteins	++	+	Yes
9	Carbohydrate	++	++	Yes

Total antioxidant capacity:

The total antioxidant capacity was determined by assessing the electron-donating capacity of the sample using the phospho-molybdenum method. In principle, this method based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of a green Mo(V) complex at a low pH with a maximal absorbance at 695 nm. A high absorbance value indicates that the sample possesses high antioxidant activity [10].

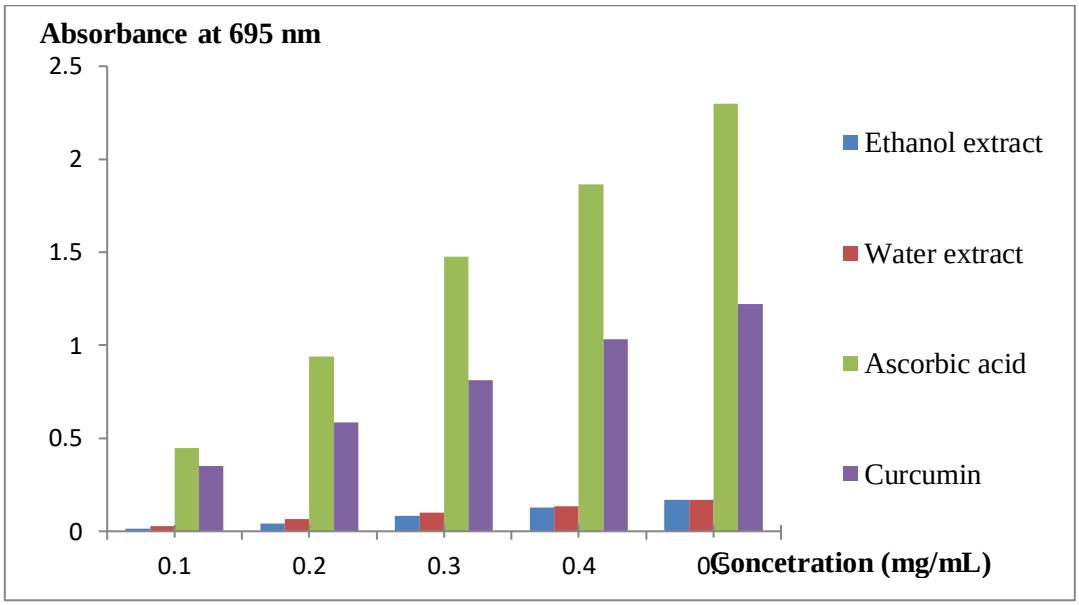

Fig 1. Antioxidant activity of ethanol extract and water extract from the *O. sobolifera*

Both ethanol extract and water extract from the *O. sobolifera* exhibited having antioxidant activity in the electron transfer model (Figure 1). However, their antioxidant activities were lower than those of gallic acid and ascobic acid. The antioxidant capacity was expressed as the number of equivalents of gallic acid or ascorbic acid (the standard curve equation of gallic acid : Abs = 0.7820 C_{GA} + 0.1648, R = 0.9966; and the standard curve equation of ascorbic acid : Abs = 4.5974 C_{AS} - 0.3231, R = 0.9952). The study revealed that the antioxidant capacity of the extracts enhanced with the increase of extract concentration and the highest capacity was observed at the concentration of 1.5 mg/mL where total antioxidant capacity of the *O. sobolifera* showed contained from 5.52 ± 0.14 to 12,71 ± 0.23 mg GA/g or from 3.55 ± 0.15 to 7.87 ± .05 µmol AS/g (Table 2).

Table 2. Total antioxidant capacity (TAC) of the ethanol extract and water extract from the *O. sobolifera*.

Extractions	TAC	
	mg GA/g	µmol AS/g
Water	12,71 ± 0.23	7.87 ± 0.05
Ethanol	5.52 ± 0.14	3.55 ± 0.15

DPPH radical scavenging activity

The values of DPPH radical scavenging activity is presented in Table 3.

Table 3. The DPPH radical scavenging activity rates of the ethanol extract and water extract from the *O. Sobolifera*

Concentration (mg/mL)	Water extraction	Ethanol extraction
2.0	72.73	72.43
1.5	67.12	67.26
1.0	52.02	58.15
0.8	43.92	55.49
0.6	33.38	44.27
0.4	19.31	14.29
IC_{50} (mg/mL)	0,95	0.70

It can be seen that the DPPH radical scavenging activities of the ethanol extract and water extract of *O. Sobolifera* enhanced along with the increasing of concentration (in Table 3). The DPPH radical scavenging activity at the concentration of 2.0 mg/mL of ethanol extract and water extract of *O. Sobolifera* was higher than that of Cordyceps sinensis [15].

From two models to evaluate the antioxidant acitivites was shown that the ethanol extracts and water extracts of *O. Sobolifera* have potential antioxidant properties.

CONCLUSIONS:

These data suggest that *O. sobolifera* is a natural source of antioxidants. The extracts of *O. sobolifera* contain significant amounts of phytochemicals with antioxidative properties and could serve as scavengers of free radicals. The antioxidant activity of extracts showed high antioxidant activity with the IC_{50} values to 0.70 from 0.95 mg/mL. Total antioxidant capacity of the *O. sobolifera* showed contained from 5.52 ± 0.14 to 12,71 ± 0.23 mg GA/g or from 3.55 ± 0.15 to 7.87 ± .05 µmol AS/g. *O. sobolifera* could be exploited as a potential source for mushroom-based pharmaceutical products. These results could form a sound basis for further investigation in the potential discovery of new natural bioactive compounds.

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