

ANTIOXIDANT AND SGC-7901 CELL INHIBITION ACTIVITIES OF *RHIZOMA DIOSCOREAE BULBIFERAE*. ETHANOL EXTRACTS

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Abstract

The objective of this research was to study the pharmacology of *Dioscorea bulbifera* L. on antioxidant and anticancer activity. Alcohol extracts of *Dioscorea bulbifera* L. were made out by different concentration alcohol; they were tested by Hydroxyl radical scavenging test, reducing capacity test and total antioxidant capacity test. In the anticancer test, MTT test was used to study the inhibition rate. The results told that 70% ethanol extracts could scavenge most DPPH· at 2mg/ml. The rate was 55.2%; 80% ethanol extract could clear the most ·OH. The clearance rate was 51.2%. 80% ethanol crude extracts possessed the strongest reducing ability per gram of the extract equal to 49.3μmol Fe²⁺. Different concentrations of the extracts could inhibit the proliferation of line SGC-7901, and with the concentration increased, the inhibition rate was gradually increased.

Key words: *Dioscorea bulbifera* L., SGC-7901, antioxidant, alcohol extracts

Introduction

Rhizoma Dioscoreae Bulbiferae are tubers of yam plants *Dioscorea bulbifera* L. Modern research has found that *bulbifera* mainly contains diterpene lactone, flavonoids, saponins, polysaccharides, diosbulbin B, diosbulbin C. and so on. Its petroleum ether extract of *bulbifera* has significant anti-tumour activity (Yu et al., 2004). When S180 rats were administered with gavages of *Bulbifera* and *Angelica*, the serum of S180 rats inhibited the proliferation of sarcoma cell and hepatoma cell well (Suo et al., 2008). Its active ingredient, 50 mg/(kg·d) and 200mg/(kg·d) *bulbifera*, could significantly inhibit acute inflammation induced by carrageenin and granuloma induced by cotton ball (Tan et al., 2003). This paper mainly studies the inhibition effects on SGC-7901 cell and the antioxidant activities of *Bulbifera* alcohol extracts.

Materials and Methods

Material

Bulbifera (purchased from Shanghai Tong Ren Tang drugstore), SGC-7901 cells (Biotech Co., Ltd. Shanghai Jin Ma), ethanol (Shanghai Haoshen Chemical Reagent Co., Ltd.), anhydrous ethanol, H₂O₂, ferrous sulphate, glacial acetic acid, potassium ferricyanide, Trichloroacetic acid, pyrogallol, HCl, DPPH (2,2-two phenyl-1-picrylhydrazyl), and Vc were used. All the materials were of analytical grade. Other materials used included UV-Vis spectrophotometer (UNICO), the sonicator (American Sonics), Rotary evaporator (Shanghai Yarong), and Human SGC-7901 cell line provided by the Department of

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Pathophysiology, Shanghai Medical University,. The cell culture medium RPMI1640 from Gibco; Calf serum was purchased from Hangzhou Evergreen Biological Engineering Materials Co., Ltd. Thiazolyl blue (MTT) and DMSO were purchased from Sigma.

Obtainment of alcohol extract (Gong Xiu Jing et al., 2002)

1.0 kg of *bulbifera* was weighted and put in 1000 ml round-bottomed flask. It was soaked in 70%, 80%, 90% ethanol solution for 13 h, refluxed for 2h, and repeated 2 times with new 70% ethanol. It was combined with filtrate vacuum recovery of ethanol, and then filtrate evaporated. The extract was weighted 0.91 kg, and the yield was 18.2%.

Preparation of 0.2 mmol·L⁻¹ ethanol solution of DPPH

weighed 4.4 mg DPPH powder was dissolved in 50 ml anhydrous ethanol contained in volumetric flask. 4 ml different concentrations of sample solution were added with 0.2 mmol/L - 1 DPPH 2 ml, and kept in dark for 30 min at room temperature. A blank and A sample were tested at 517nm wavelength. Sample clearance rate was calculated according to the following formula: $IR(\%) = (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} * 100\%$;

Activity of Radical Scavenging (Li et al., 2008; Dong et al., 2008)

Following the phenanthroline Fe²⁺ oxidation experiments, 0.2mol/L⁻¹ pH 7.4 PBS2.0ml were taken into a graduated tube. 2.5ml 5.0 mmol/L-1 phenanthroline solution and 0.5ml 7.5mmol/L-1 Fe (NH₄)₂(SO₄)₂·6H₂O solution were added. 1.0ml sample was then added (the final concentration of the work solution were 10, 20, 30, 40, 50 mg·L⁻¹). 1.0 ml 0.1%H₂O₂, distilled water was added to 10.0ml volume. The tube was placed in water bath at a constant temperature and stored for 60min at 37°. The absorbance was measured at a wavelength of 536nm and denoted by A dosing.

Using the above method, distilled water was adopted instead of anti-oxidation reagents and H₂O₂ respectively. The absorbance was measured, denoted as A damage and A is not damaged. Three parallel tests were conducted. Hydroxyl radical scavenging rate was calculated as follows: Hydroxyl radical scavenging ratio = (A dosing - A damage) / (A not damaged - A damage) × 100%.

Pipetting appropriate amount of extract, 1.0 ml 0.2 mol / L pH value of 6.6 sodium phosphate buffer and 1.0 ml 1% potassium ferricyanide were put in a 50° water bath for 20 min for rapid cooling. 1.0 ml 10% trichloroacetic acid and distilled water were adjusted to 10 ml, centrifuged at 3000 r / min for 10min. 2.5 ml supernatant was added into 0.5 ml 0.1% ferric chloride solution and mixed with distilled water to 5ml. 10mins later, the absorbance was tested at 700nm. Ferrous ammonium sulphate solution was used to finish calibration standard curve (100 to 300 mol/L). The measurement result was expressed as the mol number of equivalent of ferrous ions per gram sample.

The total antioxidant capacity assay kit was taken to determine total antioxidant capacity of the ethanol extract. The three ethanol extracts were dried and made to 0.2mg/ml. Trolox standard curve was measured in accordance with the process of the operations of the kit. To measure 80% ethanol extract concentrations of the ABTS radical scavenging ability at different times, the extracts were set in 0.2, 0.4, 1, 1.4, 1.8, 2 mg/ml concentration respectively and were detected at the 1, 4, 7, 12min to test radical scavenging rate. Absorbance of each substance was measured at 414nm; relative total antioxidant capacity was calculated as follows: Relative total antioxidant capacity = 1- Atest /Acontrol.

Anticancer test on SGC-7901 (Li et al., 2009; Gu et al., 2004; Yu et al., 2010)

The cell lines were purchased from the Shanghai Institute of Cell Bank with volume fraction 0.1 containing calf serum

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DMEM medium, 5 percent CO₂ and 95%O₂ at 37°C, and were maintained in fully saturated humidity conditions under conventional culture. The logarithmic growth phase cells were used and taken to digestion. Gastric cancer cells in logarithmic growth phase count, 1×10^4 / well, were seeded in 96-well plates, cultured for 48h with different concentrations of extract (0.1, 0.2, 0.4, 0.6, 0.8 mg / ml), in which DMSO concentration in each group was 2 %. The control group was administered with the appropriate volume containing DMSO 2% (v/v) culture medium. Each set five wells were followed at 24, 48 h detection time point, and MTT steps were observed with reference to the kit instructions. 4h before detection, 100 µL of the solution A was added to the reagent cartridge at 37°C, 5% CO₂, and cultured for 4h. Then, 50µL work solution was added and all purple crystals were dissolved. Absorbance was measured at 570nm, which is calculated as follows: Cancer cell inhibition rate (%) = $(1 - OD_{\text{dosing}} / OD_{\text{control}}) \times 100\%$.

Results

Spectrophotometric hydroxyl radicals and DPPH radical scavenging rates of different concentrations of *Bulbifera* ethanol extracts are shown in Table 1.

Table 1: Free radical scavenging rate of *Rhizoma Dioscoreae Bulbiferae* extracts

Extracts	Concentration (mg/ml)	Scavenging rate on ·OH and DPPH·	
		·OH (%)	DPPH· (%)
70% ethanol extracts	0.5	30.1±0.1	48.2±0.3
	1	32.3±0.2	53.9±0.5
	2	38.7±0.1	55.2±0.3
80% ethanol extracts	0.5	37.5±0.3	45.1±0.4
	1	42.5±0.2	47.3±0.2
	2	51.2±0.3	52.7±0.3
90% ethanol extracts	0.5	29.4±0.2	32.7±0.1
	1	35.1±0.2	36.3±0.3
	2	39.6±0.1	42.5±0.2

The results, among the three extracts, showed a concentration of 2mg/ml. 70% ethanol extract had the strongest DPPH· clearance ability, the rate was 55.2%; 80% extract clear ·OH highest clearance ability, the rate was 51.2%; scavenging rate of 70% and 90% ethanol extracts on ·OH free radicals were 38.7% and 39.6% respectively.

Table 2: Reducing ability of the ethanol extracts

Extracts	Concentration (mg/ml)	Fe ²⁺ (1ml Sample)
70% ethanol extracts	2	28.1
80% ethanol extracts	2	49.3
90% ethanol extracts	2	35.2

Reducing ability test

The tests on reducing ability of three different ethanol extracts showed that 80% ethanol crude extracts possessed the

strongest reducing ability per gram of the extract equal to $49.3\mu\text{mol Fe}^{2+}$. This can be seen in Table 2.

Total antioxidant capacity

After the tests of ABTS rapid detection kit, results showed that the scavenging capacity of 80% ethanol extract was the strongest one; the rate was nearly 72%. 70% and 90% ethanol extract showed the clearance rates of 55% and 45% respectively. This capacity could be seen in Figure 1.

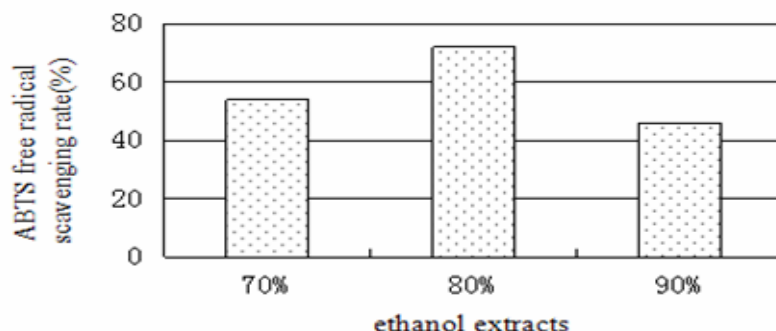


Figure 1: The total antioxidant capacity of the extracts

Total antioxidant capacity analysis

The total antioxidant capacity tended to increase gradually as the time went on and the concentration became bigger. At the lowest concentration of 0.2mg/ml, clearance rate was about 8%. At 2mg/ml, the rate was 52%. At 4min 7min, 12min, concentrations of 0.2, 0.4, 1.0mg / ml clearance rose steadily. When the concentrations were 1.4, 1.8, 2mg/ml, the changes of radical scavenging rate were not obvious, but they were generally between 55% and 67% of the fluctuations. The antioxidant activities could be seen in Figure 2.

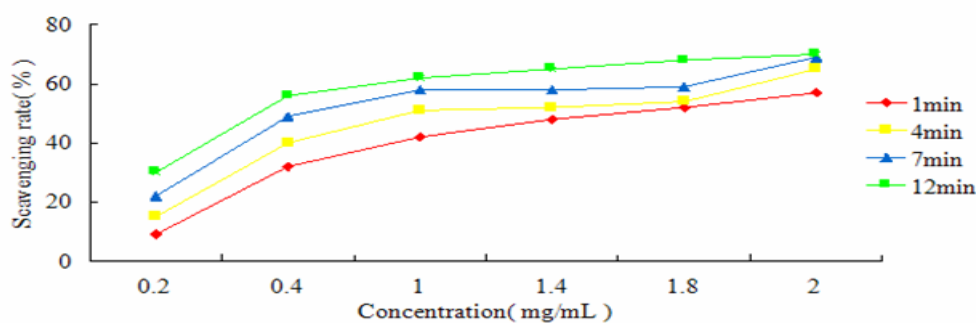


Figure 2: 80% extract total antioxidant capacity

Inhibition on SGC-7901 cells

Different concentrations of the extracts could inhibit the proliferation of line SGC-7901, and with the concentration increased, the inhibition rate was gradually increased. 0.5 mg/L inhibition rate of 80% ethanol extract was 41.3%. When the concentration was up to 2 mg/ml, the inhibition rate became 62.3%. The data can be seen in Table 3.

Table 3: Inhibition on SGC-7901 cell by *Rhizoma Dioscoreae Bulbiferae* ethanol extracts

Extracts	Concentration (mg/ml)	Anti-cancer rate (%)
70% ethanol extracts	0.5	34.5
	1	41.2
	2	45.6
80% ethanol extracts	0.5	41.3
	1	55.8
	2	62.3
90% ethanol extracts	0.5	28.7
	1	32.4
	2	34.9

Discussion

This paper mainly studied the antioxidant capacity of *bulbifera* extracts on inhibiting the proliferation of gastric cancer cell line SGC-7901, the scavenging ability on two free radical scavenging ability of DPPH and OH, and the reducing ability as well as the total antioxidant capacity of crude extracts.

The results showed that among the three extracts 70% ethanol extracts could scavenge most DPPH $\cdot$ at 2mg/ml, the rate was 55.2%; 80% ethanol extract could clear the most $\cdot$ OH, the clearance rate was 51.2%. The antioxidant activity of *bulbifera* alcohol extract exhibited stronger ability than water extracts on DPPH $\cdot$ and $\cdot$ OH scavenge; it had strong anti-lipid peroxidation and reduction ability. Its total antioxidant capacity was also strong (Liu et al., 2010).

This research proved that the ethanol extract of *bulbifera* has strong antioxidant capacity, and that generally the ability was enhanced as the concentration up-regulated, especially in ABTS detection. The total antioxidant capacity of *bulbifera* was well. The experiments also conducted ABTS scavenging comparison by testing different concentrations of the *Dioscorea bulbifera* ethanol extract at different times. We found a high inhibition on ABTS radical at a low concentration of 0.2mg/ml, and the increase of this ability had a positive correlation with the growth of action period and extracts concentration.

The formation and growth of tumours rely on nutrition supplied by micro-vessel, however, the formation of micro-vessel depends on methionine aminopeptidase II substances (Methionine aminopeptidase II, referred to as MetAP2). Huang had found a new compound which can effectively control the MetAP2 level from *bulbifera* extracts, namely 1-(3-propionyl-amino)-2- methyl-piperidine, and found *Angelica* compatible with *bulbifera* primarily through the synergy of the two components of drugs to reduce toxicity. So, *bulbifera*. could play anti-tumour activity effectively and safely (Huang, 2001). At the same time we found that *bulbifera* and different concentrations extract could inhibit the proliferation of human gastric cancer cell line SGC-7901, and with increasing concentration of the extract, the inhibition rate was gradually increased. 0.5 mg/L, 80% extract displayed 50% inhibition rate of SGC-7901. When the concentration was increased to 2 mg/L, inhibition rate was more desirable. Diosbulbin B was a novel compound which was isolated from *Dioscorea bulbifera* L., and found to be the major anti-tumour bioactive component of DB by HPLC with DAD and pharmacological tests. (Wang et al., 2012).

Bulbifera was supposed to get more compounds with anticancer activity and antioxidant activity. But it often induced liver toxicity. The hepatotoxicity would be more obvious by different periods of medicine-taken time, like aberrant

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expression of many genes in rat liver cells (Zhai et al., 2010; Chen et al., 2006). Therefore, test of a new active monomer extracted from *bulbifera*, hepatic toxicity test and the long-term drug toxicity should be carried out on rats first to ensure the safety of the new compound. As for *Dioscorea bulbifera* L., chronic toxicity testing and acute toxicity test could be used to ascertain the nature of certain side effects in traditional application process. In the next step, we would use the HPLC and UPLC skills to separate and purify the newly active compound. The ethyl acetate soluble fraction of 75 % ethanol extract of *Dioscorea bulbifera* L. showed an inhibitory effect against the tumour promotion of JB6 (Cl22 and Cl41) cells induced by a promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) (Song et al., 2010). Before we put the extracts into the clinical application, we will first finish the pharmacological screening of the new compounds to see how side effects can be appreciably minimised.

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