

# Measurement of Antioxidant Property of Coral Powder against Hydroxyl Radical Generation Using by Electron Spin Resonance (ESR) Spectroscopy

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## <Methods>

### 1. Sample preparation (distributed by First Co. Ltd.)

For drinking in the usual 1 ~ 1.5g / 200mL, the coral powder was suspended stock solution at 75mg / mL (distilled water). In addition, hydrogen was eluted from coral powder on 0, 1, 24 hours after preparation, respectively.

### 2. Hydroxyl radical generating system

Hydroxyl radical was produced by irradiation with ultraviolet rays (UVA) to hydrogen peroxide.

### 3. ESR measurement conditions

The prepared samples were collected in ESR flat cell, we performed measurements of sample using by ESR under the conditions of measurement as follows;

Center Field: 335.8 mT	Modulation Width: 0.079 mT
Sweep Width: 7.5 mT	Time constant: 0.03 sec
Seep Time: 1.0 min	Gain: 320

### 4. Hydroxyl radical measurement conditions

The measurement of scavenging activity of hydroxyl radical of the sample was performed with spin-trap as

5-(2,2-dimethyl-1,3-propoxycyclophosphoryl)-5-methyl-1-pyrroline N-oxide (CYPMPO using the following protocol

PBS (pH7.2): 140  $\mu$ L      Hydrogen peroxide (100 mM): 20  $\mu$ L

CYPMPO (0.5mM): 20  $\mu$ L      Sample: 20  $\mu$ L

UVA irradiation (100 mW, 10 sec)

After UVA irradiation, the prepared samples were collected in ESR flat cell. We carried out ESR measurement of the prepared samples, immediately.

### 5. Evaluation

Each values of added sample as % of control was evaluated obtained in the resulting 100 % control from a specific signal intensity of ESR spin adduct

### 6. Statistical Analysis

Statistical analyses were performed using the individual data. All experiments were repeated a minimum of three times.

Statistical analyses were considered significant difference of 5% less using the Tukey's analysis of variance.

( $p < 0.05$ , Tukey's multiple testing)

## <Results>

It was observed that the hydroxyl radical was significantly scavenged in sample of 1 hour and 24 hours after adjustment, respectively.

	Control	1h	24h
% of Control	103.07	85.74	90.53
	100.62	86.94	90.83
	96.31	88.11	87.52
average	100.00	86.93	89.63
SD	3.42	1.19	1.83

	Control	1h	24h
Scavenging activity (%)	-3.07	14.26	9.47
	-0.62	13.06	9.17
	3.69	11.89	12.48
average	0.00	13.07	10.37
SD	3.42	1.19	1.83

