

ELECTRON SPIN RESONANCE ESTIMATION OF HYDROXYL RADICAL SCAVENGING CAPACITY OF A MEDICINAL MOSS TEA

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As part of our ongoing investigation on the moss *Rhodobryum ontariense* (Kindb.) Kindb. (Bryaceae), a traditional Chinese herbal medicine for a wide range of cardiovascular diseases including hypertension, hydroxyl radical scavenging capacity screening of its lyophilised aqueous extract (tea) was conducted by electron spin resonance (ESR) spectroscopy. The study led to the detection of a significant activity ($94 \pm 1\%$) indicating antioxidant potential of the examined moss species. Moreover, the tea showed no cytotoxicity in the brine shrimp (*Artemia salina*) test. Since the extract was classified as rich in saccharide constituents, this group of organic compounds was suspected to be main scavengers of the hydroxyl radical.

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1. Introduction

Cardiovascular diseases account for 12 million deaths annually worldwide and are known to be number one group of 'killer diseases' [1]. The importance of hypertension lies in the fact that it forms one of the main risk factors for coronary heart disease, stroke, atherosclerosis and peripheral vascular disease [2]. On the other hand, traditional Chinese medicine suggests that some mosses of the genus *Rhodobryum* (Bryaceae) can cure cardiovascular diseases as crude drugs in form of medicinal tea [3]. Pejin et al. [4-11] have recently investigated the chemical composition and biological activity of *Rhodobryum ontariense* (Kindb.) Kindb. for the first time. It has been found that systolic, diastolic and mean arterial pressure as well as cardiac output are significantly lowered in the group of spontaneously hypertensive rats (n=7) treated intravenously with its lyophilised aqueous extract (100 mg/kg b.w. dissolved in 0.2 ml of saline) [12]. The aim of this study has been to estimate the antioxidant activity of *R. ontariense* tea *in vitro* by determining its hydroxyl radical ($\cdot\text{OH}$) scavenging capacity using electron spin resonance (ESR) spectroscopy since oxidative stress caused by this short-lived radical is directly linked to hypertension and cardiovascular disorders [13].

2. Experimental

2.1. Plant material

The sample of *R. ontariense* originated from the Fraser's Hill (Malaysia, November 2010). Voucher specimen has been deposited in the Herbarium of the Institute of Biological Sciences, Faculty of Science, University of Malaya (KT Yong 7635).

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2.2. Extraction

Before extraction the moss was carefully inspected for contaminants: soil and plant material were completely removed. The gametophyte tips were used for the extraction. Air-dried parts of *R. ontariense* (5 g) were ground and extracted with hot water for 30 min. The extract (tea) was filtered and concentrated by lyophilisation to give the residue (the yield, 10%) which was stored at + 4 °C for further use.

2.3. Biological Assays

2.3.1. Determination of hydroxyl radical scavenging capacity

For the study of the hydroxyl radical ($\cdot\text{OH}$) scavenging capacity of the moss extracts (final concentration 15 mg/mL), the Fenton reaction was initiated using H_2O_2 and FeSO_4 at final concentrations of 1 mM and 0.2 mM, respectively. The sample was prepared by adding FeSO_4 and H_2O_2 to solutions containing the extract and DEPMPO. Chemicals for ESR measurements were obtained from commercial providers: FeSO_4 (Merck, Darmstadt, Germany); H_2O_2 (Renal, Budapest, Hungary); DEPMPO (5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide) spin-trap Enzo Life Sciences International (Plymouth Meeting, PA, USA). ESR spectra were recorded after 2 min incubation. In all analyses, ultra-pure MilliQ (18 M) water was used. Samples were drawn into 10 cm long gas-permeable Teflon tubes in order to maintain constant O_2 level in the sample (wall thickness 0.025 mm and internal diameter 0.6 mm; Zeus industries, Raritan, NJ, USA), and then placed in quartz capillaries. ESR spectra were recorded using a Varian E104-A ESR spectrometer (Palo Alto, CA, USA) operating at X-band (9.572 GHz) with the following settings: modulation amplitude, 2 G; modulation frequency, 100 KHz; microwave power, 10 mW; time constant, 32 ms; field centre, 3410 G; scan range, 200 G; scanning time, 4 min. The cavity temperature was maintained at 25°C. Recordings were performed using EW software (Scientific Software, Bloomington, IL, USA). Spectral simulation of each spectrum was performed using WINEPR SimFonia computer program (Bruker Analytische Messtechnik GmbH, Darmstadt, Germany) in order to determine the signal intensity. The experiment was performed three times on three separate days. The result is presented as mean \pm S.D. (standard deviation).

2.3.2. Determination of cytotoxicity

Brine shrimp (*Artemia salina*) lethality was used as an indicator of cytotoxicity [14]. The activity was evaluated in triplicate. The extract was dissolved in water to reach final concentrations of 100, 10, and 1 ppm, in 5 mL of artificial seawater using 10 freshly hatched larvae of *A. salina*. Briefly, for each dose tested, surviving shrimps were counted after 24 h, and the data statistically analysed by the Finney program, which affords LD_{50} values with 95% confidence intervals [15].

3. Results and discussion

This study showed that *R. ontariense* extract (tea) had a high hydroxyl radical scavenging capacity ($94 \pm 1\%$) and no cytotoxicity (Figure 1). Moreover, the extract was classified as rich in different types of saccharides by means of its spectroscopic data and typical chromatographic profile (Figure 2). Though the literature pertaining to the antioxidant activity of various low-molecular weight secondary metabolites is voluminous, they are not many reports on antioxidant activity of aforementioned group of organic compounds [16]. Indeed, it has been recently shown that $\cdot\text{OH}$ radicals, the most damaging ones within the body, can be scavenged with phosphorylated fructose [17]. Therefore, *R. ontariense* saccharide constituents can be suspected as its main scavengers of this free radical which has detrimental biological activity due to very high reactivity, in particular concerning cardiovascular diseases.

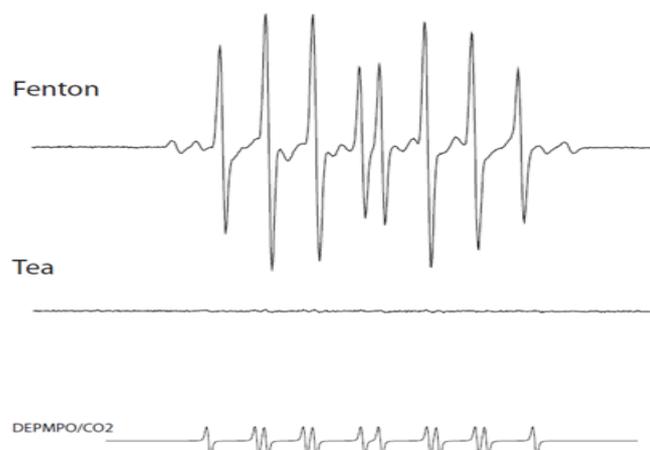


Fig. 1. ESR spectrum of *Rhodobryum ontariense* tea

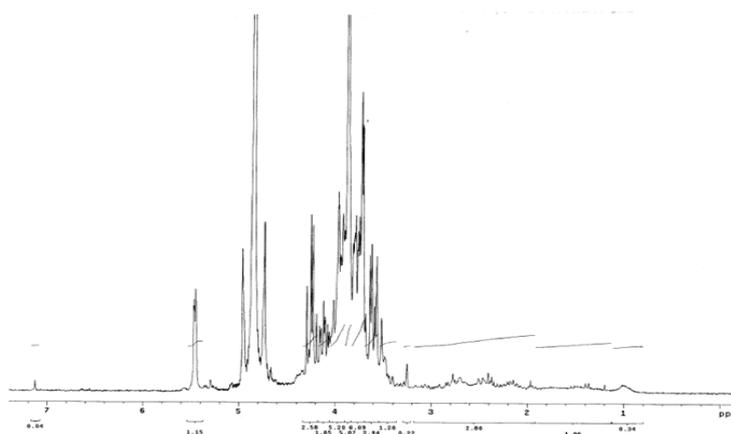


Fig. 2. ^1H NMR spectrum of *Rhodobryum ontariense* tea

4. Conclusion

Antioxidant agents can treat oxidative pathologies by neutralising ROS, chelating catalytic metals and acting as oxygen scavengers [18]. Because of the high carcinogenicity of synthetic antioxidants [19], the development of effective antioxidants of natural origin is of great interest [10,20]. Therefore, this medicinal moss tea should be the subject of further work focused on determination of its chemical composition by LC-MS analysis.

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