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Electron spin resonance, ABTS⁺ on-line HPLC and *in vitro* antioxidant activities of Sri Lankan brown alga: *Padina commersonii*

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Marine seaweeds are rich sources of biologically active secondary metabolites and have potential as lead compounds in drug discovery. However, antioxidant activities of the Sri Lankan seaweeds have not been studied extensively. Therefore, in this study, the main objective was to evaluate the antioxidant activities of the marine brown alga, *Padina commersonii* (Pc) by electron spin resonance (ESR) spectroscopy, ABTS⁺ on-line High Performance Liquid Chromatography (HPLC) and *in vitro* assay. The crude methanolic extract was partitioned into hexane, CHCl₃, EtOAc and H₂O and used for bioassays. Free radical scavenging activity was determined by ESR spectroscopy and ABTS⁺ On-line (HPLC) technique. *In vitro* antioxidant activity was determined on VERO cells by 2,7-dichlorofluorescein diacetate (DCFDA) assay and cell viability (MTT) assay. Ethyl acetate fraction (PCE) showed the highest total phenolic content (7.44 mg GAE g⁻¹) compared to the other fractions. The strongest radical scavenging activities were observed by PCE and water (PCW) fractions against alkyl radicals; IC₅₀ values were 0.017 ± 0.001 and 0.02 ± 0.003 mg mL⁻¹, respectively. The highest DPPH radical scavenging activity was identified from the PCE fraction (IC₅₀ value of 0.71 ± 0.03 mg mL⁻¹). Four different fractions were obtained from the reverse phase (RP) - ODS open column of PCE. Among them, PCEF2 fraction showed the best scavenging activity for DPPH (0.014 ± 0.001 mg mL⁻¹), alkyl (0.01 ± 0.001 mg mL⁻¹) and hydroxyl (0.100 ± 0.003 mg mL⁻¹) radicals. In addition, ABTS⁺ On-line HPLC profile showed eight very strong negative signals in the ABTS⁺ chromatogram due to trace antioxidant compounds available in PCEF2. Subsequently, three compounds were isolated from the PCEF2 fraction by High Performance Centrifugal Partition Chromatography (HPCPC). Among the isolated compounds, PCEF2-1 showed the strongest intracellular ROS scavenging activity on Vero cells under H₂O₂ oxidative stress. The results indicate that *P. commersonii* contains natural products with promising antioxidant effects.

Keywords: Antioxidant, brown algae, electron spin resonance, *Padina commersonii*