



CHEMICAL CONSTITUENTS AND ANTIOXIDANT POTENTIALS OF SEVEN PHILIPPINE MOSSES

(Juzuk Kimia dan Potensi Antioksidan bagi Tujuh Lumut Filipina)

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Abstract

Seven species of Philippine mosses, namely *Calyptothecium ramosii* Broth., *Gymnostomum recurvirostum* Hedw., *Hypnum plumiforme* Wilson, *Leucobryum scalare* Müll.Hal. ex M.Fleisch., *Meteoriopsis reclinata* (Müll.Hal.) M.Fleisch., *Mitthyridium undulatum* (Dozy & Molk.) H.Rob. and *Pelekium boniamum* (Besch) were examined in this study. The mosses were analyzed using a gas chromatography equipped with a mass spectrometer (GC-EI-MS) and an energy dispersive X-ray fluorescence spectrometer (EDX) to substantiate their possible usage as dietary supplements. EDX results displayed that calcium was the major mineral found in all the moss samples (1.199% to 11.427%). The free radical scavenging activity of the dried dichloromethane moss extracts was highest for *G. recurvirostum* indicative of the lowest IC₅₀ concentration at 0.236 mg/mL followed by *C. ramosii* at 0.306 mg/mL and *P. boniamum* at 0.315 mg/mL. *M. undulatum* (IC₅₀ = 1.360 mg/mL) and *M. reclinata* (IC₅₀ = 1.544 mg/mL) had moderate antioxidant activity, whereas, *L. scalare* (IC₅₀ = 2.120 mg/mL) and *H. plumiforme* (IC₅₀ = 2.213 mg/mL) had minimal free radical scavenging potential. The possible antioxidant capabilities of each respective bryophyte were correlated to the presence of the following major constituents from GC-EI-MS investigation: in *P. boniamum*, α -cadinol, (9.11%), caryophyllene (6.39%) and (-)-spathulenol (5.52%); in *G. recurvirostum*, pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester (56.26%), caryophyllenyl alcohol (5.96%) and α -cadinene (5.88%); in *C. ramosii*, phytol (17.86%), and phytol acetate (14.03%). 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione (9.59%), and 4,8,12,16-tetramethylheptadecan-4-olide (6.11%). Apart from *H. plumiforme*, all the samples contained considerable concentrations of n-hexadecanoic acid (range from 3.76% to 29.03%).

Keywords: bryophyte, free radical scavenging activity, gas chromatography - mass spectrometry, energy dispersive x-ray fluorescence spectroscopy

Abstract

Tujuh spesies lumut Filipina iaitu *Calyptothecium ramosii* Broth., *Gymnostomum recurvirostum* Hedw., *Hypnum plumiforme* Wilson, *Leucobryum scalare* Müll.Hal. ex M.Fleisch., *Meteoriopsis reclinata* (Müll.Hal.) M.Fleisch., *Mitthyridium undulatum* (Dozy & Molk.) H.Rob. dan *Pelekium boniamum* (Besch) telah diuji dalam kajian ini. Lumut telah dianalisa menggunakan kromatografi gas dilengkapi spectrometer jisim (GC-EI-MS) dan spektrometer pendaflour tenaga serakan sinar-X (EDX) untuk melihat potensi digunakan sebagai makanan tambahan. Keputusan EDX menunjukkan kalsium merupakan mineral utama yang dijumpai dalam semua sampel lumut (1.199% to 11.427%). Aktiviti pemerangkapan radikal bebas melalui ekstrak lumut menggunakan diklorometana telah memberikan *G. recurvirostum* hasil paling tinggi pada kepekatan IC₅₀ iaitu 0.236 mg/mL diikuti *C. ramosii* pada 0.306 mg/mL and *P. boniamum* pada 0.315 mg/mL. *M. undulatum* (IC₅₀ = 1.360 mg/mL) dan *M.*

reclinata (IC_{50} = 1.544 mg/mL) menghasilkan aktiviti antioksidan yang sederhana, manakala *L. scalare* (IC_{50} = 2.120 mg/mL) dan *H. plumiforme* (IC_{50} = 2.213 mg/mL) mempunyai potensi pemerangkapan radikal bebas yang minimum. Keupayaan antioksidan setiap briofit adalah dikaitkan dengan kehadiran juzuk utama iaitu hasil analisa GC-EI-MS seperti di dalam *P. boniamum*, mengandungi α -kadinol, (9.11%), kariofilena (6.39%) dan (-)-spathulenol (5.52%); di dalam *G. recurvirostum* seperti asid pentanoik, 2,2,4-trimetil-3-karboisopropil, isobutil ester (56.26%), kariofilenil alkohol (5.96%) dan α -kadinena (5.88%), di dalam *C. ramosii*, fitol (17.86%), fitol asetat (14.03%), 7,9-di-tert-butyl-1-oksaspiro (4,5) deka-6,9-diena-2,8-dion (9.59%), dan 4,8,12,16-tetrametillheptadekan-4-olida (6.11%). Selain dari *H. plumiforme*, semua sampel mengandungi kepekatan asid n-heksadekanoik (julat dari 3.76% hingga 29.03%).

Keywords: briofit, aktiviti pemerangkapan radikal bebas, kromatografi gas-spektrometri jisim, spektroskopi pendafklour tenaga serakan sinar-X

Introduction

There has not been much research done on non-vascular plants, particularly on mosses which belong to the division Bryophyta [1], primarily because moss samples are usually small and are considered insignificant [2]. Traditionally, Native Americans are known to have used mosses as concoctions for treating tuberculosis, pneumonia, neurasthenia, wounds and burns [3, 4]. Large mosses such as *Polytrichum commune* Hedw. have been confirmed to have antimicrobial activity on several bacteria strains (*Bacillus cereus* Frankland & Frankland 1887, *Pseudomonas aeruginosa* (Schröter 1872) Migula 1900, *Staphylococcus aureus* Rosenbach 1884, and *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919) by exhibiting inhibition zone diameters which varied from 9 to 15 mm [5]. Other mosses such as *Sphagnum magellanicum* Brid., *Dicranum polysetum* Sw., *Pleurozium schreberi* (Brid.) Mitt.) displayed high antiproliferative activity (0.9–5 μ g/mL) on rat glioma cells [5]. Some work has been specifically done on the use of mosses as biomonitors to short-term responses and antioxidant fluctuations due to heavy metal damage [6]. For instance, allelochemicals (phytoalexins in rice) momilactone A and B have been isolated from *Hypnum plumiforme* Wilson [7]. Short-term responses of H_2O_2 concentration have been found to increase in the presence of Pb and Ni induced stress in *H. plumiforme* [8]. LC-ToF-MS analysis of ethanolic moss extracts, on the other hand, revealed that the most abundant constituents are fatty acids (329–1707 mg/100 g dry moss), sterols (632–2130 mg/100 g dry moss), and amino acids (590–3266 mg/100 g dry moss) [5].

In this study, seven Philippine mosses specifically *Calyptothecium ramosii* Broth. (**CR**), *Gymnostomum recurvirostum* Hedw. (**GR**), *Hypnum plumiforme* Wilson (**HP**), *Leucobryum scalare* Müll.Hal. ex M.Fleisch. (**LS**), *Meteoriopsis reclinata* (Müll.Hal.) M.Fleisch. (**MR**), *Mitthyridium undulatum* (Dozy & Molck.) H.Rob. (**MA**), and *Pelekium boniamum* (Besch.) (**PB**) were gathered from several localities in Central Luzon Region, Philippines and were selected for investigation. Furthermore, all these mosses are essentially extra-Philippine species occurring throughout Southeast Asia and none are endemics.

Chemical characterization of the seven moss species was achieved using gas chromatograph in tandem with a mass spectrometer (GC-EI-MS) and energy dispersive X-ray spectroscopy (EDX) technique. Free radical scavenging capacity was ascertained in the dichloromethane extracts from these mosses. To the best of our knowledge, this is the first reported study using this methodology of antioxidant activity on the aforementioned crude bryophyte extracts and chemical analyses of the samples.

Materials and Methods

The mat or aerial portion taken from each moss samples was pulverized into a powdered biomass. The powder was used for the stipulated analytical techniques to characterize the aforementioned mosses gathered from Central Luzon Region, Philippines.

Solvent extraction

Each moss sample was grounded followed by soaking 3.0 grams of the powdered biomass in 20 mL of dichloromethane (DCM) for three hours. Extracts were filtered and then dried under nitrogen for one hour. The crude extracts from **PB** (0.4 mg), **HP** (0.5 mg), **GR** (0.3 mg), **LS** (0.5 mg), **MR** (0.4 mg), **CR** (0.4 mg) and **MA** (0.3 mg) were dissolved in one millilitre of dichloromethane for subsequent GC-EI-MS analyses.