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Research Article

Total Phenol Content and Antioxidant Activity of *Bryum billardieri* Schwaegr.

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ABSTRACT

Mosses contain active constituents that exhibit various biological activities. This study provides additional information on the potentials of mosses as natural sources of biologically active compounds that can be used as medicines. The antioxidant potential of *Bryum billardieri* Schwaegr. (Bryaceae) is herein studied by analyzing its total phenol content and its radical scavenging activity. Ethanolic extract of *B. billardieri* was prepared for Folin–Ciocalteau assay and 2,2'-Diphenyl-1-picrylhydrazyl radical scavenging assay. The total phenol content of *B. billardieri* ethanolic extract was 38.18 gallic acid equivalence, while the mean percentage antioxidant activity was 93.024% ± 0.023, while its EC_{50} was 0.0621%., signifying its potential as an antioxidant.

Keywords: Antioxidant, *Bryum billardieri*

INTRODUCTION

The presence of biologically active compounds in plants has been long studied. Most studies, however, focused on higher vascular plants, specifically the angiosperms. Compared to angiosperms, pharmaceutical studies on bryophytes are relatively fewer. Bryophytes are non-vascular plants which include the mosses, liverworts, and hornworts. People pay less attention to these plants due to their minute sizes. Similar to other plants, bryophytes possess antimicrobial[1-3] and antioxidant[4] properties. Some research indicated the potential of mosses as natural sources of antioxidants[5,6]. Worldwide, there is an increasing interest in the use of plants as sources of alternative medicines and as natural sources of biologically active compounds for pharmaceutical use. Evaluating the biological activities of the mosses is a significant contribution to the search for possible sources of medicines. This study aimed to establish the potential of *Bryum billardieri* Schwaegr. (Bryaceae), a moss commonly found in the Cordillera Administrative Region, Philippines, as source of antioxidants. Given that phenolics have the highest potential in neutralizing free radicals[7], the total phenol content of the plant was also measured in this study.

MATERIALS AND METHODS

Plant Material

Samples of *B. billardieri* were collected from Mt. Data, Mountain Province, Philippines. Taxonomic identification was done using taxonomic literatures, and was confirmed by a taxonomist. The samples were rinsed with distilled water, dried on filter paper, and then these were air-dried prior to grinding. Ethanolic extraction was performed following the method of Negi & Dave[8]. Ten grams of powdered plant material was thoroughly mixed with 100 ml of 95% ethanol. The mixture was kept at room temperature for 48 hours with occasional shaking or stirring. The solution was then filtered using muslin cloth and Whatman filter paper no. 1. The filtrate was then concentrated using a rotary evaporator to yield the extract.

Determination of Total Phenol Content using Folin–Ciocalteau Assay

Series of gallic acid concentrations were prepared as standard. To 0.5 ml of each gallic acid concentration and to 0.5 ml of the extract, 2.5 ml 10% of Folin–Ciocalteau reagent (v/v) and 2 ml 7.5% sodium carbonate were added. The solutions were incubated for 40 minutes at 45 °C. After incubation, the absorbance of each sample was measured at 765 nm in the spectrophotometer with Na_2CO_3 solution (2 ml of 7.5% Na2CO3 in 2.55 ml of distilled water) as blank.

Determination of Antioxidant Activity

Antioxidant activity of the moss extract was evaluated using 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay following the method of Khaing[9]. Different subsequent concentrations of plant extracts were prepared in methanol. One ml of each diluted solution was mixed thoroughly with two ml 0.16 mM DPPH (prepared in methanol) and was kept in the dark for 15 minutes. The purple-blue solution turned yellow in the presence of antioxidants. A negative control was prepared by mixing one ml methanol and two ml of the prepared DPPH. To eliminate additional absorbance due to plant extract, blank solutions were prepared per concentration. Optical density of each solution was measured at 517 nm using UV-VIS Spectrophotometer.

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The percentage scavenging activity of the plant extract was calculated using the following equation:

Antioxidant activity (%) = [(AC−AE)/AC] × 100

where AC is the absorbance of the blank solution, and AE is the absorbance of the plant extract. Similar equation was used to calculate the radical scavenging activity of the positive control, which is ascorbic acid. The procedures were performed in triplicate.

Determination of Efficient Concentration (EC_{50})

EC_{50} value is the concentration or the amount of antioxidants needed to reduce the initial concentration of radicals by 50%, which implies that the lower the EC_{50} the higher the antioxidant activity[9]. The plot of percentage antioxidant activity versus sample concentration was used to determine the EC_{50} value for each of the extract and control. The result was analyzed using Analysis of Variance.

RESULTS AND DISCUSSION

The Folin–Ciocalteau assay revealed the total phenol content of *B. billardieri* to be 38.184 gallic acid equivalence. The phenolic compounds are produced by plants in response to stress caused by biotic and abiotic factors, such as UV damage, drought, freezing, and microbial and insect attacks[10, 11]. Phenolics are also known to play significant roles in lignin and pigment biosynthesis[11]. These phenolics are known to be effective in a variety of biological activities, such as antifungal, antimicrobial, anti-inflammatory, and antioxidant[12, 13]. It is possible that the presence of such compounds influenced the free radical scavenging activity of the plant extract. The antioxidant activity of *B. billardieri* was evaluated using the DPPH radical scavenging assay, and ascorbic acid was used as the positive control. The percentage scavenging activity of *B. billardieri* and ascorbic acid were measured to be 93.024% ± 0.023 and 97.264% ± 0.035, respectively. The half maximal effective concentration of the extract was 0.0621% (v/v), significantly higher than the 0.0007% (w/v) EC_{50} of the control. Nonetheless, given that the extract was crude, this EC_{50} value indicates the potential of *B. billardieri* to be a source of antioxidants.

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