Title : Phytochemical profiles, antioxidant, and anti–cancer activities of In Vitro cultured Physcomitrium sphaericum (C.F.Ludw.) Brid. extract.

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ABSTRACT

The moss *Physcomitrium sphaericum* (C.F.Ludw.) Brid. is a rare bryophyte that remains largely unstudied regarding its chemical composition and biological properties. Investigating such novel natural sources is crucial for discovering new bioactive compounds. This study aimed to identify the phytochemical composition, evaluate the antioxidant activity, and determine the anticancer activity of *in vitro* cultured *P. sphaericum* against A549 lung cancer cells, as well as its cytotoxic effects on normal HEK293 cells. The moss was cultured in half-strength modified hydroponic (MH) media for at least three months. Harvested samples were dried at 35°C and extracted using 80% ethanol (1 g/100 mL) via ultrasonication, followed by rotary evaporation at 35–40°C. This process yielded a 3.3% w/w crude extract. Phytochemical screening via LC-MS and subsequent quantification by HPLC—using a mobile phase of water and acetonitrile, both containing 0.1% formic acid—identified nicotinic acid at the highest concentration of 2.8257 mg/g crude extract, while asarone was detected at trace amounts. The extract exhibited notable antioxidant activity, showing a DPPH IC50 of 0.971 mg/mL and a total phenolic content of 36.83 ± 1.69 mg GAE/g extract. In anticancer assays, he extract demonstrated concentration- and time-dependent cytotoxic effects against A549 cells at concentrations ranging from 6.25 to 100 µg/mL. The IC50 values for the extract were 11.81 µg/mL (24 h), 9.31 µg/mL (48 h), and 9.46 µg/mL (72 h). Comparatively, pure asarone showed potent delayed activity (IC50 ≈ 6.76 µM at 72 h), whereas nicotinic acid exhibited a rapid response (IC50 ≈ 8.26 µM at 24 h) but did not reach the IC50 threshold at later time points. These findings provide the first comprehensive chemical profile of *P. sphaericum* and highlight its time-dependent anticancer potential, presenting a promising candidate for further pharmaceutical development.

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