Phytochemical Profiling, Antioxidant Activity, and Anticancer Activities of In Vitro Cultured *Physcomitrium sphaericum* (C.F. Ludw.) Extract.

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Abstract

We investigated the phytochemical composition and bioactivity of ethanolic extracts from in vitro-cultured Physcomitrium sphaericum, a rare bryophyte with limited prior chemical and biological study. Moss tissue cultured in half-strength modified hydroponic medium was harvested after at least 3 months, dried, powdered, and extracted with 80% ethanol to obtain 1.1 g crude extract from 33 g dry weight (3.3% w/w). LC-MS screening highlighted nicotinic acid and asarone as candidate constituents, and HPLC-UV at 260 nm confirmed nicotinic acid at 2.8257 mg/g crude extract, while asarone was detected at a very low level with retention-time drift that warrants confirmation. The crude extract showed measurable antioxidant activity with DPPH IC50 = 0.971 mg/mL and total phenolic content of 36.83 ± 1.69 mg GAE/g extract. In MTT assays, the extract consistently decreased A549 lung cancer cell viability across 24-72 h while HEK293 viability remained comparatively high. Together with the pure-compound comparison, these data suggest preliminary selective anticancer potential and justify further fractionation and mechanism-based studies.

Study objectives

1) Profile major compounds by LC-MS and HPLC. 2) Measure antioxidant activity by DPPH and TPC. 3) Compare cytotoxicity in A549 and HEK293 across 24-72 h.

Yield	Major HPLC hit	DPPH	TPC	MTT pattern
1.1 g crude from 33 g DW (3.3% w/w)	Nicotinic acid 2.8257 mg/g crude extract	IC50 0.971 mg/mL	36.83 ± 1.69 mg GAE/g extract	A549 low, HEK293 high

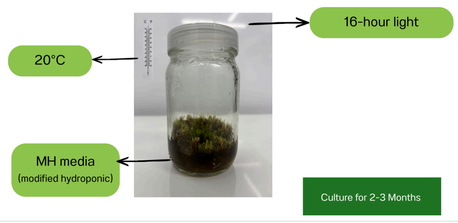
Introduction



Bryophytes remain underexplored as natural-product sources despite their ecological diversity and stress-adapted metabolism. Physcomitrium sphaericum is a vulnerable moss with little published evidence on phytochemical composition or biological activity. This work therefore asked whether an in vitro-cultured ethanolic extract contains quantifiable small molecules and whether the extract shows antioxidant and anticancer activity in a lung cancer model while sparing normal HEK293 cells.

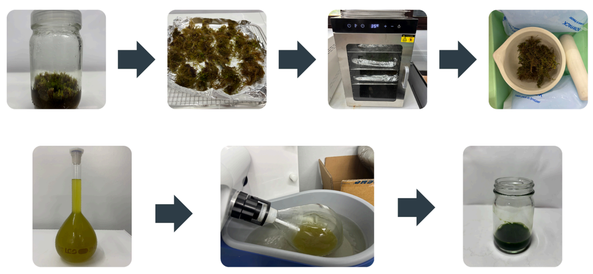
Methods

1. Tissue culture



Mosses were cultured on half-strength modified hydroponic medium at 20°C under a 16 h light photoperiod for at least 3 months before harvest.

2. Extraction workflow



Fresh biomass (335 g) was dried at 35°C to constant weight (33 g), ground cold, and extracted with 80% ethanol at 1 g/100 mL using soaking, homogenization, temperature-controlled ultrasonication, filtration, and rotary evaporation at 35-40°C.

3. Chemical and antioxidant assays

LC-MS was used for preliminary constituent screening. HPLC-UV used a C18 column at 35°C with water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B), 20 µL injection, and external standard calibration. DPPH was measured at 517 nm using a 25.0-0.391 mg/mL two-fold dilution. TPC used the Folin-Ciocalteu assay with gallic acid standards (1.000-0.0157 mg/mL) and detection at 765 nm.

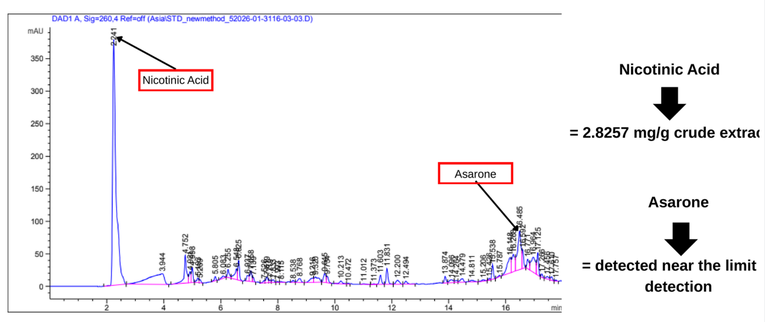
4. Cell viability assay

A549 and HEK293 cells were treated for 24, 48, and 72 h with *P. sphaericum* crude extract (6.25-100 µg/mL) or pure compounds (6.25-100 µM). Viability was quantified by MTT and IC50 values were estimated from dose-response patterns. HEK293 was included as the normal-cell comparator to assess preliminary selectivity.

Results & Discussion

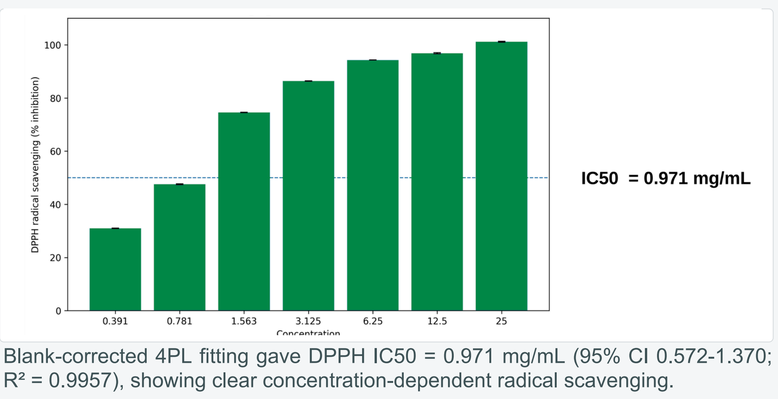
Chemical profiling and antioxidant activity

Figure 1. HPLC-UV chromatogram of *P. sphaericum* crude extract



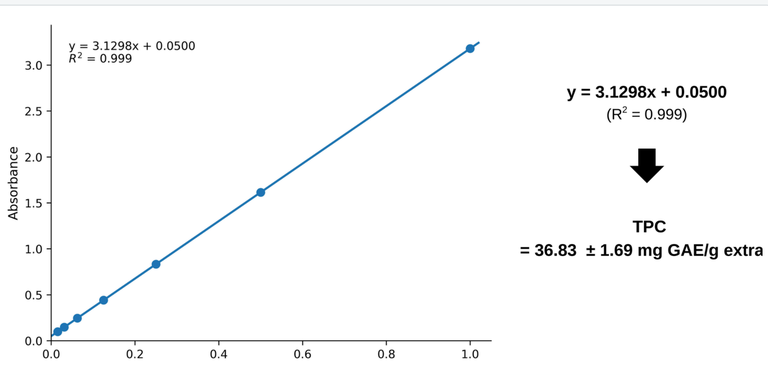
Nicotinic acid eluted at about 2.23 min and was quantified at 2.8257 mg/g crude extract. Asarone was observed only weakly and should be re-confirmed because the preliminary report noted retention-time drift at about 16 min.

Figure 2. DPPH radical scavenging activity of crude extract



Blank-corrected 4PL fitting gave DPPH IC50 = 0.971 mg/mL (95% CI 0.572-1.370; R^2 = 0.9957), showing clear concentration-dependent radical scavenging.

Figure 3. Total phenolic content by Folin-Ciocalteu assay

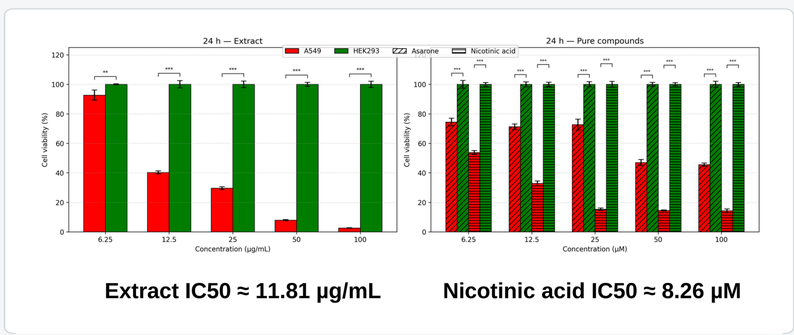


The gallic-acid calibration was highly linear (R^2 about 0.999). Mean extract absorbance was 1.2028 ± 0.0530, corresponding to 36.83 ± 1.69 mg GAE/g extract.

Interpretation: antioxidant activity is measurable but moderate for a crude bryophyte extract. Because TPC is not extremely high, non-phenolic constituents may also contribute to the observed scavenging pattern.

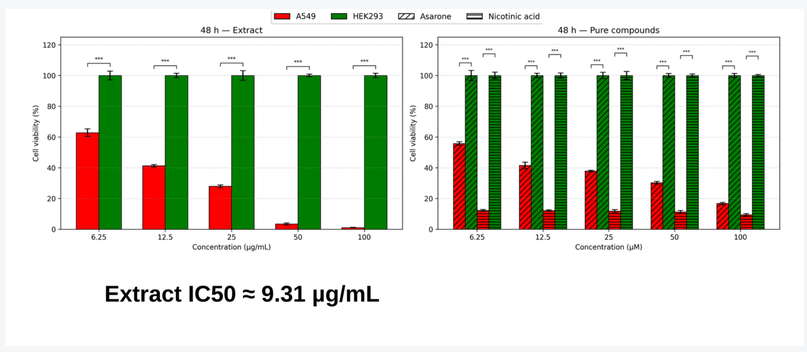
Cytotoxicity against A549 compared with HEK293

Figure 4. MTT assay at 24 h



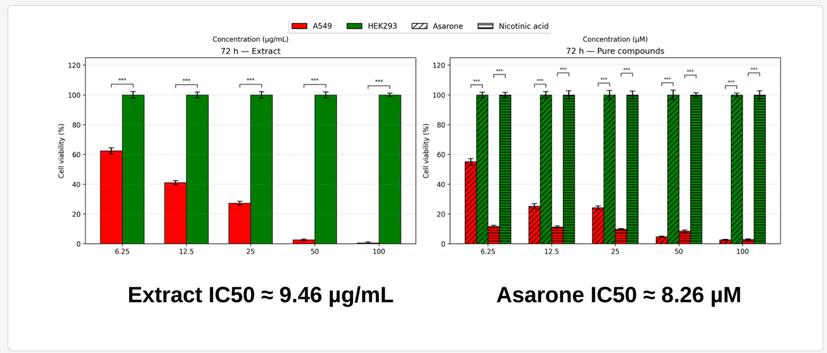
Extract IC50 = 11.81 µg/mL; nicotinic acid IC50 = 8.26 µM. Asarone showed weaker short-term activity (IC50 about 44.49 µM).

Figure 5. MTT assay at 48 h



Extract IC50 = 9.31 µg/mL. Asarone became markedly more potent at 48 h (IC50 about 7.18 µM), whereas nicotinic acid did not reach 50% viability reduction in the tested range.

Figure 6. MTT assay at 72 h



Extract IC50 = 9.46 µg/mL and asarone IC50 = 6.76 µM. Across time, A549 viability decreased strongly while HEK293 remained close to the control pattern shown in the graphs.

IC50 summary across time

Treatment	24 h	48 h	72 h
P. sphaericum extract (µg/mL)	11.81	9.31	9.46
Asarone (µM)	44.49	7.18	6.76
Nicotinic acid (µM)	8.26	NR	NR

Limitations and next steps

The study is based on a crude ethanolic extract and preliminary cell assays, so the active principle has not yet been isolated. The asarone peak should be re-confirmed because the preliminary HPLC report noted retention-time drift. The next logical step is bioassay-guided fractionation with repeated HPLC validation, followed by mechanism-focused assays and testing against additional cancer and normal cell lines.

Key discussion points

1) The crude extract maintained a narrow IC50 range from 24-72 h, indicating consistent cytotoxicity rather than a one-time transient effect. 2) Nicotinic acid alone could not explain the full extract behavior, because its activity was limited and did not remain strong beyond 24 h. 3) Asarone showed time-dependent potency, yet HPLC suggests that its abundance in the crude extract is low; therefore, additional constituents or synergistic interactions are likely involved.

Conclusions

In vitro-cultured Physcomitrium sphaericum yielded a crude ethanolic extract with quantifiable nicotinic acid, measurable antioxidant activity, and reproducible cytotoxicity against A549 lung cancer cells. The extract acted more consistently across 24-72 h than nicotinic acid alone and showed a viability pattern that remained distinctly less harmful to HEK293 cells under the tested conditions. Although asarone appears only at a low and analytically uncertain level in the crude extract, the pure compound showed strong late cytotoxicity, indicating that further confirmation and fractionation are worthwhile. Overall, the data position P. sphaericum as a promising bryophyte source for future phytochemical isolation and anticancer research.

Future work priorities

• Re-confirm asarone identity and quantify with tighter retention-time control. • Fractionate the crude extract and retest active fractions. • Add apoptosis, ROS, and cell-cycle assays to identify mechanisms. • Extend testing to more cancer and normal cell lines.

Acknowledgement

We thank the Department of Biology, Faculty of Science, Chiang Mai University, for laboratory facilities and academic support. Special thanks are extended to the bryophyte laboratory team and all collaborators who assisted with culture maintenance, analytical work, and cell-based assays.

Selected references

Narin et al., 2022; Singleton and Rossi, 1965; Singleton et al., 1999; Mosmann, 1983; Denizot and Lang, 1986.