Phytochemical profiles, antioxidant, and anti–cancer activities of Crude Extract from *In Vitro*-Cultured *Physcomitrium eurystomum* Sendtn.

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Abstract

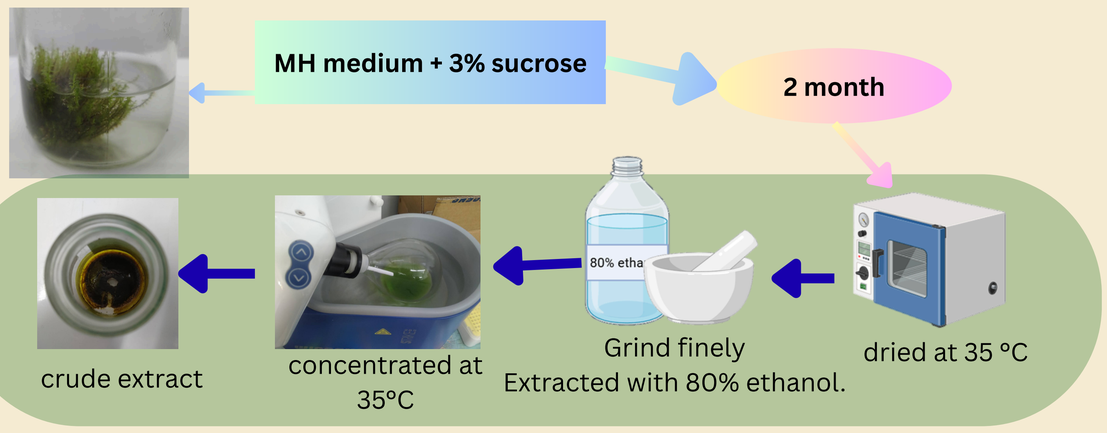
This study investigates the phytochemical properties and biological activities of the crude extract from *Physcomitrium eurystomum* Sendtn., a rare species of moss. The research aimed to evaluate its potential as a source of bioactive compounds with antioxidant and antiproliferative properties. The moss was cultivated in vitro using MH medium for a period of two months, after which the biomass was extracted using 80% ethanol. Phytochemical analysis using LC-MS and HPLC techniques revealed the presence of Nicotinic acid at a concentration of 0.235 mg/g crude extract, while Beta-Asarone was undetectable. The extract demonstrated a Total Phenolic Content (TPC) of 34.96 mg GAE/g, which is significantly high compared to many previously reported moss species. Furthermore, the antioxidant capacity was assessed using the DPPH assay, yielding an IC50 value of 0.924 mg/mL, indicating noteworthy free radical scavenging activity. The findings suggest that in vitro-cultured P. eurystomum is a promising source of phenolic compounds and Nicotinic acid, both of which are known for their health-promoting benefits. Given the concentration of Nicotinic acid and the high TPC, this moss extract holds potential for further development in pharmaceutical applications, particularly regarding its antiproliferative effects on cancer cells. This study provides a foundational framework for utilizing rare bryophytes in modern biotechnology and medicine.

Introduction

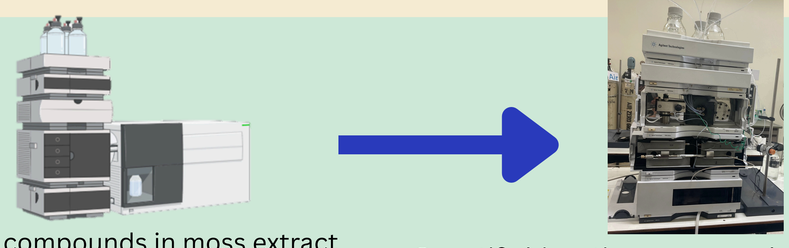
Physcomitrium eurystomum Sendtn. It is a rare species of funariaceous moss[1]. It was included in the list as Vulnerable on the IUCN Red List[2], and limited existing data regarding its phytochemical profile. The specific biological activities, particularly the antiproliferative effects of this moss remain largely unexplored.

Methods

- **Tissue culture & extraction**

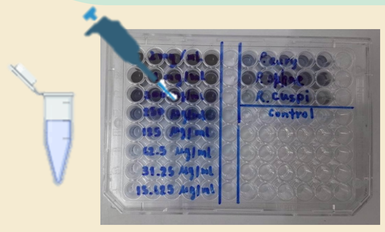


- **Identify and quantify bioactive compounds**



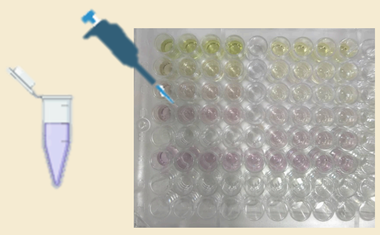
Identify compounds in moss extract with LC-MS

Quantify bioactive compounds in moss extract with HPLC



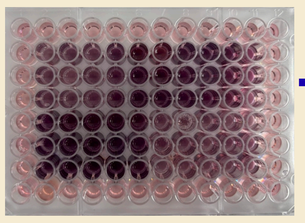
Total phenolic content (TPC) with the Folin-Ciocalteu method

Measure the absorbance at 765 nm



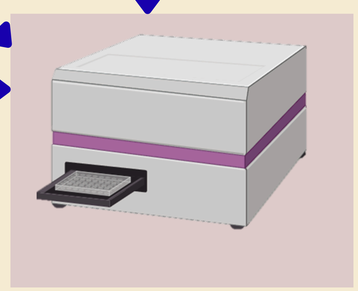
Antioxidant assay (DPPH)

Measure the absorbance at 517 nm



Cell viability assay (MTT assay)
*In process

Measure the absorbance at 570 nm



Results and Discussion

Average Mz	Reference m/z	Mass error (ppm)	Metabolite name	Adduct type	Ontology	Total score	S/N ratio	100%MeOH_Blank	P_eurystomum
231.08774	231.09000	9.78	low score: Beta-Asarone	[M+Na]+	Anisoles	1.07	437.70	961.33	21,747,142.92
124.03918	124.03850	-5.48	Nicotinic acid	[M+H]+	Pyridinecarboxylic acids	1.83	31.82	161.18	811,520.12
122.07169	122.07310	11.55	low score: Chlormequat	[M]+	Tetraalkylammonium salts	1.01	184.97	14,033.25	9,679,835.70
297.06497	297.07001	16.97	low score: clausenin methyl ether	[M+Na]+	Linear pyranocoumarins	1.20	173.08	461.11	8,936,723.41
261.14944	261.14471	-18.11	gamma-Glutamylleucine	[M+H]+	Dipeptides	1.69	286.08	5,269.01	8,601,876.34
381.08945	381.09500	14.56	low score: Gardenin B	[M+Na]+	8-O-methylated flavonoids	1.48	771.06	958.81	8,580,189.01
247.13310	247.13000	-12.54	low score: Arglabin	[M+H]+	Guaianolides and derivatives	0.99	79.81	4,109.53	5,666,561.95
543.13428	543.13220	-3.83	Melezitose	[M+K]+	Oligosaccharides	1.61	2,405.54	585.95	4,514,417.14
299.05597	299.06000	13.48	low score: Tanshinone I	[M+Na]+	Tanshinones, isotanshinones, and derivatives	1.09	462.65	345.93	4,353,091.00
281.11081	281.11499	14.87	low score: Levosimendan	[M+H]+	Phenylhydrazines	0.95	71.30	1,603.56	4,168,401.14
102.09219	102.09134	-8.33	low score: valeramide	[M+H]+	Fatty amides	0.72	1,221.41	4,842.78	4,065,963.69
297.10519	297.10001	-17.44	low score: OCP_297.1002_16.5	[M+H]+	Phenylpiperazines	0.95	172.01	129.80	3,736,504.61
144.04770	144.04828	4.03	4-Methyl-5-thiazoleethanol	[M+H]+	4,5-disubstituted thiazoles	1.73	191.53	2,182.03	3,567,763.04

Fig 1. Various compounds of P.eurystomum extract from LC-MS (AU), of particular interest are Nicotinic acid and Beta-Asarone because of their high quantity and research reports on their antioxidant and anti-cancer properties [3].

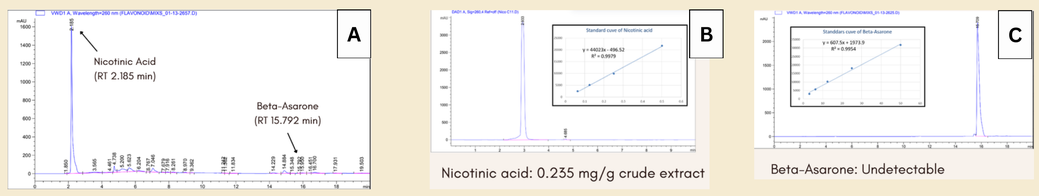


Fig 2. (A) Chromatogram of *P.eurystomum* extract from HPLC. (B) Chromatogram of the standard substance Nicotinic acid from HPLC. (C) Chromatogram of the standard substance Beta-Asarone from HPLC. This moss extract contains 0.235 mg/g of nicotinic acid in crude extract.

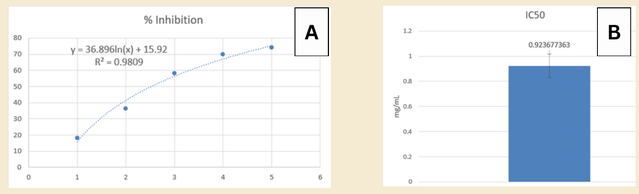


Fig 3. (A) Standard curve of DPPH. (B) The IC50 value of *P.eurystomum* extract. The IC50 value of the P. eurystomum extract was 0.924 mg/ml.

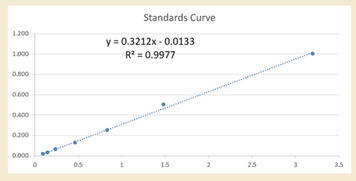


Fig 4. Standard curve of total phenolic content (TPC). The extract from *P. eurystomum* had a total phenolic content of 9.03 mg GAE/g Dry weight.

*Cell viability assay (MTT assay) and Total flavonoid content(TFC) are in progress.

Conclusion

In vitro cultures of *Physcomitrium eurystomum* Sendtn. produce essential phytochemicals, such as phenolics and nicotinic acid, with notable antioxidant.

Acknowledgement

Special thanks are due to Assistant Professor Dr. Narin Printarakul, Department of Biology, Faculty of Science, Chiang Mai University, for his guidance and support during this research.

Reference

- [1] T. Chetia and H. Roy, "Physcomitrium eurystomum Sendtn. (Funariaceae): a rare species recorded for Assam, India," Journal of Threatened Taxa, vol. 16, no. 6, pp. 25474–25477, Jun. 2024, doi: 10.11609/jott.8923.16.6.25474-25477.
- [2] The IUCN Red List of Threatened Species 2022: e.T85841752A87795278. The IUCN Red List of Threatened Species 2022: e.T85841752A87795278. Fig 3. (A) Standard curve of DPPH. (B) The IC50 value of P.eurystomum extract.
- [3] Wang, S., Li, Y., Xu, X., Wang, Z., Fan, X., Lou, M., & Zhao, J. (2017). Nicotinic acid inhibits glioma invasion by facilitating Sirt1-mediated degradation of ATF4. Scientific Reports, 7(1), 43173. https://doi.org/10.1038/srep43173