Phytochemical Profiles, antioxidant, and anti-cancer activities of *In Vitro*-Cultured *Sphagnum cuspidatulum* Müll Hal. extract

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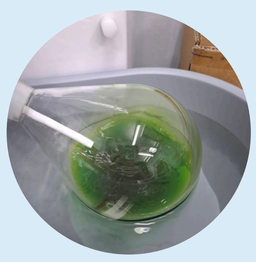
Abstract

This study aimed to investigate moss "*Sphagnum cuspidatulum*" extracts with potential medicinal benefits, with particular emphasis on their anti–lung cancer properties. In this study, Sphagnum cuspidatulum was cultured using plant tissue culture techniques prior to extraction to obtain crude extracts. The moss was cultured in 1/6 MS liquid medium (pH 5.65) for 2 months. The major chemical constituents were subsequently analyzed using liquid chromatography–mass spectrometry (LC–MS). It was found that the moss contained important compounds with levels exceeding 100,000 a.u. such as Asperulosidic acid, Nicotinic acid, Reserpine, Gardenin B, and 5-OXO-L-proline. HPLC quantification revealed the presence of nicotinic acid at 3.581 * 10^{-6} ± 6.745 * 10^{-8} mg/g DW and asperulosidic acid at 1.494±0.044 mg/g crude extract. The extract's total phenolic content was measured at 0.00966±0.00267 mg GAE/g dry sample. Furthermore, the extract exhibited concentration-dependent DPPH radical scavenging activity, with a calculated IC5050 value of 4.24 mg/mL.

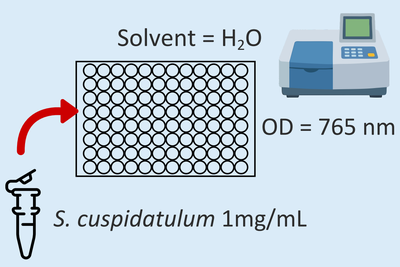
Introduction

Sphagnum mosses developed an array of morphological and anatomical peculiarities maximizing their water holding capacity. They also produce plethora of biochemicals that could prevent stresses-induced cell-damages
but these chemicals remain poorly studied. [1]

Methodology







1 Extraction

1) Air-dried 40
2) Ultrasonic 45 min
3) Evaporate 45
4) Drying 13 hours

2 LC-MS & HPLC

Mobile phase A :
0.1% Formic acid + Water
Mobile phase B :
0.1% Formic acid + Acetone

3 Total Phenolic content

1) standard : **Gallic acid**
0.015625-2 mg/mL
2) Folin reagent → 10 min
Na_2CO_3 → 30 min

4

1) standard → **Catechin**
20-100 mg/mL
2) $NaNO_2$ 5%
$AlCl_3 \cdot 6H_2O$ 10%
NaOH 1 mM

5 DPPH

1) *S. cuspidatulum*
in MeOH 100% (v/v)
(4, 2, 1, 0.5 mg/mL)
2) Control
DPPH + MeOH 100% (v/v)

6 MTT assay

1) Cultured A549 and HEK293 cells
2) Treat cells with *S. cuspidatulum* extract, asperulosidic acid and nicotinic acid
3) Compare results with standard compound



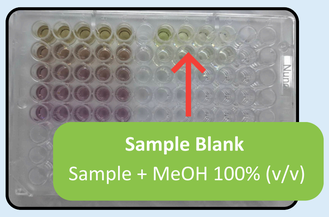
A549



HEK 293



OD = 570 nm
currently in process



OD = 517 nm



OD = 510 nm
currently in process

Results

Fig 1. *Sphagnum cuspidatulum* in liquid 1/6 MS Medium (pH 5.65)

Metabolite name	Ontology	Total score	a.u.
Gardenin B	8-O-methylated flavonoids	1.48	8,085,389.66
Reserpine	Yohinbine alkaloids	1.52	1,206,660.59
Asperulosidic acid	Iridoid O-glycosides	1.55	553,428.01
5-OXO-PROLINE	Alpha amino acids and derivatives	1.34	357,325.36
Nicotinic acid	Pyridinecarboxylic acids	1.83	112,641.67

Table 1. important compounds, selected based on high abundance (>100,000 a.u.), high total score, and anti-inflammatory or anti-cancer activity, from metabolite separation by LC-MS in the extract.

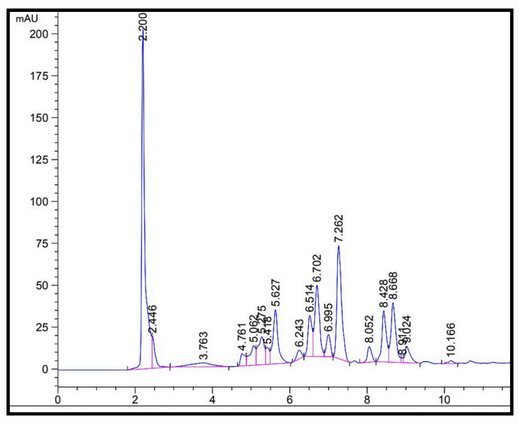


Fig 2. HPLC Chromatogram of *S. cuspidatulum* Extract

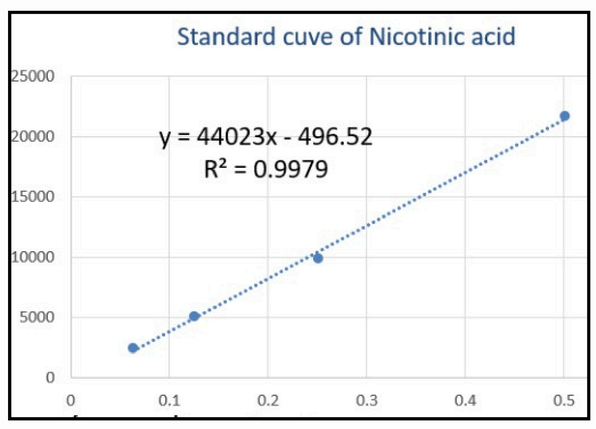
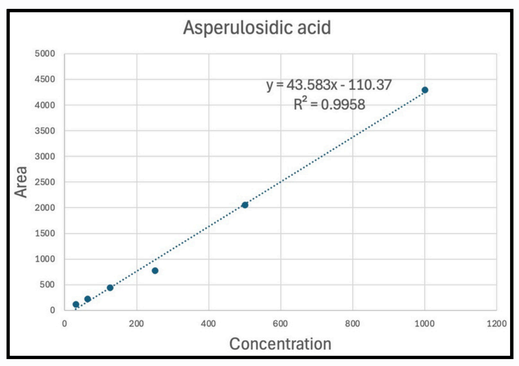


Fig 3. standard curve of nicotinic acid which is highest peak in HPLC Chromatogram of *S. cuspidatulum*, total nicotinic acid content content in *S. cuspidatulum* crude extract is 3.581 * 10^{-6} ± 6.745 * 10^{-8} mg/g DW



Fic 4. standard curve of asperulosidic acid which is 2nd major peak in HPLC Chromatogram of *S. cuspidatulum*, total asperulosidic acid content in *S. cuspidatulum* crude extract is 1.494 ± 0.044 mg/g crude extract

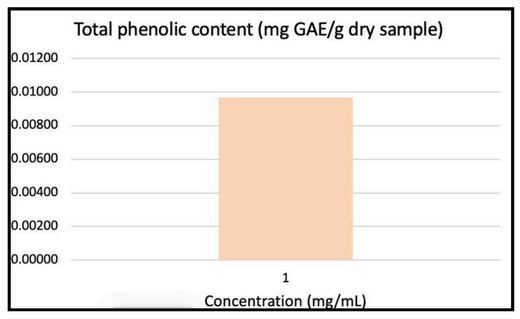


Fig 5. total phenolic content of *S. cuspidatulum* Extract is 0.00966 ± 0.00267 mg GAE/g dry sample, data are present as single measurement

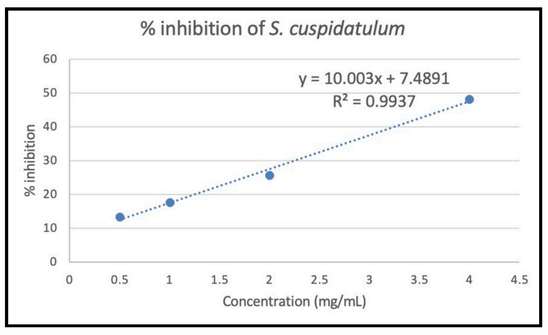


Fig 6. the graph illustrates the relationship between the extract concentration (mg/mL) and the percentage of inhibition. The IC50 value was calculated to be 4.24 mg/mL.

Disscussion & conclusion

As the total asperulosidic content that has 1.494 ± 0.044 mg/g crude extract a compound commonly found in lower concentrations in Morinda citrifolia 0.7–8.9 mg/g [2]. Previous study show total phenolic acid approximately 130 times higher than our value [3].Jamaican Ball Moss has excellent potential against PC-3 (prostate cancer cells) comparable to our IC50 value[4]. LC-MS identified several bioactive compounds in S. cuspidatulum, including asperulosidic acid, nicotinic acid, reserpine, gardenin B, and 5-OXO-L-proline. DPPH assay also showed strong antioxidant activity. Findings suggest antioxidant potential despite low phenolic and asperulosidic acid content. The extract itself has promising antioxidant properties that warrant further study, including the planned tests on lung cancer cells.

References

[1] Sytiuk, Céréghino et al., 2020. Morphological and biochemical responses of Sphagnum mosses to environmental changes. biorxiv. https://www.biorxiv.org/content/10.1101/2020.10.29.360388v1.abstract
[3] Karim, Suleiman et al., 2014 PHYTOCHEMICALS, ANTIOXIDANT AND ANTIPROLIFERATIVE PROPERTIES OF FIVE MOSS
SPECIES FROM SABAH, MALAYSIA. International Journal of Pharmacy and Pharmaceutical Sciences.

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