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Isolation, Characterization, and Antioxidant Activity of *Selliguea taeniata* Secondary Metabolites

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Abstract: This current study presents a chemical exploration of the methanolic extract of *Selliguea taeniata* leaves, a fern species endemic in certain regions in the Philippines, such as Ifugao, where it is used to treat cough for good health and well-being of the folks. Although botanical data of this fern are reported, there are still no reports regarding its chemical properties, such as phytochemicals and antioxidant activity, as of the time this study was conducted. The air-dried leaves of *S. taeniata* were extracted using absolute methanol. The crude extract was concentrated and subjected to the Folin-Ciocalteu method to determine the total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay to evaluate its antioxidant activity, and phytochemical screening. Subsequently, various chromatographic techniques and ^{1}H Nuclear Magnetic Resonance spectroscopy were applied to isolate and partially characterize its secondary metabolites partially. Methanolic extract of *S. taeniata* leaves had a TPC of 1669.11 ± 0.07 mg GAE /g of dried sample and antioxidant activity (EC_{50}= 85.79 ± 0.02 ppm). The crude extract contained alkaloids, flavonoids, cardiac glycosides, saponins, phenols, and tannins. Partial isolation of its secondary metabolites suggested that the crude extract may contain a mixture of proanthocyanidins or its monomer units and glycosides. The claims of the folks from Ifugao on the effectivity of *Selliguea taeniata* leaf decoction as an antitussive can be attributed to the plant's high antioxidant activity. The extract contained phenolic compounds that could be proanthocyanidins, inhibiting elastase that promotes inflammation.

Keywords: *Selliguea taeniata*; antioxidant activity; phenolic compounds; free radical scavenging activity; good health and well-being.

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1. Introduction

Ferns are any of several nonflowering vascular plants and are characterized by pteridophytes. They are one of the oldest plant species on Earth, with a fossil record dating back to the Middle Devonian, about 383-393 million years ago. However, today's diversity of ferns evolved relatively [1]. It has been observed that ferns are resistant to microbial pathogens, contributing to their successful evolution and survival [2]. Ferns are the second-largest vascular plant species having about 13,000 species and more than 250 different genera distributed worldwide. They are commonly found in tropical countries with warm and damp areas. Some

ferns are limited to particular soil acidity or alkalinity ranges, and some grow well on acidic rocks like granites and sandstones and quantities [3].

Notably, the Philippines is the habitat of about 1,100 species of ferns and lycophytes distributed among 154 genera and 34 families, and 296 species are known to be native [4-6]. Although many fern species are used as traditional medicines for human diseases and consumed as vegetables, ferns remain unexplored for their phytochemical and bioactivity [7-9].

Figure 1. *Selliguea taeniata* leaves.

One of which is *Selliguea taeniata* (Figure 1), also called ag-agfa by the locals of Banaue, Ifugao, Philippines. This fern is first classified as *Polypodium taeniatum* [10]. It is currently known in the eastern part, particularly in Peninsular Malaysia, Borneo, Philippines, Indonesia, Vietnam, and Taiwan, and is said to be predominant in the Philippines. It was first reported to be abundant in Mt. Bali-it, Balbalasang, Kalinga, and Northern Luzon [11]. It has 25-50 cm long fronds, and its stipe is usually longer than its lamina [12]. *Selliguea*, a fern member of the Polypodiaceae family, can be found mostly in tropical countries, particularly in Asia, from India eastward to Japan and southward to New Guinea, actively growing from the second quarter of the year [13,14]. These plants mainly were epiphytic and epilithic, and a few terrestrial.

Although botanical data are available for *S. taeniata*, there are still no reports regarding its chemical properties, such as phytochemicals and biological activities, to our knowledge. Thus, the present study is primarily designed to chemically explore the leaves of *S. taeniata* by evaluating its antioxidant properties and isolating and characterizing its secondary metabolites.

2. Materials and Methods

2.1. Collection and preparation of extract.

All extractions and bioactivity tests were carried out under atmospheric room conditions. All reagents and solvents used in the analysis were analytical grades, and spectral grade solvents were used for spectroscopic studies. Thin-layer chromatography plates were procured from Merck. All glassware was washed with dishwashing liquid and rinsed with water, followed by washing with distilled water and methanol and subsequent drying. The plant material used in this study was collected at Brgy, Ducligan, Banaue, Ifugao last December 2018. It was authenticated by a botanist from the Department of Biological Sciences, Central Luzon State University, to be *S. taeniata*. The leaves of the plant *S. taeniata* were rinsed with clean tap water to make them dust- and debris-free. Then, the leaves were dried under room conditions for 20 days. The dried sample (138 grams) was ground using a blender and soaked

in 1400 mL of methanol for 48 hours in a tightly closed amber container. The mixture was filtered, and the filtrate was collected and concentrated using a rotary evaporator under reduced pressure at 40°C to yield the crude extract. Subsequently, the crude extract was subjected to fractionation, phytochemical screening, and bioactivity tests, namely antimicrobial and antioxidant activities, as described in the next section.

2.2. Phytochemical screening.

Phytochemical screening for various phytochemical constituents was accomplished following the standard test tube method [15]. Crude extract of the plant *S. taeniata* leaves was used to screen for alkaloids, carbohydrates, flavonoids, cardiac glycosides, phlobatannins, saponins, phenols, and tannins.

2.3. Determination of total phenolic content.

The extract's total phenolic content (TPC) was determined following the Folin-Ciocalteu spectrophotometric method [16,17]. For calibration, different concentrations (10, 20, 40, 60, 80, 100, and 120 ppm) of gallic acid in ethanol were prepared. The crude extract (400 µL) was diluted with 800 µL distilled water before adding an mL of Folin-Ciocalteu reagent. The mixture was shaken vigorously and allowed to stand for five minutes at room temperature. Then, two mL of 7.5% Na_2CO_3 solution was added. The mixture was shaken thoroughly and left to stand in the dark for 90 minutes. Absorbance was obtained at 765 nm using Cole-Parmer Unico 1201 UV-Vis spectrophotometer. The test was carried out in triplicate. The TPC was expressed as gallic acid equivalent (GAE) milligrams per gram dried sample, calculated according to equation 1:

$$\text{TPC} = \frac{\text{concentration of gallic acid x mass of crude extract}}{\text{mass of dried sample}} \quad (1)$$

2.4. Antioxidant activity using DPPH free radical scavenging assay.

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay is a widely used method to evaluate the antioxidant activity of natural compounds. This assay measures the scavenging ability of antioxidant substances toward the stable radical [18]. The free radical scavenging activity of the extract was examined *in vitro* following the slightly modified method described by [19]. A 1.5 mL of 0.1, 10, 100, 300, and 1000 ppm of the crude extract were separately added to 2.5-mL DPPH solution (6.0 mg DPPH/100 mL methanol). The mixture was shaken vigorously and left to stand without light for 30 minutes at room temperature. The absorbance of the mixture was read at 517 nm using Cole-Parmer Unico 1201 UV-Vis spectrophotometer. Ascorbic acid was used as a reference and methanol as a blank. The inhibition percentage for scavenging DPPH radical was calculated according to equation 2:

$$\%\ \text{scavenging DPPH radical} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}}\ \text{x}\ 100\% \quad (2)$$

The test solutions were prepared in triplicates for three trials. Graphpad Prism 7 was used to estimate the median effective concentration of the sample (EC_{50}), which was the concentration required to scavenge radical by 50%.

2.5. Partial isolation and characterization of compounds.

The crude extract was defatted using hexane before fractionation using various chromatographic techniques. Then, it was subjected to Sephadex LH-20 column chromatography eluted with methanol. Thin-layer chromatography (TLC) was used to study the profile of the produced fractions. Fractions with the same TLC profile were grouped before further purification using Silica gel column chromatography, and preparative TLC was applied until a potential pure compound was isolated. Isolated compound/s was partially characterized using 300 MHz ^{1}H Nuclear Magnetic Resonance spectroscopy using a Bruker Advance 300 and CD_3OD as solvent.

2.6. Characterization using thin-layer chromatography (TLC).

Thin-layer chromatography was used to identify which fractions are to be mixed and determine the solvent system that will cause good separation of the components. This method was composed of (a) preparation of the plate, (b) development of the chromatogram, and (c) visualization of the chromatogram. Strips of plastic-back normal-phase TLC sheets were prepared by cutting a 20 cm x 20 cm plate into 5 cm x 1 cm. Solvents used were dichloromethane, methanol, and ethyl acetate. The fraction was dropped at the center, about 1 cm above the bottom of the strip, using a capillary tube. The solvent was allowed to evaporate before the strip was developed using various solvent systems until the solvent reached about 1 cm from the top of the strip completely. TLC strips containing fractions were subjected separately to pure solvents, then to a mixture of solvents to observe separation. The developed chromatograms were visualized by exposing them to a long-wave UV lamp and placing it in a jar containing silica gel and iodine mixture for about 5 seconds. The spots were traced and labeled. Fractions showing similar profiles were then mixed. The solvent system that offered the best separation was used in column chromatography and preparative thin-layer chromatography.

2.7. Preparation of the column and separation and isolation by column chromatography.

After extraction, the separation of components was done by column chromatography. A 20-inch high cylindrical tube with a diameter of 1 inch packed with 50 grams of Sephadex LH-20 in methanol was used to separate crude extract. Fractions were collected in different 30-mL test tubes and were concentrated. Chosen fractions were subjected to Silica gel column chromatography. Different columns were prepared for each fraction. First, a 12-inch high cylindrical tube with a diameter of 1.2 inches was packed with 50 grams of Silica gel in dichloromethane. Gradient elution starts from 250 mL portions 20:80, 30:70, 40:60, 50:50 of dichloromethane-ethyl acetate; followed by 50:50, 40:60, 30:70, 20:80 ethyl acetate-methanol; and lastly, 350 mL of dichloromethane-methanol-ammonia solution (90:9:1). Secondly, a 19-inch column with a diameter of 0.5-inch was packed with 21-grams Silica gel and then eluted with 80 mL portions of 10:90, 20:80, and 30:70 of methanol-ethyl acetate; 90:9:1 dichloromethane-methanol-ammonia solution. Fractions were collected in different 10-mL test tubes and concentrated using a rotary evaporator under reduced pressure at 40°C.

2.8. Preparation of plates and separation and isolation by preparative thin-layer chromatography (TLC).

Preparative thin-layer chromatography was used to purify further the fractions produced from column chromatography. Glass plates with dimensions 20 cm x 20 cm were used. Silica gel and $CaSO_4$ (20:1) mixture were dissolved in distilled water (1 g mixture/3 mL water) to produce a slurry consistency. The mixture was poured onto plates and spread evenly into the glass plate. It was left to stand for about one hour and then activated in an oven at 120°C for 45 minutes. Each crude extract fraction was applied to the preparative TLC plates about 1 cm from the bottom and edge. The solvent was allowed to evaporate. Afterward, the TLC plate was developed in an appropriate solvent system until the solvent ascended to about 1 cm from the top of the plate. After developing, the plates were left dry and exposed to short- and long-wave UV lamps for visualization. Bands were traced and collected in a 50-mL beaker. The fractions were then extracted from the silica gel using ethyl acetate or methanol.

2.9 Statistical analyses.

Results obtained were reported as mean ± SD. The differences between test solutions were statistically analyzed using a non-parametric t-test (Kolmogorov-Smirnov test) using GraphPad Prism 7 software.

3. Results and Discussion

3.1. Phytochemical screening.

The concentrated crude extract was viscous and deep green. After concentration using a rotary evaporator under reduced pressure at 40°C, 18.61 grams of crude extract were obtained, with a percent yield of 13.47%. Phytochemical constituents identified in the crude extract were alkaloids, flavonoids, cardiac glycosides, saponins, phenols, and tannins. Phytochemical surveys revealed that *Selliguea* species has a wide range of alkaloids, flavonoids, polyphenols, terpenoids, and steroids, which are usually distinct from those produced by other higher plants (19-23). The above results make *Selliguea taeniata* a potentially important source of chemical diversity for drug development.

3.2. Antioxidant activity.

3.2.1 Total phenolic content.

The *S. taeniata* crude extract analysis using the Folin-Ciocalteu method resulted in a TPC of 1669.11±0.07 mg GAE/g of dried sample. This value of TPC can be ascribed to phytochemicals such as flavonoids, steroids, and tannins, which contain phenol groups. Plants produce phenolic compounds in response to environmental stress [24]. These compounds protect the plants against UV-B damage and subsequent cell death by protecting DNA from dimerization and breakage [25-28]. Moreover, the production of flavonoids, especially anthocyanins and flavones by phenylalanine ammonia-lyase, is triggered by light [29]. Thus, plants in high areas, like Ifugao, which are undoubtedly exposed to several stress factors, may have produced antioxidants such as flavonoids.

3.2.2. DPPH free radical scavenging activity.

The free radical scavenging activity of the methanolic leaf extract of *S. taeniata* showed a concentration-dependent response (Figure 2). That is, the antioxidant activity of the extract increases with increasing concentration.

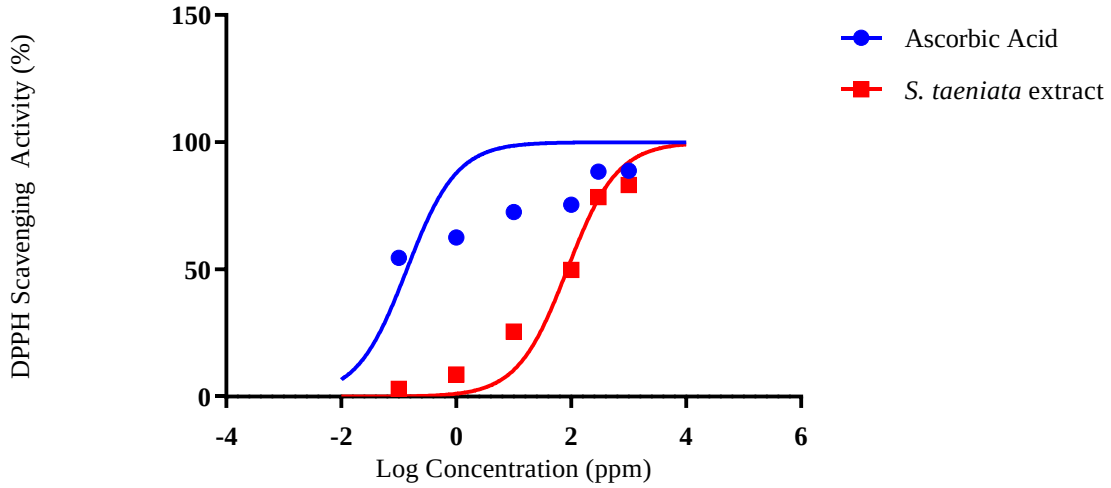


Figure 2. DPPH scavenging activity of the ascorbic acid and crude extract of *S. taeniata*.

The crude extract exhibited a median effective concentration (EC_{50}) of 85.79 ± 0.02 ppm. EC_{50} is the concentration needed to scavenge 50% of free radicals. It is inversely proportional to the free radical scavenging activity, suggesting lower EC_{50} correlates with higher antioxidant activity. *S. taeniata* crude extract is considered a strong antioxidant, while that of the ascorbic acid (0.51±0.66 ppm) is a very strong antioxidant. However, ascorbic acid was expected to possess higher antioxidant activity as it is already pure compared to the crude extract of *S. taeniata*. Thus, isolating and identifying its bioactive secondary metabolites may significantly increase the observed antioxidant activity. The antioxidant activity of *S. taeniata* can be attributed to the phenolic compounds. Its presence in the crude extract was confirmed and supported by the results of this study's phytochemical and total phenolic content analyses. Phenolic groups possess antioxidant activity as they can donate a proton to stabilize the DPPH radical [22,29], as shown in Figure 2.

3.3. Fractionation and partial isolation of compounds.

The crude extract was extracted to isolate and characterize the bioactive secondary metabolites of *S. taeniata* (Figure 3). The concentrated crude extract (18 g) was subjected to Sephadex LH-20 column chromatography and eluted with methanol yielding 24 25-mL fractions. Fractions obtained range from deep green (Fraction 1) to light orange (Fraction 24). Thin-layer chromatography (TLC) was done to determine the profile of each fraction, which was used to reduce the 24 fractions into five (5) fractions (F1.1-F1.5). Figure 2 shows the fractionation scheme. F1.1 and F1.3 were subjected to Silica gel column chromatography eluted with 20:80 dichloromethane-ethyl acetate and 10:90 methanol-ethyl acetate, respectively. F1.1 produced 135 15-mL fractions that were reduced to eight (8) fractions (Fraction I - Fraction VIII), while F1.3 yielded 31 10-mL fractions that were reduced to three (3) fractions (Fraction IX - Fraction XI). Fraction VII showed a single spot with an Rf value of 0.6 in TLC with methanol as solvent. It was then subjected to preparative TLC to afford Fraction A. Fractions IX, and X were also subjected to preparative TLC using 70:10:20

methanol-ethyl acetate-dichloromethane as the solvent system. A brownish-orange spot under UV (Rf=0.64) was collected in Fraction IX to yield Fraction B. In contrast, the blue spot under UV was acquired in Fraction X to produce Fraction C. F1.4 was also subjected to preparative TLC, and the green spot under UV (Rf=0.73) was collected as Fraction D.

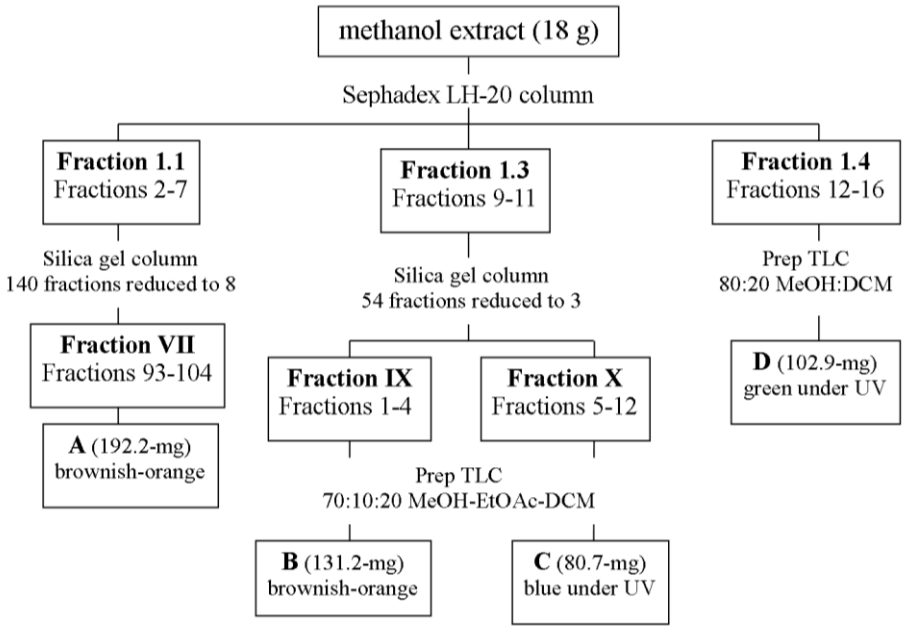


Figure 3. Isolation scheme followed for the fractionation of *S. taeniata* crude extract (Legend: MeOH = Methanol, EtOAc = Ethyl acetate, DCM = Dichloromethane).

3.4. Partial characterization of S. taeniata secondary metabolites.

Fractions A to D were subjected to ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy to characterize and identify the secondary metabolites of *S. taeniata* partially. The ^{1}H NMR spectra of Fractions A to D revealed that the fractions were not yet pure and contained multiple compounds with varying concentrations. The ^{1}H NMR spectrum of Fraction A (Figure 4) revealed signals of aliphatic hydrogens (CH_3-, CH_2-, and/or CH-) at δ_H 0.7-2.5 ppm. Proton signals were observed at δH 3.0-4.0 ppm, attributed to alkyl groups attached to heteroatoms like O. Moreover, the proton signal at around δ_H 5.5 ppm suggested the presence of olefinic proton/s.

Overall, the ^{1}H NMR of Fraction A resembled characteristic peaks of a sugar molecule with multiple alkyl groups linked to O (at δ_H 3.0-4.0 ppm). This result suggested that Fraction A may be a glycoside, supported further by the phytochemical analysis.

The same proton signals were presented in the ^{1}H NMR spectrum of Fraction C (Figure 5). It also revealed the presence of heteroatom-linked alkyl proton signals at δ_H 3.0-4.0 ppm, which suggested Fraction C may be a glycoside that may be the same secondary metabolite in Fraction A. However, additional aromatic protons resonating at δ_H 7.5-7.8 ppm with relatively lower intensity than those at δ_H 3.0-4.0 ppm were also detected, implying another compound containing an aromatic ring was present.

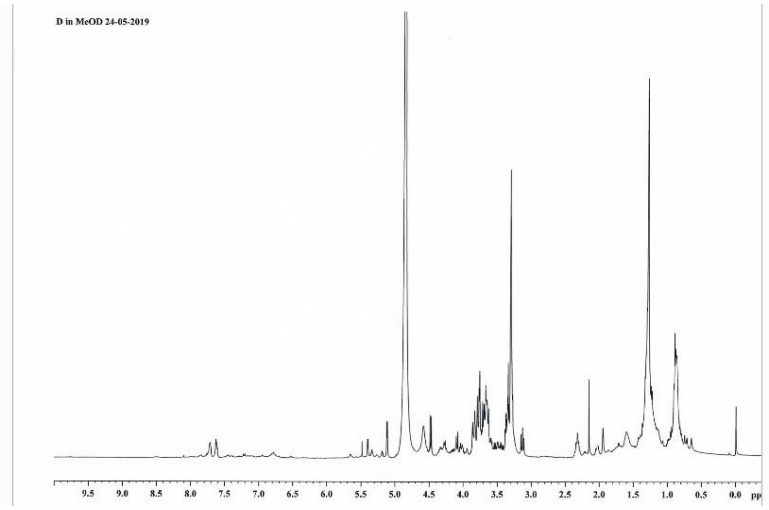


Figure 4. 300 MHz ^{1}H-NMR spectrum of **Fraction A** in CD_3OD.

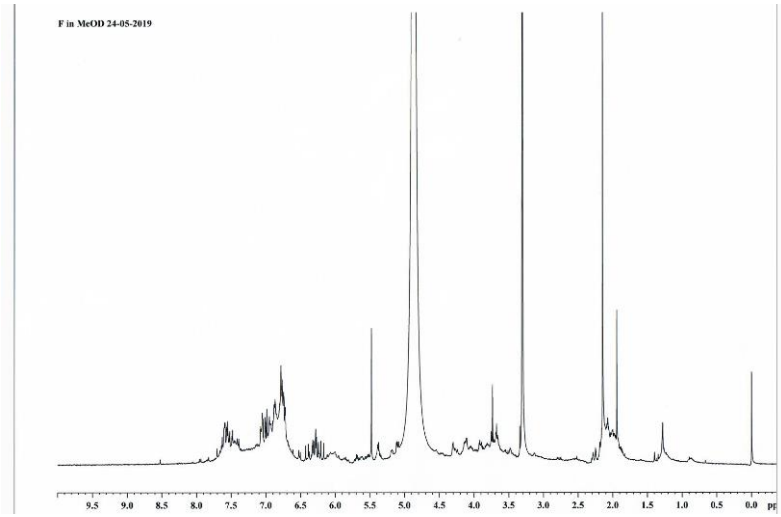


Figure 5. 300 MHz ^{1}H-NMR spectrum of **Fraction C** in CD_3OD.

The ^{1}H NMR spectra of Fractions B (Figure 6) and D (Figure 7) revealed comparable proton signals indicating these fractions contain similar compounds or groups of compounds. The low-field aliphatic protons at δ_H 1.9-2.2 ppm can be attributed to aliphatic protons near an electron-withdrawing carbonyl group. Heteroatom-linked aliphatic protons were also revealed at δ_H 3.5-4.5 ppm. Additionally, aromatic protons were also detected at δ_H 6.0-7.8 ppm.

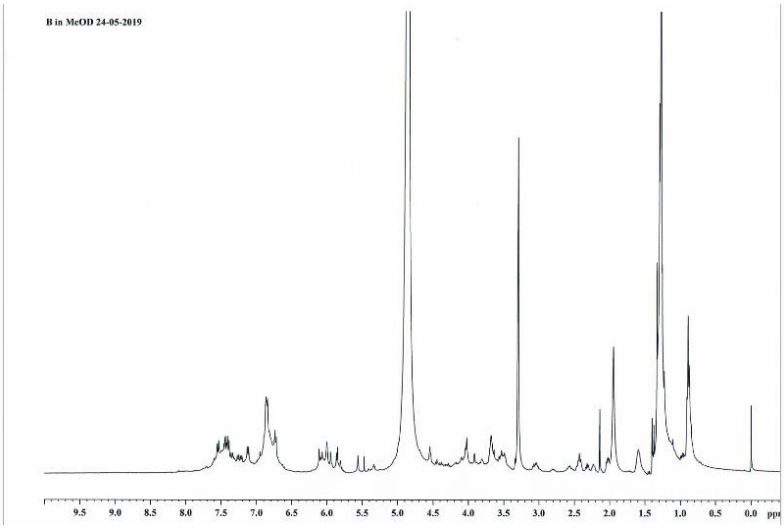


Figure 6. 300 MHz ^{1}H-NMR spectrum of **Fraction B** in CD_3OD.

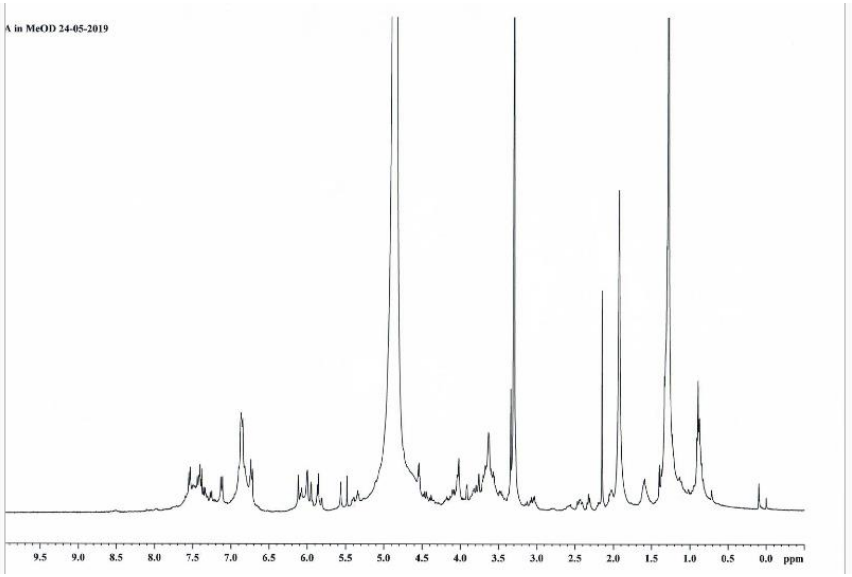


Figure 7. 300 MHz ^{1}H-NMR spectrum of **Fraction D** in CD_3OD.

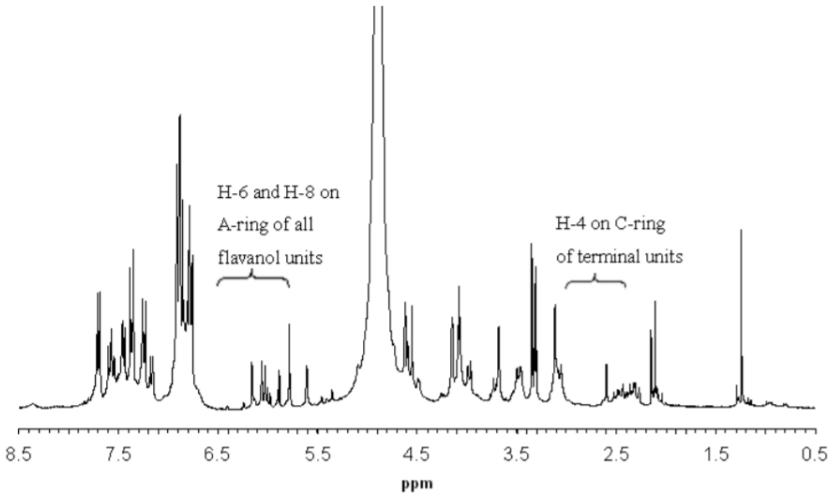


Figure 8. Room temperature 300 MHz ^{1}H-NMR spectrum in CD_3OD of proanthocyanidins from the rhizomes of *Selliguea feei* [30].

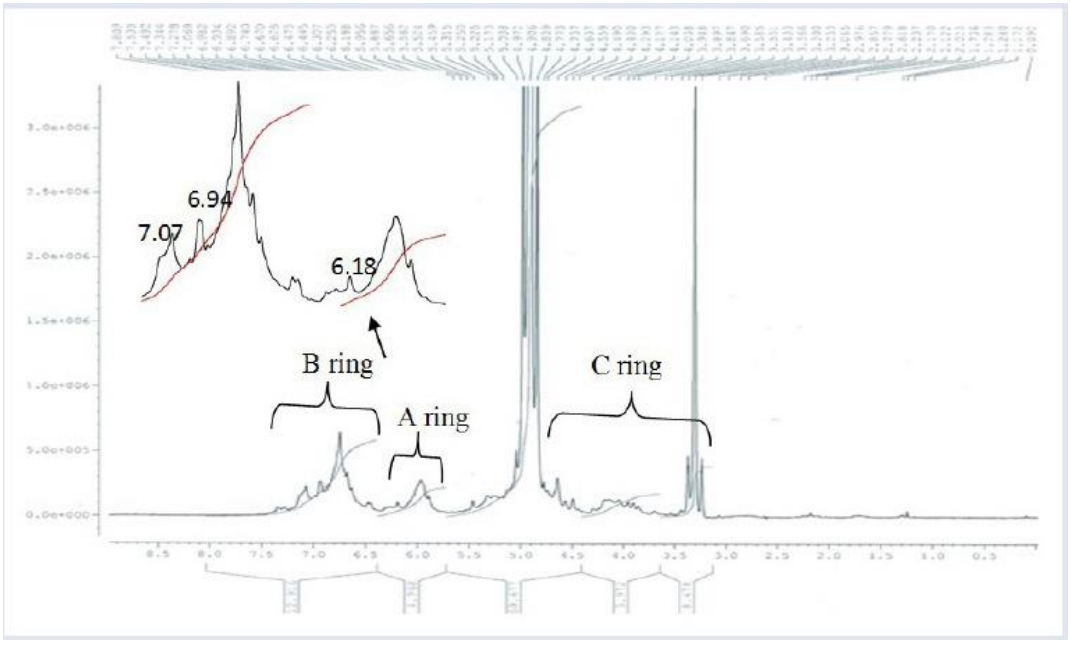
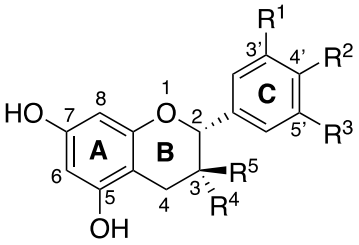


Figure 9. ^{1}H-NMR spectrum of proanthocyanidin from *S. feei* [19].



FLAVAN-3-OLS	R^1	R^2	R^3	R^4	R^5
Afzelechin	H	OH	H	H	OH
Epiafzelechin	H	OH	H	OH	H
Catechin	H	OH	OH	H	OH
Epicatechin	H	OH	OH	OH	H
Gallocatechin	OH	OH	OH	H	OH
Epigallocatechin	OH	OH	OH	OH	H

Figure 10. The general structure of monomer units of proanthocyanidins [19].

The ^{1}H NMR spectra of Fractions B and D were comparable to those reported by Fu and colleagues [30] (Figure 8) and Lai and co-workers [19] (Figures 9 & 10) for proanthocyanidins. Fu and colleagues extracted a mixture of proanthocyanidins from *S. feei*, which belongs to the same genus as *S. taeniata* [30]. They reported the extraction of proanthocyanidin consisting of afzelechin and/or epiafzelechin units with A-type and B-type interflavanyl linkages from *S. feei*, which had a similar ^{1}H HMR spectrum to Fractions B and D. Additionally, the proanthocyanidin consisting of catechin and/or epicatechin units, was isolated from *Blechnum orientale* by [31]. Moreover, as early as 1997, flavonoids and proanthocyanidins were detected in the rhizomes of *S. feei* [32].

Based on these reports, it can be inferred that Fractions B and D were composed of a mixture of proanthocyanidins and/or their monomer units. This inference was also supported by the phytochemical analysis for flavonoids in the crude extract. Proanthocyanidins are also reported to exhibit antioxidant activity [33,34]; thus, these may contribute to the antioxidant activity of *S. taeniata*. Moreover, proanthocyanidins also showed various biological activities such as anti-inflammatory [35], anti-carcinogenic [36,37], and vasodilatory actions [38]. In addition, they also demonstrate inhibition of lipid peroxidation, platelet aggregation, capillary permeability, and fragility and affect enzyme systems, including phospholipase A2, cyclooxygenase, and lipoxygenase [33].

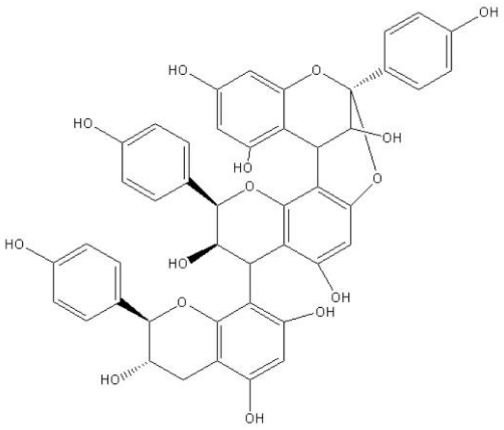


Figure 11. Chemical Structure of Selligueain A.

Studies reported the isolation of Selligueain A, a sweet-tasting proanthocyanidin, which can be a source of commercial sweetener [39,40]. The compound was synthesized from fluorobenzene as the starting reagent to produce the benzyl alcohol, and after several steps, Selligueain A (Figure 11) with a molecular formula of $C_{45}H_{35}O_{15}$ and m/z 815.1981 was obtained [41].

Selligueain A was not acutely toxic nor mutagenic to mice. When dissolved in water, the compound is 35 times sweeter than a 2 percent w/v aqueous sucrose solution, without appreciable off-taste or after-taste [40,41]. The study of Suwandi *et al*. [38] on the analgesic and anti-inflammatory of this compound suggests that selligueain A had an inhibitory effect on cyclooxygenase and the elastase release in humans neutrophils as reported by Vasänge *et al*. [43]. Studies on the regulation of enzyme activity by elastase inhibition can lead to the development of medicine for emphysema, pulmonary diseases, and cancers [44].

4. Conclusions

The claims of the folks from Ifugao on the effectivity of *Selliguea taeniata* leaf decoction as an antitussive can be attributed to the plant's high antioxidant activity. The crude extract exhibited a median effective concentration (EC_{50}) of 85.79±0.02 ppm, thus considered a strong antioxidant. The extract contained a high number of phenolic compounds that could be proanthocyanidins and selligueain A, which can inhibit elastase that promotes inflammation.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Revilla, M. A.; Fernandez, H. *In vitro* culture of ornamental ferns. *Plant Cell Tissue Organ Cult.* **2003**, *73*, 1-13, https://doi.org/10.1023/A:1022650701341.
2. Singh, M., Singh, N., Khare, P. B., & Rawat, A. K. S. Antimicrobial activity of some important Adiantum species used traditionally in indigenous systems of medicine. *J Ethnopharmacol* **2008**, *115*, 327-329, https://doi.org/10.1016/j.jep.2007.09.018.
3. Cano-Mangaoang, C.; Bretaña, B.L.P.; Amoroso, V.B. Rapid assessment of epiphytic pteridophyte biodiversity in Mt. Apo Natural Park, North Cotabato Province, Philippines: a comparison of disturbed and undisturbed forests. *Philipp. J. Syst. Biol* **2020**, *14*, 1-13, https://doi.org/10.26757/pjsb2020c14004.
4. Lillo, E.; Malaki, A.B.; Alcazar, S.M.T.; Rosales, R.; Redoblado, B.R.; Pantinople, E.; Almirante, A. Diversity and distribution of ferns in forest over limestone in Cebu Island Key Biodiversity Areas (KBAs), Philippines.*Biodivers J.* **2020**,*21*. 413-421, https://doi.org/10.13057/biodiv/d210148.
5. Barrogo, K.N.; Delos Santos, M.P.; Montes, A.A.T.; Quiben, A.D.; Rotaquio Jr., E.L.; Valete, E.J.P. Fern and fern allies as non-timber forest product in Baler, Aurora, Philippines. *International Journal of Agricultural Technology* **2021**, *17*, 423-432.
6. Salgado, A.E. Taxonomic and Nomenclatural Notes on Philippine ferns. V. On the identity of *Asplenium*

lepturus, *A. militare*, and *A. nigrescens* (Polypodiales, Aspleniaceae) *Philipp. J. Sci.* **2022**, *151*, 291-301.
7. Ding, Z.T.; Fang, Y.S.; Tai, Z.G.; Yang, M.H.; Xu, Y.Q.; Li, F.; Cao, Q.E. Phenolic content and radical scavenging capacity of 31 species of ferns.*Fitoterapia* **2008**, *79*, 581-583, https://doi.org/10.1016/j.fitote.2008.01.011.
8. Raj, R.L.; Bupesh, G.; Gayatri, V.; Tamizharasi, P. Bioactivity prediction and molecular docking of phytocompounds from Drynaria quercifolia against osteoarthritis receptors. *J App Biol Biotech* **2022**, *10*, 65-69, https://doi.org/10.7324/JABB.2022.100309
9. Ultari, A.; Handayani, D.; Eriadi, A. A Review: Study of Chemical Content, Bioactivity of Mangrove Fern Plants (*Acrostichum aureum* L.). *EAS J Pharm Pharmacol* **2021**, *3*, 7-12, https://doi.org/10.36349/easjpp.2021.v03i01.002.
10. Hovenkamp, P. An account of the malay-pacific species of *Selliguea* (Polypodiaceae). *Blumea Journal of Plant Taxonomy and Plant Geography* **1998**, *43*, 1-108.
11. Barcelona, J.F. The Taxonomy and Ecology of the Pteridophytes of Mt. Iraya and Vicinity, Batan Island, Batanes Province, Northern Philippines. *Pteridology in the New Millennium* **2003,** 299-325,https://doi.org/10.1007/978-94-017-2811-9_22.
12. Qin, H.; Yang, Y.; Dong, S.; He, Q.; Jia, Y.; Zhao, L.; Yu, S.; Liu, H.; Liu, B.; Yan, Y.; Xiang, J.; Xia, N.; Peng, H.; Li, Z.; Zhang, Z.; He, X.; Yin, L.; Lin, Y.; Liu, Q.; Hou, Y.; Liu, Y., Liu, Q.; Cao, W.; Li, J.; Chen, S.; Jin, Z.; Gao, T.; Chen, W.; Ma, H.; Geng, Y.; Jin, X.; Chang, C.; Jiang, H.; Cai, L.; Zang, C.; Wu, J.; Ye, J.; Lai, Y.; Liu, B.; Lin, Q.; Xue, N. Threatened Species List of China's Higher Plants.*Biodivers. Sci.* **2017**, *25*, 696, https://doi.org/10.17520/biods.2017144.
13. Cudal, M.; Calimbo, L.G.L.; Sumibay Jr, D.; Salise, J.; Gomez, J.; Pagaling, M.; Locson, J.; Cahayagan, J.; Seno, J.; Daculio, J.N. Diversity of Pteridophytes in Mount Timolan Protected Landscape, Zamboanga del Sur, Philippines.*J. Trop. Life Sci.* **2021**, *11*, 161-170, https://doi.org/10.11594/jtls.11.02.05
14. Fan, X.P.; Zhang, L.; Zhang, L.B.; Zhang, L. *Selliguea wusugongii* (Polypodiaceae), a new fern species from southeastern Xizang, China based on morphological and molecular evidence. *Phytotaxa* **2021**, *480*, 57-68, https://doi.org/10.11646/phytotaxa.480.1.5.
15. Sayed, H.; Issa, M.; Mahmoud, M.; Abdelkader, E.; Ismail, H. Phytochemical Screening and Antioxidant Activity of some Egyptian Medicinal Plants. *Journal of Modern Research* **2022**, *4*, 14-20. https://doi.org/doi: 10.21608/jmr.2021.83376.1075.
16. Mahdi-Pour, B.; Jothy, S.L.; Latha, L.Y.; Chen, Y.; Sasidharan, S. Antioxidant activity of methanol extracts of different parts of Lantana camara. *Asian Pac. J. Trop. Biomed.* **2012**, *2*, 960-965, https://doi.org/10.1016/S2221-1691(13)60007-6.
17. Hsieh, C.Y.; Tsai, J.R.; Lee, C.J.; Ciou Y.R.; Li, J.S.; Tayo, L.L.; Tsai, P.W. Biological Properties and Phytonutrient Profile of Beans: *Vigna angularis*, *Vigna radiat*a, and *Glycine max*. *Biointerface Res. Appl. Chem.* **2023**, *13*, 53, https://doi.org/10.33263/BRIAC131.053.
18. Pavithra, K.; Sathibabu Uddandrao, V.V.; Chandrasekaran, P.; Brahmanaidu, P.; Sengottuvelu, S.; Vadivukkarasi, S.; Saravanan, G. Phenolic fraction extracted from Kedrostis foetidissima leaves ameliorated isoproterenol-induced cardiotoxicity in rats through restoration of cardiac antioxidant status. *J. Food Biochem.* **2020**, *44*, e13450, https://doi.org/10.1111/jfbc.13450.
19. Lai, H.Y.; Lim, Y.Y.; Kim, K.H. Isolation and Characterisation of a Proanthocyanidin With Antioxidative, Antibacterial and Anti-Cancer Properties from Fern Blechnum orientale. Pharmacogn. Mag. **2017**, *13*, 31-37, https://doi.org/10.4103/0973-1296.197659.
20. Valizadeh, H.; Sonboli, A.; Kordi, F.M.; Dehghan, H.; Bahadori, M.B. Cytotoxicity, antioxidant activity and phenolic content of eight fern species, from north of Iran. *Pharm. Sci.* **2016**, *21*, 18-24, https://doi.org/10.15171/PS.2015.12.
21. Cao, H.; Chai, T.T.; Wang, X.; Morais-Braga, M.F.B.; Yang, J.H.; Wong, F.C.; Wang, R.; Yao, H.; Cao, J.; Cornara, L.; Burlando, B.; Wang, Y.; Xiao, J. Coutinho, H.D.M. Phytochemicals from fern species: potential for medicine applications. *Phytochem Rev.* **2017**, *16*, 379-440, https://doi.org/10.1007/s11101-016-9488-7
22. Ding, Z.T.; Fang, Y.S.; Tai, Z.G.; Yang, M.H.; Xu, Y.Q.; Li, F.; Cao, Q.E. Phenolic content and radical scavenging capacity of 31 species of ferns. *Fitoterapia* **2008**, *79*, 581–583, https://doi.org/10.1016/j.fitote.2008.01.011.
23. Deden Winda Suwandi, D.W.; Rostnawati, T.; Muchtaridi, M.; Subarnas, A. In vitro evaluation of selligueain A effects on the proinflammatory mediators production in RAW264.7 murine macrophages. *J Herbmed Pharmacol*. **2021**, *10*, 313-318, https://doi.org/10.34172/jhp.2021.36.
24. Barbaza, M.Y.U.; De Castro-Cruz K.A.; Hsieh, C.L.; Tsai, P.W. Determination of the Chemical Constituent Contents and Antioxidation Properties of *Asystasia Gangetica*. *Indian J of Pharmaceutical Education and Research* **2021**, *55*, 863-871, https://doi.org/10.5530/ijper.55.3.160.
25. Song, W.; Zhu, X.F.; Ding, X.D.; Yang, H.B.; Qin, S.T.; Chen, H.;Wei, S.D. Structural features, antioxidant and tyrosinase inhibitory activities of proanthocyanidins in leaves of two tea cultivars. *Int. J. Food Prop*. **2017**, *20*, 1348-1358, https://doi.org/10.1080/10942912.2016.1209682.
26. Chen, S.; Hongtao, L. How plants protect themselves from ultraviolet-B radiation stress, *Plant Physiology* **2021**, *187*, 1096-1103, https://doi.org/10.1093/plphys/kiab245.

27. Mavrič Čermelj, A.; Golob, A.; Vogel-Mikuš, K.; Germ, M. Silicon Mitigates Negative Impacts of Drought and UV-B Radiation in Plants. *Plants* **2022**, *11*, 91, https://doi.org/10.3390/plants11010091.
28. Darré, M.; Vicente, A.R.; Cisneros-Zevallos, L.; Artés-Hernández, F. Postharvest Ultraviolet Radiation in Fruit and Vegetables: Applications and Factors Modulating Its Efficacy on Bioactive Compounds and Microbial Growth. *Foods* **2022**, *11*, 653, https://doi.org/10.3390/foods11050653.
29. Baschieri, A.; Amorati, R. Methods to Determine Chain-Breaking Antioxidant Activity of Nanomaterials beyond DPPH•. A Review. *Antioxidants* **2021**, *10*, 1551, https://doi.org/10.3390/antiox10101551.
30. Fu, C.; Wang, H.; Ng, W.L.; Song, L.; Huang, D. Antioxidant activity and proanthocyanidin profile of *selliguea feei* rhizomes. *Molecules* **2013,** *18*, 4282-4292. https://doi.org/10.3390/molecules18044282.
31. Liu, Y.; Wujisguleng, W.; Long, C. Food uses of ferns in China: A review. *Acta Soc.Bot.Pol.* **2012**, *81*, 263-270, https://doi.org/10.5586/asbp.2012.046.
32. Baek, N.; Kennelly, E.; Leonardus, K.; Tsauri, S.; Padmawinata, K.; Soejarto, D.D.; Kinghorn, A.D. Flavonoids and a proanthocyanidin from rhizomes of *Selliguea feei*, Phytochemistry **1994**, *36*, 6–11, https://doi.org/10.1016/S0031-9422(00)97105-X.
33. Dudnik, A., Gaspar, P., Neves, A. R., & Forster, J. Engineering of microbial cell factories for the production of plant polyphenols with health-beneficial properties.*Curr.Pharm.Des.* **2018**, *24*, 2208-2225, https://doi.org/10.2174/1381612824666180515152049.
34. Song, W., Zhu, X. F., Ding, X. D., Yang, H. B., Qin, S. T., Chen, H., & Wei, S. D. Structural features, antioxidant and tyrosinase inhibitory activities of proanthocyanidins in leaves of two tea cultivars. *Int. J. Food Prop*. **2017**, *20*, 1348-1358, https://doi.org/10.1080/10942912.2016.1209682.
35. Galarraga-Vinueza, M.E.; Dohle, E.; Ramanauskaite, A.; Al-Maawi, S.; Obreja, K.; Magini, R.; Schwarz, F. Anti-inflammatory and macrophage polarization effects of Cranberry Proanthocyanidins (PACs) for periodontal and peri-implant disease therapy. *J. Periodontal Res.* **2020**, *55*, 821-829, https://doi.org/10.1111/jre.12773.
36. Unusan, N. Proanthocyanidins in grape seeds: An updated review of their health benefits and potential uses in the food industry.*J. Funct. Foods* **2020**, *67*, 103861, https://doi.org/10.1016/j.jff.2020.103861.
37. Holkem, A.T.; Favaro-Trindade, C.S.; Lacroix, M. Study of anticancer properties of proanthocyanidin-rich cinnamon extract in combination with Bifidobacterium animalis subsp. lactis BLC1 and resistance of these free and co-encapsulated materials under in vitro simulated gastrointestinal conditions.*Food Research International* **2020**, *134*, 109274, https://doi.org/10.1016/j.foodres.2020.109274.
38. Suwandi, D.W.; Rostnawati, T.; Muchtaridi, M.; Subarnas, A. *In vitro* evaluation of selligueain A effects on the proinflammatory mediators production in RAW264. 7 murine macrophages.*J. Herbmed Pharmacol.* **2021**, *10*, 313-318, https://doi.org/10.34172/jhp.2021.36.
39. Kinghorn, A. D., & Kennelly, E. J. Discovery of highly sweet compounds from natural sources. *J. Chem. Educ.***1995**, *72*, 676, https://doi.org/10.1021/ed072p676.
40. Kinghorn, A.D.; Soejarto, D.D. Discovery of terpenoid and phenolic sweeteners from plants.*Pure Appl. Chem.* **2002**, *74*, 1169-1179, https://doi.org/10.1351/pac200274071169.
41. Noguchi, Y.; Takeda, R.; Suzuki, K.; Ohmori, K. Total synthesis of selligueain A, a sweet flavan trimer.*Org. Lett.* **2018**, *20*, 2857-2861, https://doi.org/10.1021/acs.orglett.8b00873.
42. Subarnas, A.; Wagner, H. Analgesic and anti-inflammatory activity of the proanthocyanidin shellegueain A from *Polypodium feei* METT. *Phytomedicine* **2000**, *7*, 401-405, https://doi.org/10.1016/S0944-7113(00)80061-6.
43. Vasänge, M.; Liu, B.; Welch, C.J.; Rolfsen, W.; Bohlin, L. The flavonoid constituents of two Polypodium species (Calaguala) and their effect on the elastase release in human neutrophils.*Planta medica* **1997**, *63*, 511-517, https://doi.org/10.1055/s-2006-957753.
44. Jakimiuk, K., Gesek, J., Atanasov, A. G., & Tomczyk, M. Flavonoids as inhibitors of human neutrophil elastase.*J. Enzyme Inhib. Med. Chem.* **2021,** *36*, 1016-1028, https://doi.org/10.1080/14756366.2021.1927006.