

Photoprotective Capacity of Pheophytin a isolated from Caribbean Sea brown algae

Capacidad fotoprotectora de feofitina a aislada de algas pardas del Mar Caribe

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ABSTRACT

Background: Due to its high energy and deep penetration capacity, solar radiation possesses significant risks to skin health, including sunburn, hyperpigmentation, the inhibition of the skin's immune system, and the development of melanoma and nonmelanoma skin cancers, among others. Photoprotection includes an array of strategies to prevent the harmful effects of solar radiation on the skin and other parts of the body, becoming crucial in preventing skin damage and associated pathologies. Although there are various methods to prevent sunlight's negative effects, there is a serious growing concern about the environmental and health impacts of traditional synthetic UV filters, underscoring the need for new, effective photoprotective products. Due to their photoprotective capacity, natural products from algae have been proposed for skin protection in cosmetic applications. **Objectives:** This work aimed to evaluate the photoprotective capacity of pheophytin a isolated from the brown algae *Dictyota pinnatifida* collected in the Colombian Caribbean Sea. **Methods:** Pheophytin a was isolated from the brown seaweed *Dictyota pinnatifida*. Three *in vitro* spectrophotometric activity assays (SPF: Sun Protection Factor; UVA_r: UVA ratio; and λ_c : critical wavelength) were used to evaluate this compound's photoprotection capacity. **Results:** This chlorophyll derivative is a magnesium-free pigment, with multiple conjugated double bonds featuring a porphyrin ring esterified with a phytol chain. Such structural features make this marine natural product optimal for absorbing solar radiation. *In vitro* photoprotective activity assays revealed promising results for skin photoprotection with SPF of 2.916 ± 0.355 and 1.411 ± 0.138 , UVA_r of 6.815 ± 2.296 and 4.629 ± 1.347 , and λ_c of 394.639 ± 0.493 nm and 394.072 ± 0.912 nm, at concentrations of 30 and 10 ppm, respectively. The last two parameters, together with this compound UV-Vis spectrum (absorption maxima at 409 nm), demonstrate its potential for UVA and visible light skin protection. **Conclusions:** Pheophytin a has the potential for skin-photoprotection-related applications, particularly against UVA radiation.

KEYWORDS: *Dictyota pinnatifida*, photoprotection, Ochrophyta, *in vitro* assay, radiation, UV.

JOURNAL VITAE

School of Pharmaceutical and
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ISSN 0121-4004 | ISSN 2145-2660
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Received: 25 January 2025

Accepted: 19 May 2025

Published: 04 June 2025



RESUMEN

Antecedentes: Considerando la alta energía y capacidad de penetración, la radiación solar puede representar riesgos significativos para la salud de la piel, incluyendo quemaduras, hiperpigmentación, la inhibición del sistema inmunológico de la piel y el desarrollo de cáncer de piel, entre otros. La fotoprotección son todas las estrategias para prevenir los efectos nocivos de la radiación solar sobre la piel y otras partes del cuerpo, siendo clave para prevenir daños cutáneos y patologías asociadas. Aunque hay varios métodos para prevenir los danos nocivos que puede generar la radiación solar, hay una creciente preocupación por el impacto que tiene el uso de filtros sintéticos UV tradicionales en la salud humana y los ecosistemas. Debido a su capacidad fotoprotectora, los productos naturales derivados de las algas han resultado prometedores para la protección de la piel en aplicaciones cosméticas. **Objetivos:** En este trabajo se evaluó la actividad fotoprotectora de la feofitina a aislada a partir del alga parda *Dictyota pinnatifida* colectada en el Caribe Colombiano. **Metodología:** La feofitina a fue aislada a partir del alga parda *Dictyota pinnatifida* y su capacidad fotoprotectora fue evaluada a partir de la determinación *in vitro* de tres parámetros de protección solar (SPF, Factor de protección solar; UVA_r radiación UltraVioleta; and λ_c , Longitud de onda crítica). **Resultados:** La feofitina a es un pigmento derivado de la clorofila a que contiene un anillo porfirínico esterificado con una cadena de fitol. Estas características estructurales hacen que este producto marino natural sea óptimo para absorber la radiación solar. La evaluación *in vitro* de la capacidad fotoprotectora de este compuesto mostró resultados prometedores para la protección de la piel con valores de SPF de 2.916 ± 0.355 y 1.411 ± 0.138 , UVA_r de 6.815 ± 2.296 y 4.629 ± 1.347 , y λ_c de 394.639 ± 0.493 nm y 394.072 ± 0.912 nm, a concentraciones de 30 y 10 ppm, respectivamente. Estos dos últimos parámetros, junto con el espectro UV-Vis del compuesto (máximo de absorción en 409 nm), demostraron su potencial como fotoprotector para la piel frente a la radiación UVA y luz visible. **Conclusiones:** La feofitina a tiene capacidad de protección frente a la radiación UVA, siendo un compuesto prometedor para productos para la fotoprotección de la piel.

PALABRAS CLAVE: *Dictyota pinnatifida*, fotoprotección, Ochrophyta, ensayo *in vitro*, radiación, UV.

1. INTRODUCTION

UV radiation constitutes about 10% of solar radiation reaching the Earth's surface and poses significant risks to the skin due to its high energy and deep penetration (1). Within the UV spectrum, UVC radiation ($\lambda = 100\text{--}290$ nm)(2) is entirely absorbed by the ozone layer, whereas UVB radiation ($\lambda = 290\text{--}320$ nm)(2) accounts for 4–5% of the UV radiation that reaches the Earth's surface. The majority, 95%, is UVA radiation ($\lambda = 320\text{--}400$ nm) (3) and can cause sunburn, DNA damage, and skin cancer (1). The remaining solar radiation includes visible light (50%) and infrared radiation (40%), which, along with artificial sources, contribute to photoaging, inflammation, overheating, hyperpigmentation, collagen degradation, and oxidative damage (1,4).

Photoprotection is crucial in preventing skin damage and associated pathologies, primarily caused by oxidative stress, sunburn, dryness, and other effects of sun exposure (2,3,5). There are two photoprotection mechanisms. The primary mechanism comprises organic or inorganic sun filters (sunscreens) that absorb or reflect radiation (2,5)The secondary mechanism includes molecules that can neutralize reactive oxygen (ROS) and nitrogen (NOS) species when they are produced as a consequence of the transfer of high energy from light to skin biomolecules (2,3).

Although many sunscreens exist, some traditional synthetic organic UV-Vis filters can have adverse effects on both human health and the environment (6).

Photoprotective metabolites such as mycosporine-like amino acids, phenolic compounds, flavonoids, sulfated polysaccharides, and fucoidans have been studied as photoprotective compounds through both primary and secondary mechanisms, implying fewer secondary effects (6–8). The structures of these marine-derived metabolites feature aromatic systems or long-conjugated double bonds with electron-donating or electron-withdrawing groups, making them structurally similar to synthetic filters. This allows them to absorb high-energy radiation, thus protecting the skin from UV radiation (9).

To assess photoprotection, assays are required to cover both the UVA and UVB spectra. While *in vivo* assays may be necessary for commercialization, *in vitro* assays are helpful for research purposes as they reduce time and test requirements, providing a good approach to the expected *in vivo* tests. The parameters used to evaluate photoprotection include: SPF (Sun Protection Factor) for UVB photoprotection, UVA_r (UVA ratio) for protection against UVA radiation, and λ_c (critical wavelength) as a measure of photoprotection capability within the UVA and UVB spectrum. These parameters cover the bioactivity of the compounds thoroughly and can be simultaneously analyzed through *in vitro* tests using a single spectrophotometric measurement (10,11).

The Colombian Caribbean has 15 recognized species from the *Dictyota* genus. Their metabolic production consists mainly of diterpenes, which enables

them to survive in different marine environments (12,13). Despite the potential applications of this algal biomass, such as antifouling, antiviral, and antimicrobial activities (8,14,15), research in the cosmetic industry with species that are part of Colombia's marine biodiversity is still limited. This study presents the chemical characterization of pheophytin a isolated from the Caribbean brown algae *Dictyota pinnatifida* and the *in vitro* evaluation of its photoprotective activity.

2. MATERIALS AND METHODS

2.1 General experimental procedures

HPLC-grade methanol (MeOH) (99.9%), hexane (95%), silica gel 60 (70-230 MESH ASTM), and RP-18 (40-63 μ m) were purchased from Merck Millipore, USA. Dichloromethane (DCM) (99.8%), and ethyl acetate (EtOAc) (99.8%) were purchased from Panreac AppliChem, USA. Ethanol (EtOH) (99.8%) and 2-hydroxy-4-methoxybenzophenone (BP-3) (98%) were purchased from Sigma Aldrich, USA. Ultrapure Type I Milli-Q water (H_2O , membrane 2.2 μ m) was used.

UV-Vis spectra of the crude extracts were recorded in a Shimadzu UV-1800 UV-Vis spectrophotometer using a quartz cell (1 cm). NMR analysis was performed in a 400 MHz Bruker Advance Neo device, using deuterated chloroform ($CDCl_3$) (99.5%) from Merck Millipore, USA.

2.2 Algae Collection

A sample of *Dictyota pinnatifida* was collected in May 2015 by SCUBA diving in Providencia, Colombia. Sample identification was conducted by biologist Freddy Duque using morphological identification keys provided by De Clerk (16) and Litter and Litter (17). A sample has been registered for preservation and comparison under the code 596747 in the collection of the Instituto de Ciencias Naturales, Universidad Nacional de Colombia.

2.3 Extraction

172 g of algae was extracted three times with 300 mL of DCM/MeOH (1:1) for 24 hours each. Solvents were dried under vacuum to obtain crude extracts and then partitioned between DCM/ H_2O . The organic extract was fractionated by column chromatography (Silica Gel 60, 70-230 MESH ASTM, Merck) using a discontinuous gradient of increasing polarity

(hexane, EtOAc, and MeOH), resulting in twelve fractions (F1 to F12). Fraction 5 was separated by column chromatography using RP-18 (40-63 μ m, Merck) and a MeOH/ H_2O gradient from 3:7 to 1:0, resulting in nine subfractions (F5.1 to F5.9). Fraction F5.9 yielded 217 mg of pheophytin a.

2.4 Compound identification

1H - and ^{13}C -NMR experiments were conducted on a Bruker Avance Neo 400MHz NMR instrument, using $CDCl_3$ (99.5%, Merck). Compound identification was achieved through analysis of this information and comparison with previously reported spectra, as depicted in the Supporting Information (18–20).

Pheophytin a: dark-green oil. ^{13}C -NMR (101 MHz, $CDCl_3$): 189.8 (C-9), 173.1 (C-7c), 172.4 (C-18), 169.7 (C-10a), 161.4 (C-17), 155.8 (C-13), 151.1 (C-14), 149.8 (C-16), 145.3 (C-4), 143.0 (C-P3), 142.2 (C-11), 138.0 (C-15), 136.6 (C-12), 136.4 (C-2), 136.3 (C-3), 132.0 (C-1), 129.2 (C-2a), 129.1 (C-5), 129.1 (C-6), 122.9 (C-2b), 117.8 (C-P2), 105.4 (C- γ), 104.5 (C- β), 97.6 (C- α), 93.3 (C- Δ), 64.8 (C-10), 61.6 (C-P1), 53.0 (C-10b), 51.3 (C-7), 50.2 (C-8), 39.9 (C-P4), 39.5 (C-P6), 37.5 (C-P10), 37.4 (C-P8), 37.4 (C-P12), 36.7 (C-P14), 32.9 (C-P7), 32.7 (C-P11), 31.3 (C-7a), 29.8 (C-7b), 28.1 (C-P15), 25.1 (C-P5), 24.9 (C-P9), 24.5 (C-P13), 23.2 (C-8a), 22.8 (C-P16), 22.7 (C-P15a), 19.8 (C-P7a), 19.8 (C-P11a), 19.6 (C-4a), 17.5 (C-4b), 16.4 (C-P3a), 12.2 (C-1a), 12.2 (C-5a), 11.4 (C-3a).

1H -NMR (400 MHz, $CDCl_3$): Δ 9.49 (s, H- β), 9.35 (s, H- α), 8.54 (s, H- Δ), 7.96 (dd, J = 18.0, 11.6 Hz, H-2a), 6.27 (dd, J = 17.8, 1.5 Hz, H-2b trans), 6.25 (s, H-10), 6.16 (dd, J = 11.6, 1.6 Hz, H-2b cis), 5.11 (H-P2), 4.47 (H-P1a), 4.44 (H-8), 4.42 (H-P1b), 4.20 (H-7), 3.86 (s, H-10b), 3.67 (s, H-4a), 3.65 (s, H-5a), 3.38 (s, H-1a), 3.20 (s, H-3a), 2.61 (H-7a), 2.48 (H-7b), 2.33 (H-7a'), 2.18 (H-7b'), 1.86 (H-P4), 1.80 (d, J = 7.3 Hz, H-8a), 1.70 (H-4b), 1.60 (H-P5), 1.55 (H-P3a), 1.52 (H-P15), 1.32 (H-P7), 1.27 (H-P13), 1.24 (H-P8), 1.24 (H-P10), 1.24 (H-P12), 1.14 (H-P14), 1.10 (H-P9), 0.99 (H-P6), 0.87 (H-P7a), 0.87 (H-P15a), 0.84 (H-P11), 0.80 (H-P11a), 0.78 (H-P16).

2.5 *In vitro* photoprotection activity assays

2 mg/mL ethanolic solutions of pheophytin a and benzophenone-3 were prepared. From this, 30 and 10 ppm dilutions were further prepared in ethanol (> 99.8%, Sigma Aldrich). The absorbance for each solution was measured in a quartz cell (1cm) using a Shimadzu UV-1800 UV-Vis spectrophotometer, scanning between 290 and 400 nm in 1nm intervals.

2.5.1 Sun Protection Factor (SPF)

SPF, a measure of UVB photoprotection capacity, was determined using Mansur's spectrophotometric methodology (21) with some modifications as proposed by Nunes and coworkers (22). The spectrophotometric SPF was calculated based on absorbance readings in 5 nm increments, utilizing Mansur's equation (1):

$$\text{SPF}_{\text{Mansur}} = \text{CF} \times \sum_{290}^{320} \text{ABS}(\lambda) \cdot \text{EE}(\lambda) \cdot \text{I}(\lambda) \quad (1)$$

Where CF stands for correction factor = 10, EE (λ) corresponds to the erythral effect spectrum, and I (λ) stands for solar intensity spectrum, in which the EE X I product are constants (Table 1)(23). Abs (λ) corresponds to the recorded absorbance at the studied wavelength.

Table 1. Constants for Mansur SPF determination(21).

Wavelength [nm]	EE X I
290	0,015
295	0,082
300	0,287
305	0,328
310	0,186
315	0,084
320	0,018

2.5.2 UVA ratio (UVA_r)

The UVA ratio, a quantitative measure assessing the balance between total UVA and UVB absorbances for evaluating UVA-photoprotection, was determined following the methodology outlined by Rojas and colleagues (24). Absorbances between 290 to 400 nm at 1 nm intervals were used, employing the following equation (2):

$$\text{UVA}_r = \frac{\int_{320}^{400} \text{Abs}(\lambda) d\lambda}{\int_{290}^{320} \text{Abs}(\lambda) d\lambda} \quad (2)$$

Here Abs (λ) corresponds to the recorded absorbance within the studied UVB (290-320 nm) and UVA range (320-400 nm) at 1 nm interval readings.

2.5.3 Critical wavelength (λ_{crit})

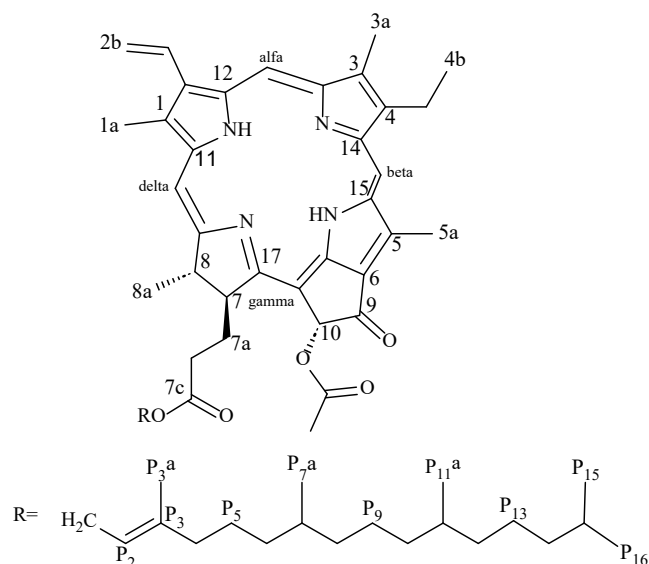
Critical wavelength evaluates the balance between UVA and UVB photoprotection, representing the overall photoprotection capacity within this spectral range. It corresponds to the wavelength at which 90% of the total absorbance curve is reached. Mathematically, it is determined using equation (3):

$$\int_{290}^{\lambda_c} \text{Abs}(\lambda) d\lambda = 0.9 \times \int_{290}^{400} \text{Abs}(\lambda) d\lambda \quad (3)$$

Abs (λ) represents the recorded absorbance between 290 to 400 nm in 1nm increments. Following Rojas and co-workers' procedure(24), the total area under the absorbance curve is designated as 100%, and the critical wavelength is determined by interpolation as the wavelength at which 90% of the curve is reached.

3. RESULTS

F.5.9 (>200 mg) from the *Dictyota pinnatifida* sample was isolated as a dark green oil that was identified as pheophytin a (Figure 1) through ¹H-NMR and ¹³C-NMR analyses, as well as two-dimensional COSY, HMBC, and HSQC experiments and comparisons with literature reports. Details on NMR spectra and full assignments are presented in the **Supplementary Information**.



visible regions (Vis) at 272, 326, 409, 506, and 536 nm (Figure 1a), characterizing this compound as UVA photoprotector. In contrast, benzophenone-3 used

as reference material, has maxima absorbance peaks at 241, 288, and 325 nm, which corresponds to a UVB filter (Figure 1b).

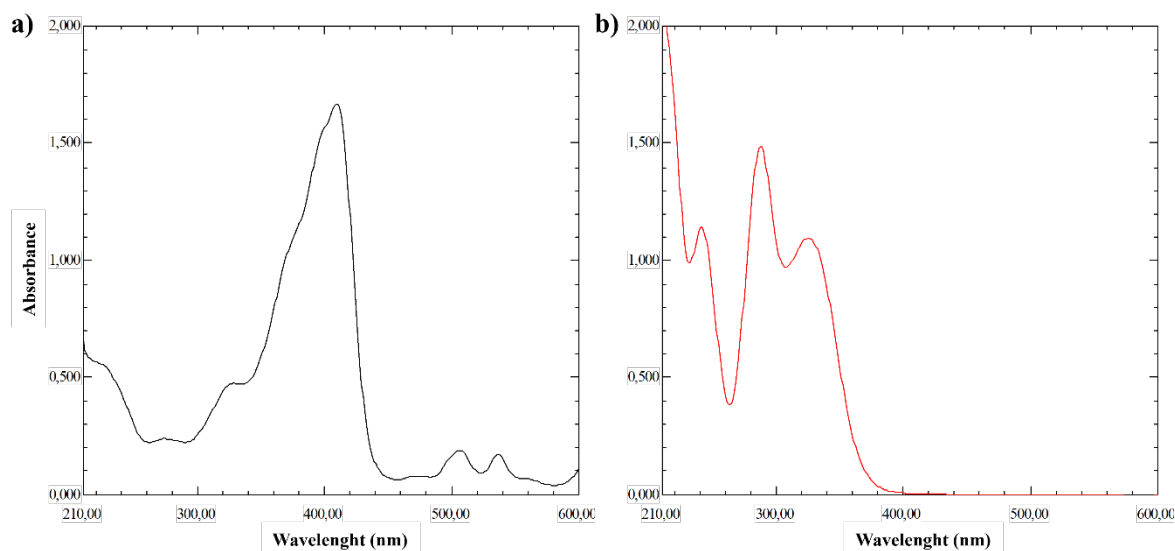


Figure 2. UV-Vis spectra of a) pheophytin a and b) benzophenone-3

SPF assessment for pheophytin a showed concentration-dependent values ranging from 1.4 and 2.9, UVA_r between 4.6 and 6.8, and λ_c of 394 nm for 10 and 30 ppm solutions, respectively (Table 2). As a reference, the same determination was made for BP-3, a recognized UV filter, obtaining SPF values of 4.2 and 10.3, 0.9 and 1.1 for UVA_r, and 353 nm and 349 nm for λ_c , in solutions of 10 and 30 ppm, respectively.

Table 2. Spectrophotometric SPF values obtained for pheophytin a

Parameter (units)	Concentration (ppm)	BP-3	pheophytin a
SPF $\pm$ SD	30	10.310 $\pm$ 0.130	2.916 $\pm$ 0.355
	10	4.208 $\pm$ 1.201	1.411 $\pm$ 0.138
UVA _r $\pm$ SD	30	1.054 $\pm$ 0.011	6.815 $\pm$ 2.296
	10	0.915 $\pm$ 0.029	4.629 $\pm$ 1.347
λ_c $\pm$ SD (nm)	30	349.674 $\pm$ 0.767	394.639 $\pm$ 0.493
	10	353.539 $\pm$ 0.922	394.072 $\pm$ 0.912

BP-3: benzophenone -3 (standard); SPF: sun protection factor; UVA_r: UVA ratio; λ_c : critical wavelength; SD: standard deviation (n=3; mean of three technical replicates).

4. DISCUSSION

Colombia's sea harbors a diverse array of macroalgae; however, their chemical and cosmetic potential remains largely unexplored (25). One of the

few chemical studies of Colombia's macroalgae came from *Dictyota pinnatifida*. The metabolite composition includes prenylated germacrene and xeniane diterpenes. Of those compounds, a few exhibited inhibitory activities against *Pseudomonas aeruginosa* and *Escherichia coli* biofilms (26).

Dictyota pinnatifida study showed a significant amount (>200 mg) of a pigment that was isolated and identified as pheophytin a through 1D and 2D NMR analysis. The structure of this brown algae pigment includes multiple conjugated double bonds that allow it to absorb and stabilize UV and visible radiation. There are reports of similar structures in photosynthetic organisms with photoprotective effects, including different types of chlorophylls, carotenoids, and phycobilins (14). Some of these compounds are fucoxanthin, the main pigment in brown algae, chlorophylls a and b, differently distributed in brown and green algae, and phycobilins, principal pigments in red algae. Therefore, we anticipated that pheophytin a, a chlorophyll derivative, would exhibit mainly protective activity through primary photoprotection mechanism.

The UV-Vis spectra showed absorbance within the 210 and 600 nm range, confirming its potential as a photoprotector (Figure 2). This observation was confirmed through the *in vitro* photoprotective

activity test in which SPF, UVA_r, and λ_c values were determined (Table 2). SPF showed concentration-dependent-values ranging between 1.4 and 2.9 for 10 and 30 ppm solutions, respectively, lower than those determined for BP-3 used as a sunscreen standard. In comparison, UVA_r and λ_c for pheophytin a were 6.815 and 394.6 nm for 30 ppm solutions, respectively, higher than those calculated for BP-3. These results showed that pheophytin a can be used as a sun filter for the UVA region, which could protect skin from sunburn, DNA damage, and skin cancer, among others.

Even though there are no *in vitro* tests to assess visible light protection, the absorbance maxima observed between 400 and 600 nm demonstrate that this pigment could be also used as a photoprotector for this region (infrared and visible light), making it suitable for skin-photoprotection applications preventing issues such as photoaging, inflammation, overheating, hyperpigmentation, collagen degradation, and oxidative damage, among others.

It is noteworthy that BP-3 and pheophytin a exhibit photoprotection in different UV regions; however, results demonstrate that rather than substituting, they can be used as complementary sunscreens in cosmetic formulas, protecting skin in the whole UV spectrum. Also, once pheophytin a preliminary photoprotection activity test has been addressed, further comparisons with dedicated UVA commercial filters, as well as stability, toxicity, and validated standard photoprotection activity tests must be assessed in the future to confirm this compound's suitability for cosmetic and other photoprotection-related applications.

CONCLUSIONS

The algae pigment pheophytin a was isolated from the Colombian Caribbean from *Dictyota pinnatifida*. This magnesium-free compound features a porphyrinic ring esterified with a phytol group, with a multiple conjugated double bond structure that makes it suitable as a photoprotector. *In vitro* evaluation of its photoprotective activity indicates potential for skin-photoprotection-related applications, particularly in UVA, visible, and infrared light. Further assessment through *in vivo* tests will be conducted. This is the first reported evaluation of pheophytin a photoprotective capacity, being relevant for the cosmetic industry that can explore its potential as an alternative or complement to

traditional widespread sunscreens and as a more sustainable photoprotective ingredient.

ACKNOWLEDGMENTS

Ministerio de Ambiente y Desarrollo Sostenible for granted permission to collect and carry out this research (Contrato Marco de Acceso a Recursos Genéticos y sus productos derivados No. 121, otrosí No. 7). Ministerio de Ciencias and "BALCAR-Q: Bioprospección y Química de Algas del Caribe" program (Code: 1101-852-69964. Contract 739-2020) and "Programa Jóvenes investigadores", which provided financial support for this project.

SUPPLEMENTARY INFORMATION

This text contains supplementary information.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

S.P.A and P-A.R. analyzed and interpreted the results. S.P.A designed the photoprotection experiments and prepared data visualizations. S.P.A, P-A.R., F.A.R., and L.C contributed to writing, reviewing, and editing the manuscript. L.C. and F.A.R. acquired funding and supervised the research. All the authors have read and approved the final version of the manuscript.

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IDENTIFICATION OF PHEOPHYTIN A BY 1D AND 2D NMR SPECTROSCOPY

Pheophytin a was obtained as a dark-green oil. The APT experiment (**Supplementary Figure S.1**) revealed 55 carbons comprising 11 methyl, 14 methylenes, 11 methines, and 19 quaternary carbons. The signal at Δ_C 189.8 is characteristic of an α,β -unsaturated ketone. Chemical shifts at Δ_C 136.6, 138.0, 142.2, 149.8, 151.1, 155.8, 161.4, 169.7, 172.4, 173.1 corresponded to sp^2 carbons bonded to heteroatoms in esters, imines, and amines. 14 signals were assigned to sp^2 carbons, including one methylene at Δ_C 122.9, five methines at Δ_C 93.3, 97.6, 104.5, 117.8, and 129.2, and 8 quaternary carbons at Δ_C 105.4, 129.1, 129.1, 132.0, 136.3, 136.4, 143.0, and 145.3. Additionally, ten methyl groups at Δ_C 11.4, 12.2, 12.2, 16.4, 17.5, 19.8, 19.8, 22.7, 22.8, and 23.2, and two oxygenated carbons, one corresponding to a methyl at Δ_C 53.0 and the other to a methylene at Δ_C 61.6 were identified. Another 18 signals corresponded to sp^3 carbons, six corresponding to methines at Δ_C 28.1, 32.9, 32.7, 50.2, 51.3 and 64.8, and 12 corresponding to methylenes at 19.6, 24.5, 24.9, 25.1, 29.8, 31.3, 36.7, 37.4, 37.4, 37.5, 39.5 and 39.9.

1H - NMR experiment (**Supplementary Figure S.2**) showed low-field signals in Δ_H 8.54, 9.35 y 9.49, characteristic of olefinic protons bonded

to nitrogen-heterocyclic systems. Two signals at Δ_H 4.44 and 4.20 indicated methine protons in a heterocycle, while four signals at Δ_H 3.20, 3.38, 3.65 and 3.86 suggested the presence of methyl groups near heteroatoms.

Dereplication of these spectroscopic data with the information reported by Iglesias¹⁴, Islam¹⁵ y Friday¹⁶, reveals that it corresponds to a compound with a porphyrinic ring, resembling chlorophyll derivatives. Shift values of the porphyrinic type nuclei correspond to those reported for pheophytin a, a non-magnesium chelated chlorophyll-a analog. HSQC (**Supplementary Figure S.3**), HMBC (**Supplementary Figure S.4**), and COSY (**Supplementary Figure S.5**) correlations confirm the presence and assignments of the porphyrinic ring. Additionally, HSQC correlation between H_{P_2} (5.12 ppm) and C_{P_2} (118.04 ppm), $H_{P_{1a}}$ (4.40 ppm) and $C_{P_{1a}}$ (61.65 ppm), the HMBC correlation between H_{P_1} (4.50 ppm) and C_{P_2} (117.83 ppm), and the COSY correlation between $H_{P_{1a}}$ (4.48 ppm) and H_{P_2} (5.10 ppm), confirm that this porphyrin-type ring is substituted with a phytol group ($C_{20}H_{40}O$), confirming the complete molecule identity. Complete assignments for pheophytin a are fully describe in **Supplementary Table S.1**.

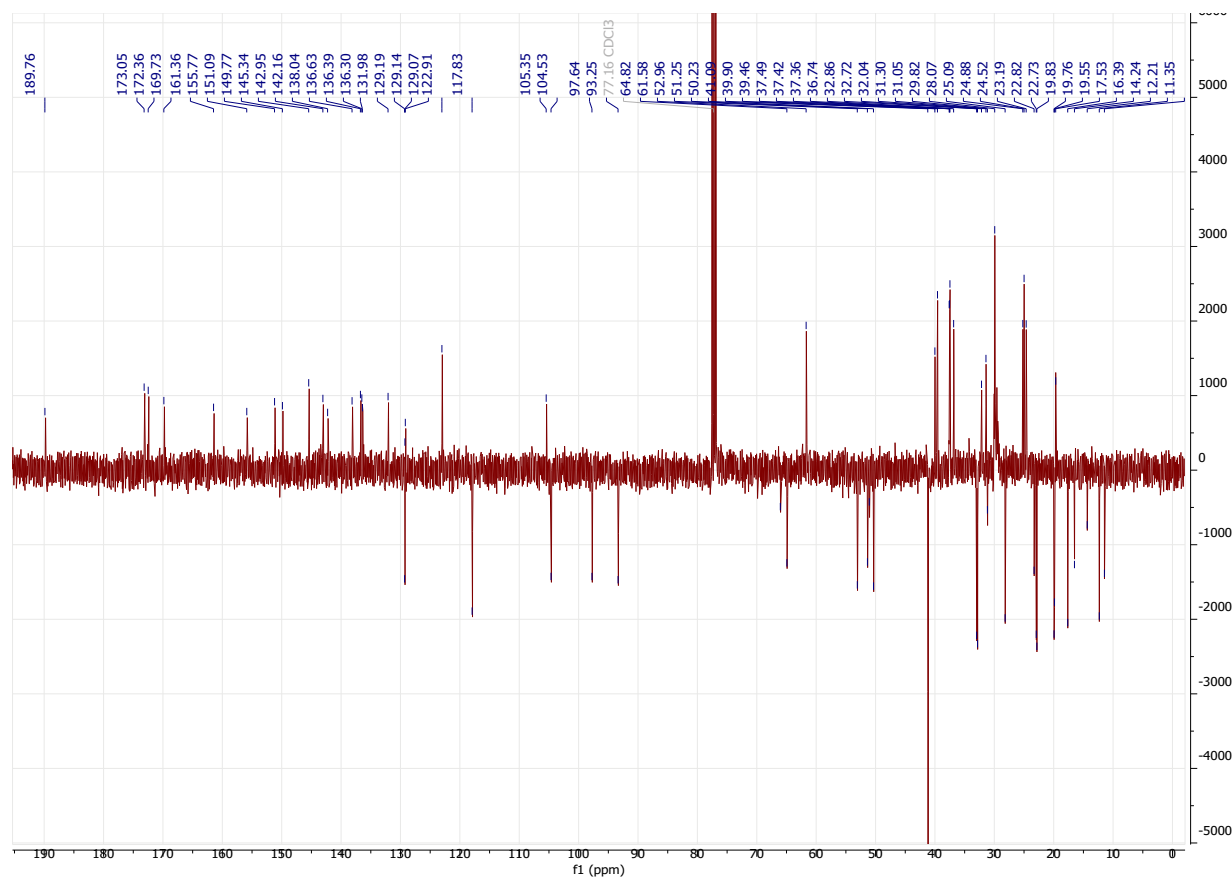
Supplementary Table S. 1. NMR data for pheophytin a identified in fraction F5.9 and literature reports.

Position	1H -NMR (δH (ppm), m, (J (Hz)))		^{13}C -NMR (δC (ppm))	
	Iglesias, 2019 (CDCl ₃ , 600 MHz); Friday, 2021a (CDCl ₃ , 500 MHz)	F5.9 (CDCl ₃ , 400 MHz)	Islam, 2013 (CDCl ₃ , 100 MHz)	F5.9 (CDCl ₃ , 100 MHz)
α	9,4 s	9,4 s	97,3	97,6
β	9,5 s	9,5 s	104,2	104,5
γ	-	-	105,2	105,4
δ	8,6 s	8,5 s	93,0	93,3
1	-	-	131,8	132,0
2	-	-	136,0	136,4
3	-	-	135,9	136,3
4	-	-	145,0	145,3
5	-	-	128,9	129,1
6	-	-	128,8	129,1
7	4,2	4,2	51,1	51,3
8	4,5	4,4	50,1	50,2
9	-	-	189,6	189,8
10	6,3 s	6,3 s	64,7	64,8
11	-	-	141,9	142,2

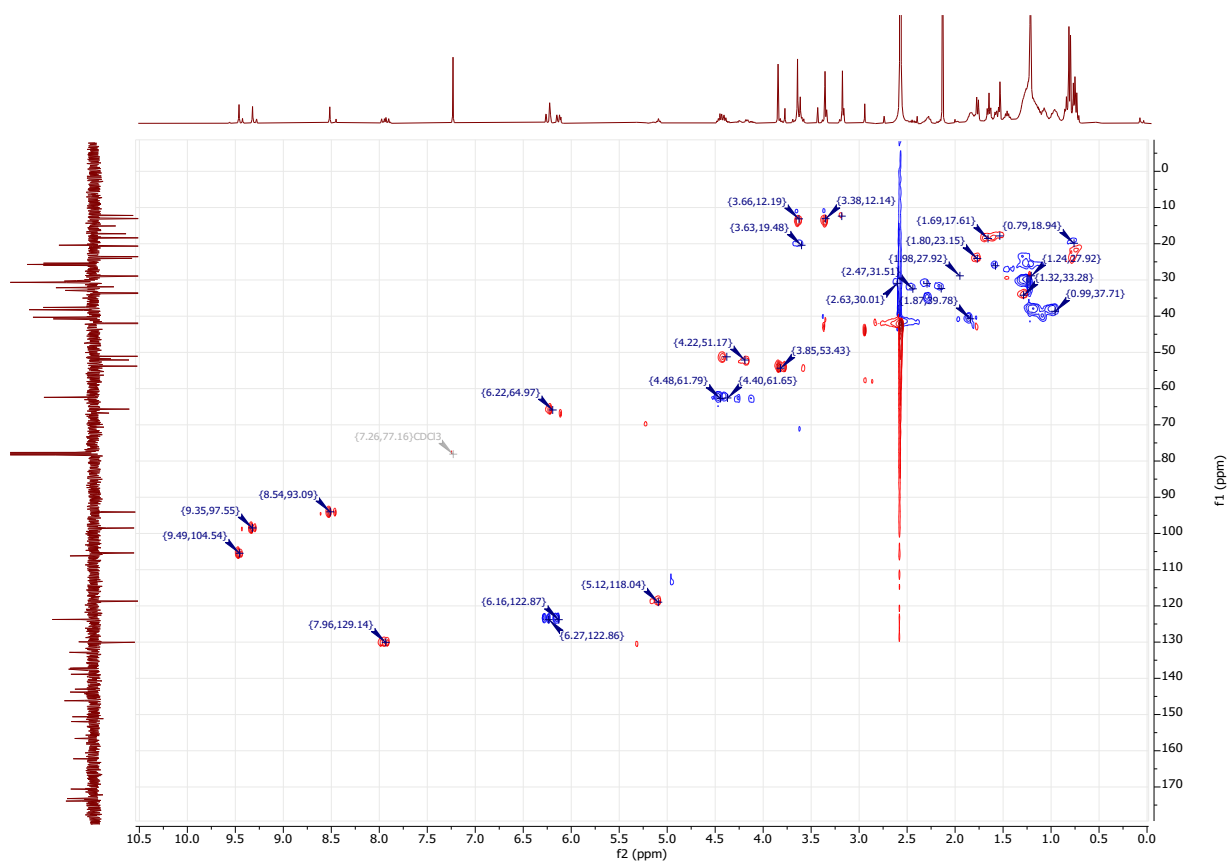
Position	¹ H-NMR (δH (ppm), m, (J (Hz)))		¹³ C-NMR (δC (ppm))	
	Iglesias, 2019 (CDCl ₃ , 600 MHz); Friday, 2021a (CDCl ₃ , 500 MHz)	F5.9 (CDCl ₃ , 400 MHz)	Islam, 2013 (CDCl ₃ , 100 MHz)	F5.9 (CDCl ₃ , 100 MHz)
12	-	-	136,3	136,6
13	-	-	155,4	155,8
14	-	-	150,8	151,1
15	-	-	137,8	138,0
16	-	-	149,6	149,8
17	-	-	161,2	161,4
18	-	-	172,1	172,4
1a	3,4 s	3,4 s	12,0	12,2
2a	8,0 dd (J= 17.8; 11.5 Hz)	8,0 dd (J=11.6, 18)	128,8	129,2
2b cis	6,2 dd (J= 11.6; 1.2 Hz)	6,2 dd (J= 11.6, 1.6)	122,6	122,9
2b trans	6,3 dd (J=17.8; 1.2 Hz)	6,3 dd (J= 17.8, 1.5)		
3a	3,3 s	3,2 s	11,0	11,4
4a	3,7 s	3,7 s	19,2	19,6
4b	1,7	1,7	17,3	17,5
5a	3,7 s	3,7 s	12,0	12,2
7a	2,6	2,6	31,2	31,3
7a'	2,3	2,3		
7b	2,5	2,5	29,8	29,8
7b'	2,2	2,2		
7c	-	-	172,9	173,1
8a	1,8 d (J=7.4 Hz)	1,8 (J=7.3)	23,1	23,2
10a	-	-	172,9	169,7
10b	3,9 s	3,9 s	52,8	53,0
p1a	4,6	4,5	61,5	61,6
p1b	4,5	4,4		
p2	5,1	5,1	117,7	117,8
p3	-	-	142,8	143,0
p3a	1,6	1,6	16,3	16,4
p4	1,9	1,9	39,8	39,9
p5	1,6	1,6	24,9	25,1
p6	1,0	1,0	39,3	39,5
p7	1,3	1,3	32,7	32,9
p7a	0,9	0,9		
P8	1,2	1,2	37,3	37,4
p9	1,1 a	1,1	24,7	24,9
p10	1,2 a	1,2	37,3	37,5
p11	0,9	0,8	32,6	32,7
p11a	0,8 a	0,8		
p12	1,2 a	1,2	37,2	37,4
p13	1,3 a	1,3	24,4	24,5
p14	1,1 a	1,1	36,6	36,7
p15	1,5 a	1,5	27,9	28,1
p15a	0,9 a	0,9	22,6	22,7
p16	0,8	0,8	22,7	22,8



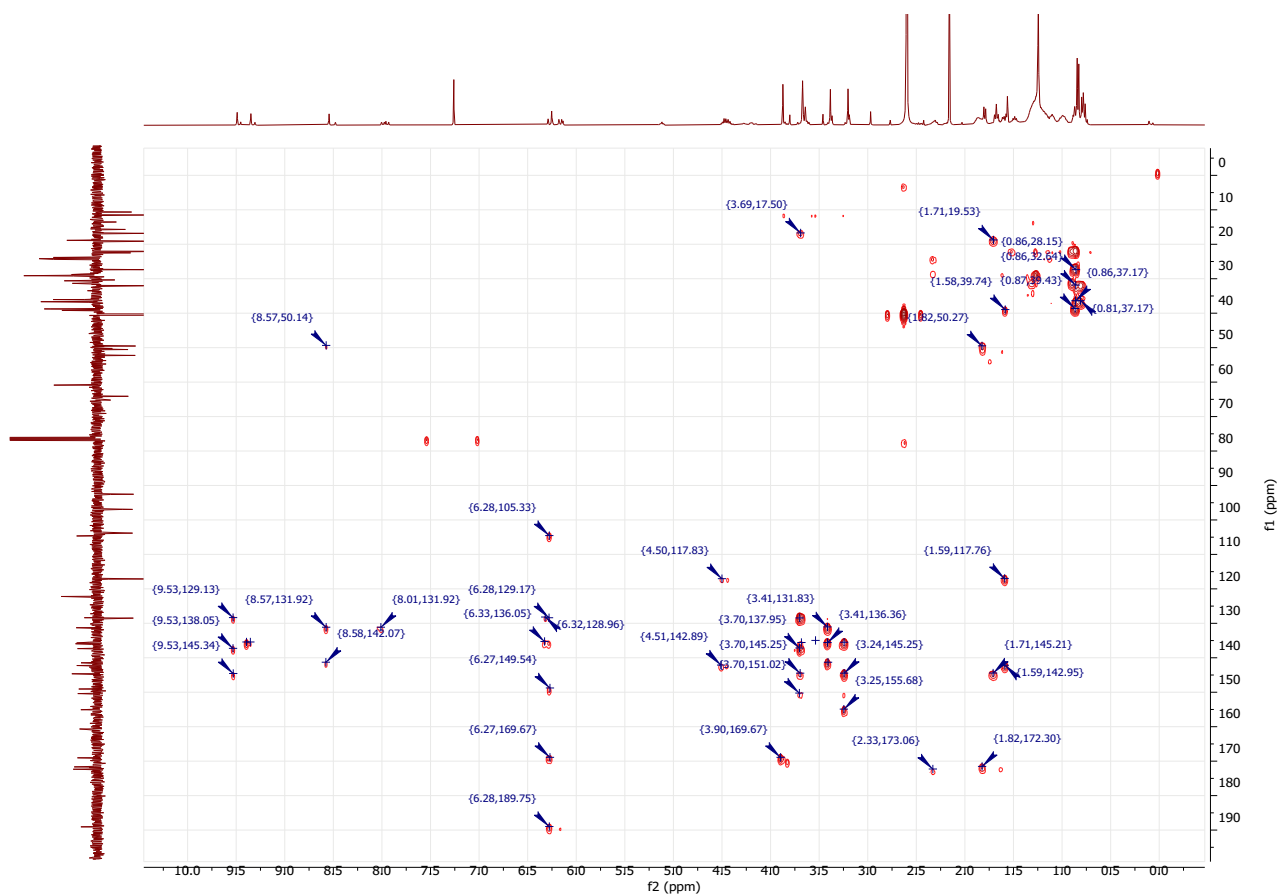
Supplementary Figure S. 1. ^1H -NMR spectra (CDCl_3 ; 400MHz) of pheophytin a.



Supplementary Figure S. 2. APT spectra (CDCl_3 ; 400MHz) of pheophytin a.



Supplementary Figure S. 3. HSQC (CDCl₃; 400MHz) of pheophytin a.



Supplementary Figure S. 4. HMBC (CDCl₃; 400MHz) of pheophytin a.



Supplementary Figure S. 5. COSY (CDCl₃; 400MHz) of pheophytin a.