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Nutritional Value, Major Chemical Compounds, and Biological Activities of *Petromarula pinnata* (Campanulaceae)—A Unique Nutraceutical Wild Edible Green of Crete (Greece)

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Citation: Dimitriadis, K.M.; Karavergou, S.; Tsiftoglou, O.S.; Karapatzak, E.; Paschalidis, K.; Hadjipavlou-Litina, D.; Charalambous, D.; Krigas, N.; Lazari, D. Nutritional Value, Major Chemical Compounds, and Biological Activities of *Petromarula pinnata* (Campanulaceae)—A Unique Nutraceutical Wild Edible Green of Crete (Greece). *Horticulturae* **2024**, *10*, 689. <https://doi.org/10.3390/horticulturae10070689>

Academic Editor: Antonio Ferrante

Received: 2 May 2024

Revised: 13 June 2024

Accepted: 21 June 2024

Published: 28 June 2024



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Abstract: The Mediterranean diet is mostly based on high intakes of olive oil, vegetables, legumes, and fruits, with limited amounts of red meat and sweets, and is related to lower risk of cardiovascular diseases, mainly due to the health benefits of antioxidants of wild greens, fruits, and vegetables. *Petromarula pinnata* (L.) A. DC. is a unique (monotypic) and threatened local endemic wild edible green of Crete which is consumed raw in salads or cooked as an ingredient of the Mediterranean (Cretan) diet. In this study, we aimed to examine the nutritional value of *P. pinnata* with reference to wild-growing material; moreover, we investigated its chemical composition with ¹H NMR spectra and its in vitro total phenolics and flavonoids (TPC, TF assays), and we evaluated the antioxidant (TAC, DPPH, and inhibition of lipid peroxidation), antimicrobial (MIC), and anti-inflammatory (inhibition of soybean lipoxygenase) in vitro activity during two different developmental stages (winter and summer collections), all referring to ex situ cultivated material (air-dried or frozen in liquid nitrogen). Our results showed that *P. pinnata* has good nutritional value, being rich in terpenoids and poor in phenolic compounds and flavonoids. Furthermore, the extracts showed high antioxidant activity for TAC and DPPH and some of the extracts had higher antioxidant activities than the standard compounds. The summer plant materials had higher antioxidant activity than the winter ones. The methanol/water extracts were the strongest inhibitors of the lipid peroxidation, and the methanol extracts were not found to be active. None of the extracts inhibited the soybean lipoxygenase, and thus they were devoid of anti-inflammatory activity. Finally, the extracts showed a moderate to strong bacterial inhibition. These findings consolidate that *P. pinnata* has a novel nutraceutical interest with high nutritional value and high antioxidant activity combined with bactericidal effect, thus updating the evaluation of its exploitation potential in the medicinal sector from below average (37%) to high (67%).

Keywords: Greece; Mediterranean diet; antioxidant; anti-inflammatory; conservation; medicinal; agro-alimentary

1. Introduction

In modern times, people tend to eat less healthy and/or nutritious foods and prefer to consume ready-made fast food, processed food, and sweets more often, which results in

higher body weights and concomitant diseases. At the same time, such trends highlight the need to follow healthier diets locally, regionally, and globally [1]. Consolidated since the 1960s, the Mediterranean diet (MD) reflects the original traditional dietary habits of the countries of the Mediterranean Basin such as Greece, Italy, and Spain; the MD is based on high intakes of olive oil, vegetables, greens, fruits, and legumes combined with low intakes of sweets and red meat. The health advantages of the MD are due to the antioxidant vitamins, phytochemicals, or micronutrients contained in vegetables, fruits, and greens [1–3]. These compounds can interact with the free radicals or other oxidants, preventing oxidative stress and helping against several diseases such as cancer, heart issues, and cardiovascular diseases [2–4]. In Greece and especially in Crete, people follow the MD diet closely, consuming daily fruits, vegetables, and greens that are cultivated or are wild-growing, being native and/or confined to Greece; among the latter, some belong to the family Campanulaceae and are traditionally sourced directly from the wild. In Greece alone, 95 taxa (species and subspecies) in eight genera of Campanuloideae (Campanulaceae) can be found, i.e., *Campanula* spp., *Asyneuma* spp., *Edraianthus* spp., *Halacsyella* spp., *Jasione* spp., *Legousia* spp., *Phyteuma* spp., and the monotypic *Petromarula pinnata* (L.) A.DC., which is a threatened local endemic plant of Crete [5] traditionally used as wild edible green.

Many plants of the Campanulaceae family are known as culinary herbs not only in the Mediterranean area but also in other places across the world. Some of the most widely used Campanulaceae edible plants are *Codonopsis lanceolata* (Siebold & Zucc.) Benth. & Hook.f. ex Trautv. (roots and leaves) [6], *Canarina canariensis* (L.) Vatke (ripe fruits) [7], *Platycodon grandiflorus* (Jacq.) A.DC. (roots) [8,9], *Phyteuma spicatum* L., *P. michielii* All., *P. orbiculare* L., *P. ovatum* Honck., *Campanula rapunculus* L., *C. trachelium* L., and *Legousia speculum-veneris* (L.) Chaix. (leaves) [10]. In Greece and especially in Crete, people have consumed many plant-based foods daily since ancient times and they still source wild-growing edible greens from the natural environment. Due to the high richness in wild-growing native and endemic species on the island of Crete, there is a plethora of species that are known to be edible, and thus domestic people taste them regularly and use them yearly in continuation of ancient traditions. Among those of the Campanulaceae family found in the Cretan area (21 species of four genera), the threatened local Cretan endemics *Campanula pelviformis* Lam. and *Petromarula pinnata* (L.) A.DC. are included among the frequently used edible greens [11–15].

Species-wise, the investigation herein is focused on *P. pinnata*, which is a local endemic plant of Crete representing a relict floristic element of this distinct genus (monotypic), which can be naturally found in calcareous cliffs, rocks, and rocky places in phrygana or on old walls and fortresses from lowlands occasionally up to 1200 m [11,15]. The Latin name *Petromarula* comes from the Greek words ‘petra’, which means rock, and ‘maruli’, which means lettuce, thus rendering it as rock-lettuce in English. In Crete, it is commonly known as ‘petrofiliá’ (meaning rock-dwelling or petrophilous) and ‘petromarulida’ (meaning rock-lettuce). Despite being widely scattered across Crete [5], *P. pinnata* is considered threatened (Vulnerable) [15] and represents an edible wild plant species directly sourced from the wild to be consumed raw or boiled in salads or stirred in olive oil (‘tsigarolachana’), a culinary preparation to fill handmade dough pot pies or to accompany fish, seafood, or meat [12–14]. Despite its high ornamental value and strong agro-alimentary interest [13], *P. pinnata* has not been extensively studied to date in phytochemical terms, thus still being a neglected and underutilized plant species. Some scattered studies examined to date its phylogenetics [11], the seed germination and/or ex situ propagation [15,16], while some others focused on screening the antioxidant capacity and the total phenolic content of either the wild plant material itself [17] or as basic ingredient in admixture with wild-growing edible greens and cultivated vegetables used for traditional ‘kaltsounia’, i.e., a small halfmoon-shaped pastry filled with edible greens (wild-growing and cultivated greens) along with a mixture of local cheeses (‘tiromálama’ or ‘maláka’ and pungent ‘mizíthra’) [14]. It is noteworthy that only a couple of these traditional healthy snacks (ca. 100 g of food) may cover almost 40% of the estimated daily intake of strong antioxidants (flavonols and flavones) [14].

Therefore, context-wise, the current study aimed to consolidate the nutraceutical interest of *P. pinnata* by evaluating the nutritional value of the wild material and by examining the chemical composition of *P. pinnata*. We further aimed to assess in vitro experiments the antioxidant, antimicrobial, and anti-inflammatory activity of the ex situ cultivated plant material of *P. pinnata* harvested in two developmental stages (collections of plant material in December and June).

2. Materials and Methods

2.1. Plant Material

Using a special collection permit issued by the Greek competent authorities, the original seed collection of *P. pinnata* was performed on 29 August 2019 in Spilia (N35.286489 and E25.167267), next to Agia Eirini gorge, Heraklion, Crete, Greece. After taxonomic identification by Dr. N. Krigas, the original collection obtained the IPEN (International Plant Exchange Network) accession number GR-1-BBGK-19,125. From the same wild-growing population of *P. pinnata*, non-flowering plant material was collected in early June 2019 for nutritional value analysis (Figure 1).



Figure 1. Wild-growing *Petromarula pinnata* on rocky substrates (left) and ex situ cultivated individual (right).

The collected seeds were germinated in vivo after a couple of months and plant seedlings were cultivated ex situ for two consecutive years [15] at the premises of the Institute of Plant Breeding and Phytogenetic Resources, Hellenic Agriculture Organization Demeter (Thessaloniki, Greece). The aerial parts of ex situ cultivated hemicryptophyte *P. pinnata* GR-BBGK-1-19,125 were collected from the ex situ cultivation in December 2019 and June 2020. Only non-flowering 1- or 1.5-year-old plants were harvested, since the major edible part of *P. pinnata* consists of young leaves (rosettes of leaves). For the experiments we used frozen plant material in liquid nitrogen and air-dried plant material from both collections. The code name PSK was given to the examined plant material. PSK1 was harvested in December 2019 and was ground with liquid nitrogen, PSK2 was harvested in December 2019 and was air-dried (both from 1-year-old plant individuals), PSK3 was

harvested in June 2020 and was ground with liquid nitrogen, and PSK4 was harvested in June 2020 and was air-dried (both from 1.5-year-old plant individuals).

2.2. Extractions

All the plant materials were extracted successively with hexane (A), Dichloromethane (B), methanol (C) and methanol:water 70:30 (D). The extractions with each solvent were performed three times at room temperature for 48 h each time. The solvent-to-sample ratio was 2:1.

2.3. Nuclear Magnetic Resonance (NMR)

The ^1H NMR spectra were recorded on an AGILENT DD2 500 spectrometer (500.1 MHz). CD_3OD and CDCl_3 were used as solvents. Chemical shifts are reported in δ (ppm) values relative to TMS (3.31 ppm for CD_3OD and 7.26 ppm for CDCl_3).

2.4. Nutritional Value

The nutritional value of the aerial parts of wild-growing *P. pinnata* was measured by NIR methodology, namely Perten DA 7250 NIR Analyzer (Perten–PerkinElmer, New York, NY, USA), which is designed specifically for analysis in the food and agriculture industries.

The carbohydrate percentage and the nutritive value were calculated using the following equations [18]:

$$\text{Carbohydrate percentage (\%)} = 100 - (\text{ash\%} + \text{moisture\%} + \text{fat\%} + \text{protein\%})$$

$$\text{Nutritive value} = 4 \times \text{protein percentage (\%)} + 9 \times \text{fat percentage (\%)} + 4 \times \text{carbohydrate percentage (\%)}$$

2.5. Total Phenolic Content (TPC)

The TPC was measured by the Folin-Ciocalteu method as described by Papagrigoriou et al. [19]. The extracts were dissolved in Dimethyl Sulfoxide (DMSO) at a concentration of 20 mg/mL. Then, 20 μL of the samples was mixed with 2.5 mL of deionized water and 400 μL of Folin-Ciocalteu reagent (F9252, Sigma-Aldrich, Darmstadt, Germany) and was left in the dark for 8 min at room temperature. Afterwards, 0.500 mL of Na_2CO_3 7% (*w/v*) was added, and the mixture was incubated in the dark for 30 min at 40 °C. For the blank, we used 20 μL of DMSO instead of sample. The absorbance was recorded at 750 nm using a Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). The TPC was measured using a gallic acid standard curve ($\text{absorbance} = 0.00226x + 0.03727$, $R^2 = 0.94694$) and the results were expressed in milligrams of gallic acid equivalents per 100 g of extract.

2.6. Total Flavonoid (TF) Assay

The TF was carried out with the AlCl_3 method as described in previous studies [20]. The extracts were dissolved in deionized water at a concentration of 10 mg/mL. In brief, 0.5 mL of the samples or water for the blank, 2 mL of distilled water, and 0.15 mL of 5% NaNO_2 (*w/v*) were added in a test tube and were incubated at room temperature for 6 min. To the mixture, 0.15 mL of 10% AlCl_3 (*w/v*) was added and was incubated at room temperature for 6 min. Then, 2 mL of 4% NaOH (*w/v*) and 0.2 mL of deionized water were added to the reaction mixture and incubated for another 15 min at room temperature. The absorbance was recorded at 510 nm using a Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). The TF was measured using a rutin (Sigma-Aldrich, Darmstadt, Germany) standard curve ($y = 0.9736 \times \text{Absorbance} + 0.0013$, $R^2 = 0.9991$) and the results were expressed in milligrams of rutin equivalents per 100 g of extract.

2.7. Total Antioxidant Capacity (TAC)

The TAC was measured with the phosphomolybdate method as described by Jan et al. [21]. The extracts were dissolved in deionized water at a concentration of 10 mg/mL. In a testing tube, 20 μL of the samples or water for the blank and 1 mL of the reagent mixture (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were added. The mixtures were incubated at 95 °C for 90 min. The reduction of the Mo

(VI) to Mo(V) and a formation of a green phosphate/Mo(V) complex were measured at 765 nm using a Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). Ascorbic acid was used as a standard (1 mg/mL).

2.8. DPPH Scavenging Assay

The DPPH (1,1-diphenyl-2-picryl-hydrazyl) (Sigma-Aldrich, Darmstadt, Germany) scavenging assay was carried out as described by Tsiftoglou et al. [22]. Briefly, a solution of 0.1 mM of DPPH in methanol was prepared and the test samples, dissolved in DMSO, were added (100 µM). After 20 min, the optical density was recorded at 517 nm using a Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). NGDA (nordihydroguaiaretic acid) (Sigma-Aldrich, Darmstadt, Germany) was used as a reference. The Percentage (%) Reduction was calculated using the following formula:

$$\text{Percentage (\%) Reduction} = [(OD_{\text{control}} - OD_{\text{sample}}) / OD_{\text{control}}] \times 100\%$$

2.9. Inhibition of Linoleic Acid Lipid Peroxidation

The inhibition of linoleic acid lipid peroxidation assay was carried out as described by Hodaj-Çeliku et al. [23]. The test samples were dissolved in DMSO (20 mg/mL). 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) (Sigma-Aldrich, Darmstadt, Germany) was used as a free radical initiator. The ability of the tested samples to prevent the oxidation of linoleic acid in an aqueous dispersion and the production of conjugated diene hydroperoxide were recorded at 234 nm using a Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). Trolox (Sigma-Aldrich, Darmstadt, Germany) was used as a reference compound.

2.10. Inhibition of Soybean Lipoxygenase (LOX)

The inhibition of soybean lipoxygenase assay was performed as described by Peperidou et al. [24]. The test samples were dissolved in DMSO (20 mg/mL). The samples were incubated with sodium linoleate (0.1 mM), 0.2 mL of soybean lipoxygenase (Sigma-Aldrich, Darmstadt, Germany) solution ($1/9 \times 10^{-9}$ w/v in saline) in a Tris buffer of pH 9. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm using a Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). Nordihydroguaiaretic acid (NDGA) (Sigma-Aldrich, Darmstadt, Germany) was used as a reference compound.

2.11. Antimicrobial Activity

The antimicrobial activity was performed as described by Charalambous et al. [25], using the microbroth dilution method. The test samples were dissolved in DMSO (10 mg/mL) and diluted further with Tryptic Soy Broth (TSB) (Sigma-Aldrich, Darmstadt, Germany). Bacterial cultures at a concentration of 1×10^6 cfu/mL of *Escherichia coli* (NCTC9001), *Staphylococcus aureus* (NCTC 6571), *Enterococcus faecalis* (NCTC775), and *Salmonella enteritidis* (WDCM00030) were incubated with the test sample for 18 h at 37 °C. Ampicillin (0.526 mg/mL) (Sigma-Aldrich, Darmstadt, Germany) and Gentamycin (0.064 mg/mL) (Sigma-Aldrich, Darmstadt, Germany) were used as positive controls. The minimum inhibitory concentration (MIC) of each sample was detected after the addition of 30 µL (0.2 µg/mL) of p-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, Darmstadt, Germany).

2.12. Statistical Analysis

IBM SPSS Statistics for Windows, v. 29.0.01 (IBM Corp., Armonk, NY, USA) was used for the Duncan and three-way analysis of variance (three-way ANOVA) of the data for all in vitro tests to determine significant differences ($p < 0.05$). Three-way ANOVA was used to determine if there is an interaction between the extract solvent (hexane, dichloromethane, methanol, or methanol:water 70:30), the processing method (ground with liquid nitrogen or air-dried) and the time period of the collection (winter or summer) for each in vitro experiment.

3. Results

3.1. Nutritional Value of Wild-Growing *Petromarula pinnata*

The nutritional value of wild-growing *P. pinnata* GR-1-BBGK-19,125 is shown in Table 1. According to the standards of the European Commission, *P. pinnata* is low in fat ($2.98 \pm 0.01\%$), is a source of protein ($13.27 \pm 0.03\%$), carbohydrates ($64.54 \pm 0.12\%$), and fiber ($4.14 \pm 0.04\%$), and contains $0.4 \pm 0.00\%$ calcium and $0.32 \pm 0.00\%$ phosphorus. All the measurements were within the guidelines for the daily requirements for human consumption. The high ash content ($9.02\% \pm 0.04\%$) indicated that the plant material is easily digestible.

Table 1. Nutritional value of the aerial parts of wild-growing *Petromarula pinnata*.

Parameter	Results
Neutral detergent fiber (NDF As)	$40.04 \pm 0.11\%$
Acid detergent fiber (ADF As)	$21.44 \pm 0.06\%$
Moisture content	$10.19 \pm 0.08\%$
Ash content	$9.02 \pm 0.04\%$
Crude fiber	$4.14 \pm 0.04\%$
Protein content	$13.27 \pm 0.03\%$
Fat content	$2.98 \pm 0.01\%$
Carbohydrate content	$64.54 \pm 0.12\%$
Calcium	$0.40 \pm 0.00\%$
Phosphorus	$0.32 \pm 0.00\%$
Nutritive value	338.08 ± 0.55 cal/100 g plant material

3.2. Extraction Yields of Ex Situ Cultivated *Petromarula pinnata* Harvested in December and June

As seen in Table 2, the extract yields of the ex situ raised *P. pinnata* GR-1-BBGK-19,125 plants that were air-dried and then ground were higher, almost ten times higher, than the extract yields of the plants that were frozen in liquid nitrogen and then ground with a mortar and pestle. This was expected because the frozen plant material contained a lot of moisture, unlike the air-dried plant material. The methanol extracts (C) had the highest yield, followed by the methanol: water 70:30 (D) extracts. The hexane extracts (A) had the lowest yields. The plants harvested in December had slightly higher yields in all solvents compared to the plants harvested in June that were processed with the same method.

Table 2. Extraction yields of all the extracts of ex situ cultivated *Petromarula pinnata* per 100 g of plant material (mean $\pm$ SD; $n = 3$) (PSK1-PSK2: harvested in December 2019; PSK3-PSK4: harvested in June 2020).

Processing Method	Code Name	Extraction Solvent	Weight of Extracts	Extraction Yield
Ground with liquid nitrogen	PSK1-A	Hexane	0.413 ± 0.09 g	$0.35 \pm 0.07\%$ ^g
	PSK1-B	Dichloromethane	0.973 ± 0.12 g	$0.82 \pm 0.10\%$ ^{f,g}
	PSK1-C	Methanol	4.053 ± 0.25 g	$3.52 \pm 0.21\%$ ^d
	PSK1-D	Methanol:H ₂ O 70:30	3.245 ± 0.34 g	$2.73 \pm 0.28\%$ ^{d,e,f}
Air-dried and then ground	PSK2-A	Hexane	3.799 ± 0.20 g	$2.45 \pm 0.13\%$ ^{d,e,f}
	PSK2-B	Dichloromethane	5.595 ± 0.29 g	$3.61 \pm 0.18\%$ ^d
	PSK2-C	Methanol	45.923 ± 2.95 g	$29.61 \pm 1.90\%$ ^a
	PSK2-D	Methanol:H ₂ O 70:30	35.825 ± 2.50 g	$23.10 \pm 1.61\%$ ^b
Ground with liquid nitrogen	PSK3-A	Hexane	0.332 ± 0.07 g	$0.30 \pm 0.06\%$ ^g
	PSK3-B	Dichloromethane	0.789 ± 0.11 g	$0.71 \pm 0.10\%$ ^{f,g}
	PSK3-C	Methanol	3.273 ± 0.26 g	$2.92 \pm 0.23\%$ ^{d,e}
	PSK3-D	Methanol:H ₂ O 70:30	1.174 ± 0.17 g	$1.05 \pm 0.15\%$ ^{e,f,g}

Table 2. Cont.

Processing Method	Code Name	Extraction Solvent	Weight of Extracts	Extraction Yield
Air-dried and then ground	PSK4-A	Hexane	1.303 ± 0.12 g	2.98 ± 0.27% ^{d,e}
	PSK4-B	Dichloromethane	1.153 ± 0.04 g	2.63 ± 0.10% ^{d,e,f}
	PSK4-C	Methanol	10.876 ± 0.92 g	24.83 ± 2.09% ^b
	PSK4-D	Methanol:H ₂ O 70:30	6.314 ± 0.68 g	14.41 ± 1.55% ^c

^{a–g} values with the same letter are not significantly different ($p < 0.05$).

3.3. ¹H NMR of the Extracts

The ¹H NMR spectra of the extracts of ex situ cultivated *P. pinnata* GR-1-BBGK-19,125 (Figure 2) did not show significant differences between the two different processing methods (air-dried/frozen in liquid nitrogen) or the two harvesting periods. The hexane extracts had peaks around $\delta = 0.5$ –2.5 and 4.0–5.0, which indicated the presence of fatty acids and terpenoids. The dichloromethane extracts had peaks around $\delta = 0.5$ –2.5 and 3.0–5.0, which indicated the presence of terpenoids. The peaks at $\delta = 9.5$ indicated the presence of aldehyde groups. The methanol extracts had terpene peaks ($\delta = 0.5$ –2.0) and sugar peaks ($\delta = 3.0$ –5.0). Also, there were low peaks at $\delta = 6.5$ –7.5, which indicated the presence of phenolic compounds in low concentrations. The ¹H NMR of the methanol/water extracts were like the methanol extracts.

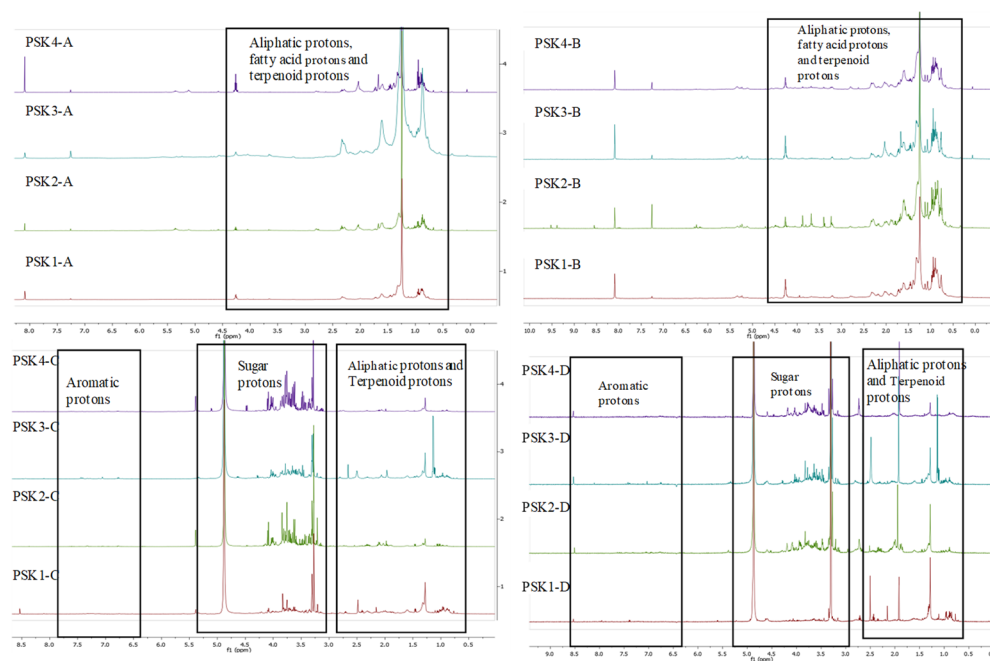


Figure 2. ¹H NMR spectra of all the extracts (PSK1–PSK2: harvested in December 2019; PSK3–PSK4: harvested in June 2020) of ex situ cultivated *Petromarula pinnata* (CDCl₃ for A and B, CD₃OD for C and D, 500 MHz).

3.4. In Vitro Tests

All the results of the in vitro tests performed in *P. pinnata* to provide insight into its biological activities are shown below in Tables 3 and 4. These tests employed the total phenolic content, total flavonoids, TAC percentage (%), percentage (%) interaction with the DPPH (20 min) free radical, percentage (%) inhibition of lipid peroxidation, and percentage (%) inhibition of LOX (Table 3). Their antimicrobial activity against four common bacterial pathogens was also evaluated and the results are presented in Table 4.

Table 3. Results of the in vitro tests from all the extracts of ex situ cultivated *Petromarula pinnata* (mean $\pm$ SD; $n = 3$) (PSK1-PSK2: harvested in December 2019; PSK3-PSK4: harvested in June 2020).

Sample	Total Phenolic Content (mg of Gallic Acid Equivalents per 100 g of Extract)	Total Flavonoids (mg of Rutin Equivalents per 100 g of Extract)	Percentage (%) TAC	Percentage (%) Interaction with the DPPH (20 min) Free Radical	Percentage (%) Inhibition of Lipid Peroxidation	Percentage (%) Inhibition of LOX
PSK1-A	1267.10 $\pm$ 34.22 ^b	363.18 $\pm$ 16.69 ^f	57.34 $\pm$ 1.90 ^{g,h}	67.03 $\pm$ 8.81 ^e	2.53 $\pm$ 0.34 ⁱ	na
PSK1-B	424.20 $\pm$ 12.30 ^d	421.56 $\pm$ 15.04 ^e	114.64 $\pm$ 8.95 ^d	10.71 $\pm$ 0.26 ^h	30.8 $\pm$ 2.13 ^e	17.69 $\pm$ 1.46 ^c
PSK1-C	45.85 $\pm$ 2.98 ^l	322.03 $\pm$ 20.69 ^{fg}	72.75 $\pm$ 0.80 ^{ef}	86.81 $\pm$ 3.31 ^{a,b,c}	2.99 $\pm$ 0.77 ⁱ	6.88 $\pm$ 0.85 ^e
PSK1-D	382.15 $\pm$ 6.83 ^e	83.73 $\pm$ 5.38 ^h	12.02 $\pm$ 1.29 ⁱ	22.73 $\pm$ 2.25 ^g	65.53 $\pm$ 6.39 ^d	na
PSK2-A	280.40 $\pm$ 10.62 ^g	490.46 $\pm$ 25.81 ^d	104.72 $\pm$ 2.42 ^d	93.4 $\pm$ 0.10 ^{a,b}	83.12 $\pm$ 4.29 ^b	na
PSK2-B	185.25 $\pm$ 5.76 ⁱ	955.56 $\pm$ 36.58 ^a	151.37 $\pm$ 5.48 ^a	3.29 $\pm$ 1.61 ^h	11.95 $\pm$ 3.45 ^h	na
PSK2-C	149.85 $\pm$ 12.56 ^j	464.62 $\pm$ 27.33 ^e	102.32 $\pm$ 8.48 ^d	94.16 $\pm$ 0.81 ^{a,b}	na	11.79 $\pm$ 1.01 ^d
PSK2-D	222.85 $\pm$ 9.74 ^h	802.44 $\pm$ 44.63 ^a	79.57 $\pm$ 4.59 ^e	93.83 $\pm$ 0.57 ^{a,b}	17.01 $\pm$ 1.87 ^g	19.9 $\pm$ 1.38 ^b
PSK3-A	877.70 $\pm$ 24.71 ^c	331.60 $\pm$ 17.63 ^a	100.88 $\pm$ 3.04 ^d	36.26 $\pm$ 11.44 ^f	29.43 $\pm$ 2.12 ^e	na
PSK3-B	426.40 $\pm$ 10.04 ^d	489.50 $\pm$ 18.77 ^d	124.28 $\pm$ 1.83 ^c	76.92 $\pm$ 11.24 ^{c,d,e}	29.43 $\pm$ 2.97 ^e	na
PSK3-C	304.70 $\pm$ 8.34 ^{fg}	622.53 $\pm$ 33.03 ^c	109.71 $\pm$ 4.38 ^{c,d}	86.81 $\pm$ 2.90 ^{a,b,c}	2.53 $\pm$ 2.41 ⁱ	11.55 $\pm$ 0.86 ^d
PSK3-D	333.45 $\pm$ 4.19 ^f	349.78 $\pm$ 6.23 ^f	66.33 $\pm$ 1.30 ^{fg}	82.42 $\pm$ 6.49 ^{b,c,d}	91.32 $\pm$ 4.32 ^a	20.93 $\pm$ 1.34 ^b
PSK4-A	1377.70 $\pm$ 20.94 ^a	284.70 $\pm$ 8.34 ^g	102.44 $\pm$ 9.24 ^d	89.01 $\pm$ 0.17 ^{a,b}	na	32.19 $\pm$ 2.13 ^a
PSK4-B	81.25 $\pm$ 5.64 ^k	486.63 $\pm$ 11.23 ^d	129.06 $\pm$ 11.98 ^{b,c}	71.75 $\pm$ 4.65 ^{d,e}	24.6 $\pm$ 1.97 ^f	na
PSK4-C	39.25 $\pm$ 1.23 ^l	421.56 $\pm$ 24.81 ^e	135.28 $\pm$ 5.28 ^b	97.8 $\pm$ 0.03 ^a	na	na
PSK4-D	180.8 $\pm$ 9.09 ⁱ	770.86 $\pm$ 39.56 ^a	52.58 $\pm$ 1.29 ^h	91.21 $\pm$ 0.14 ^{a,b}	71.69 $\pm$ 5.86 ^c	na
NGDA	-	-	-	81.00	93.00	-
Trolox	-	-	-	-	-	95.00
Ascorbic acid (1 mg/mL)	-	-	100	-	-	-

na: not active; NGDA: Nordihydroguaiaretic acid; Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. ^{a-l} values with the same letter are not significantly different ($p < 0.05$).

Table 4. Results of the in vitro antimicrobial activity expressed as minimum inhibitory concentration (MIC $\pm$ SD; $n = 3$) from all the extracts of *Petromarula pinnata* (PSK1-PSK2: harvested in December 2019; PSK3-PSK4: harvested in June 2020).

MIC (mg/mL)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Salmonella enteritidis</i>
PSK1-A	2.50 $\pm$ 0.025 ^b	5.00 $\pm$ 0.010 ^e	2.50 $\pm$ 0.046 ^c	2.50 $\pm$ 0.026 ^b
PSK1-B	5.00 $\pm$ 0.103 ^c	5.00 $\pm$ 0.032 ^e	5.00 $\pm$ 0.030 ^d	5.00 $\pm$ 0.035 ^c
PSK1-C	2.50 $\pm$ 0.025 ^b	1.25 $\pm$ 0.015 ^c	2.50 $\pm$ 0.021 ^c	2.50 $\pm$ 0.015 ^b
PSK1-D	5.00 $\pm$ 0.025 ^c	2.50 $\pm$ 0.035 ^d	5.00 $\pm$ 0.035 ^d	5.00 $\pm$ 0.036 ^a
PSK2-A	2.50 $\pm$ 0.025 ^b	2.50 $\pm$ 0.010 ^d	5.00 $\pm$ 0.010 ^d	5.00 $\pm$ 0.038 ^c
PSK2-B	5.00 $\pm$ 0.020 ^c	5.00 $\pm$ 0.035 ^e	5.00 $\pm$ 0.035 ^d	5.00 $\pm$ 0.050 ^c
PSK2-C	2.50 $\pm$ 0.025 ^b	1.25 $\pm$ 0.025 ^c	2.50 $\pm$ 0.030 ^c	2.50 $\pm$ 0.025 ^b
PSK2-D	2.50 $\pm$ 0.040 ^b	2.50 $\pm$ 0.064 ^d	5.00 $\pm$ 0.040 ^d	5.00 $\pm$ 0.055 ^c
PSK3-A	1.25 $\pm$ 0.025 ^a	1.25 $\pm$ 0.040 ^c	1.25 $\pm$ 0.025 ^b	1.25 $\pm$ 0.035 ^a
PSK3-B	5.00 $\pm$ 0.021 ^c	2.50 $\pm$ 0.020 ^d	2.50 $\pm$ 0.031 ^c	2.50 $\pm$ 0.051 ^b
PSK3-C	1.25 $\pm$ 0.046 ^a	0.63 $\pm$ 0.010 ^b	1.25 $\pm$ 0.035 ^b	1.25 $\pm$ 0.015 ^a
PSK3-D	1.25 $\pm$ 0.025 ^a	1.25 $\pm$ 0.046 ^c	2.50 $\pm$ 0.044 ^c	2.50 $\pm$ 0.035 ^b
PSK4-A	1.25 $\pm$ 0.015 ^a	1.25 $\pm$ 0.025 ^c	2.50 $\pm$ 0.040 ^c	2.50 $\pm$ 0.025 ^b
PSK4-B	2.50 $\pm$ 0.055 ^b	2.50 $\pm$ 0.055 ^d	2.50 $\pm$ 0.046 ^c	2.50 $\pm$ 0.020 ^b
PSK4-C	1.25 $\pm$ 0.025 ^a	0.31 $\pm$ 0.015 ^a	0.63 $\pm$ 0.025 ^a	1.25 $\pm$ 0.015 ^a
PSK4-D	2.50 $\pm$ 0.032 ^b	2.50 $\pm$ 0.040 ^b	2.50 $\pm$ 0.040 ^c	2.50 $\pm$ 0.020 ^b

Table 4. Cont.

MIC (mg/mL)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Salmonella enteritidis</i>
Ampicillin	0.03 ± 0.001	-	-	0.02 ± 0.002
Gentamycin	-	0.02 ± 0.001	0.02 ± 0.003	-

Ampicillin: positive control for *E. coli* and *S. enteritidis*; gentamycin: positive control for *S. aureus* and *E. faecalis*.
^{a-e} values with the same letter are not significantly different ($p < 0.05$).

3.4.1. Total Phenolic Content

Even though the phenolic compounds were mostly polar, the hexane extracts (280.4–1377.7 mg of gallic acid equivalents per 100 g of extract) were the richest in total phenolic content (Table 3 and Figure 3), while the methanol extracts (39.25–304.7 mg of gallic acid equivalents per 100 g of extract) were the poorest. The hexane extracts seemed to contain twice the quantity of phenolic compounds than the rest of the extracts. There was a statistically significant three-way interaction between the collection, the processing method, and the extract, i.e., $F(3,32) = 5317.172$, $p < 0.001$.

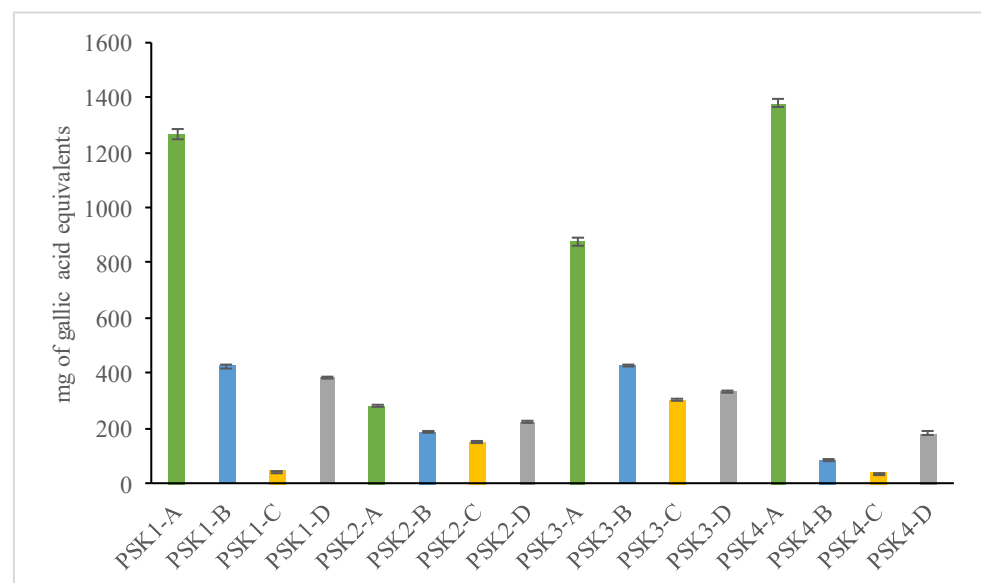


Figure 3. Total phenolic content results (mean ± Standard Error; $n = 3$) of the extracts (PSK1-PSK2: harvested in December 2019; PSK3-PSK4: harvested in June 2020) of *Petromarula pinnata*.

3.4.2. Total Flavonoid Assay

P. pinnata was poor in flavonoids (Table 3 and Figure 4). For the winter collection, PSK1 and PSK2, the dichloromethane extracts were the richest in flavonoids (421.56 ± 15.04 and 955.56 ± 36.58 mg of rutin equivalents per 100 g of extract, respectively), while for the summer collection, the methanol (PSK3-C = 622.53 ± 33.03 mg of rutin equivalents per 100 g of extract) or the methanol:water (PSK4-D = 770.86 ± 39.56 mg of rutin equivalents per 100 g of extract) were the richest. PSK1-D (83.73 ± 5.38 mg of rutin equivalents per 100 g of extract) was the poorest in flavonoids. There was a statistically significant three-way interaction between the collection, the processing method, and the extract, i.e., $F(3,32) = 27.597$, $p < 0.001$.

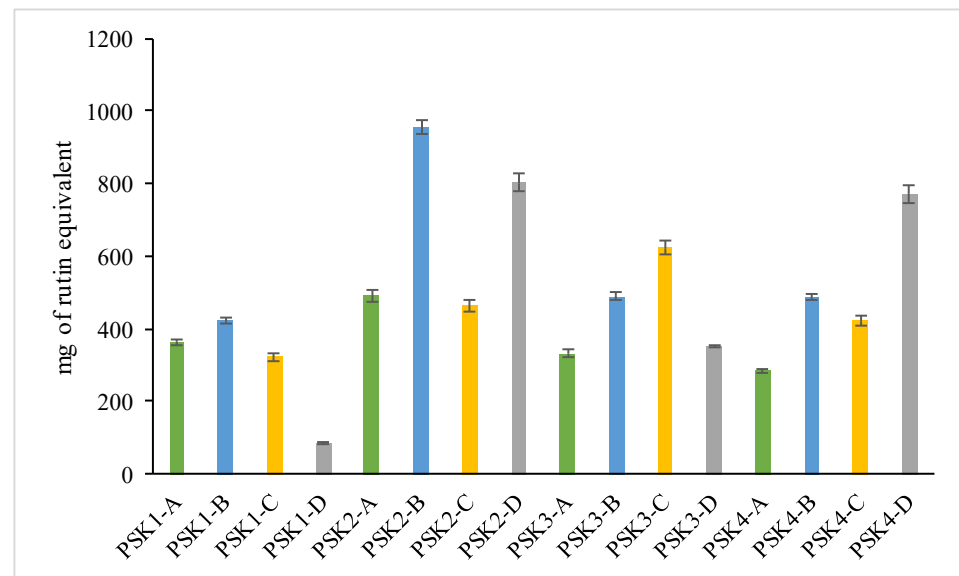


Figure 4. Results of total flavonoids (mean $\pm$ Standard Error; $n = 3$) of the extracts of *Petromarula pinnata* (PSK1-PSK2: harvested in December 2019; PSK3-PSK4: harvested in June 2020).

3.4.3. Phosphomolybdate Assay

The dichloromethane extracts in all the cases had the highest total antioxidant capacity (Table 3 and Figure 5), which was higher than the standard (ascorbic acid, 1 mg/mL). The hexane and methanol extracts also had a high total antioxidant capacity (Table 3 and Figure 5). The methanol:water extracts showed the lowest TAC percentage (%). PSK2-B had the higher ability ($151.37 \pm 5.48\%$ TAC) and PSK1-D had the lowest ($12.02 \pm 1.29\%$ TAC). The PSK1 sample showed the lowest TAC percentage (%) in general (Table 3 and Figure 5). There was a statistically significant three-way interaction between the collection, the processing method, and the extract, i.e., $F(3,32) = 24.433$, $p < 0.001$.

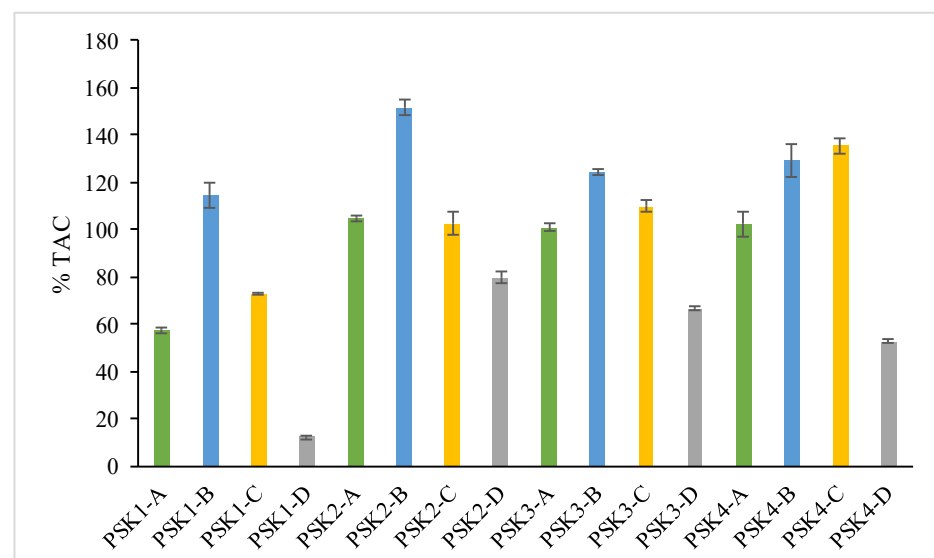


Figure 5. TAC percentage (%) of the extracts of *Petromarula pinnata* (mean $\pm$ Standard Error; $n = 3$) (PSK1-PSK2: harvested in December 2019; PSK3-PSK4: harvested in June 2020).

3.4.4. DPPH

The methanol extracts had high reducing activity, ranging from 76.92 to 97.80% (Table 3 and Figure 6). The hexane and methanol:water extracts also showed high activity. The

winter collection (PSK1 and PSK2) showed a lower percentage (%) reduction of the DPPH free radical as compared to the summer collection (PSK3, PSK4) that had more than 80% activity (Table 3 and Figure 6). PSK1-B and PSK2-B were not found active ($10.71 \pm 0.26\%$ and $3.29 \pm 1.61\%$). There was a statistically significant three-way interaction between the collection, the processing method, and the extract, i.e., $F(3,32) = 81.813$, $p < 0.001$.

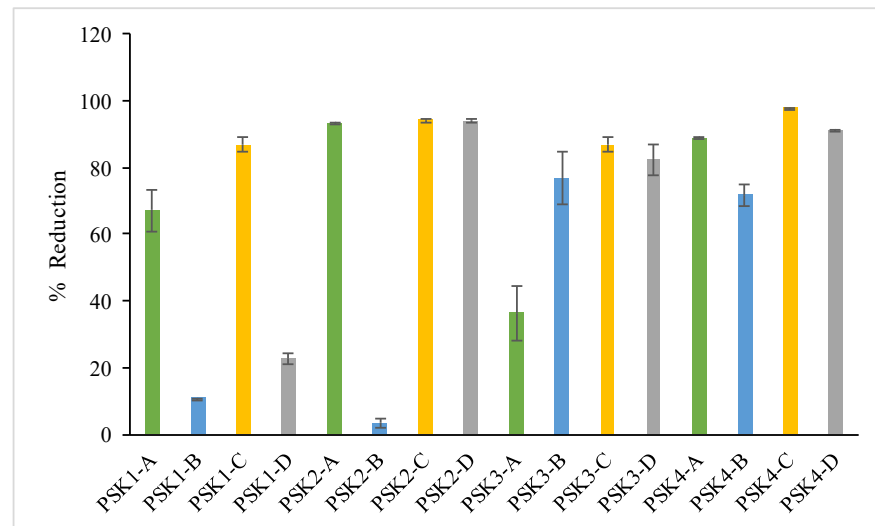


Figure 6. Percentage (%) reduction of the DPPH free radical (mean $\pm$ Standard Error; $n = 3$) of the extracts (PSK1-PSK2: harvested in December 2019; PSK3-PSK4: harvested in June 2020) of *Petromarula pinnata*.

3.4.5. Inhibition of Linoleic Acid Lipid Peroxidation

The plant extracts showed low inhibition of linoleic acid peroxidation while most of the extracts had lower than 50% activity (Table 3 and Figure 7). PSK1-D ($65.53 \pm 6.39\%$), PSK2-A ($83.12 \pm 4.29\%$), PSK3-D ($91.32 \pm 4.32\%$), and PSK4-D ($71.69 \pm 5.86\%$) were the strongest inhibitors of linoleic acid peroxidation (Table 3 and Figure 7), although their activity was lower than the reference drug NGDA (93%). There was a statistically significant three-way interaction between the collection, the processing method, and the extract, i.e., $F(3,32) = 7.940$, $p < 0.001$.

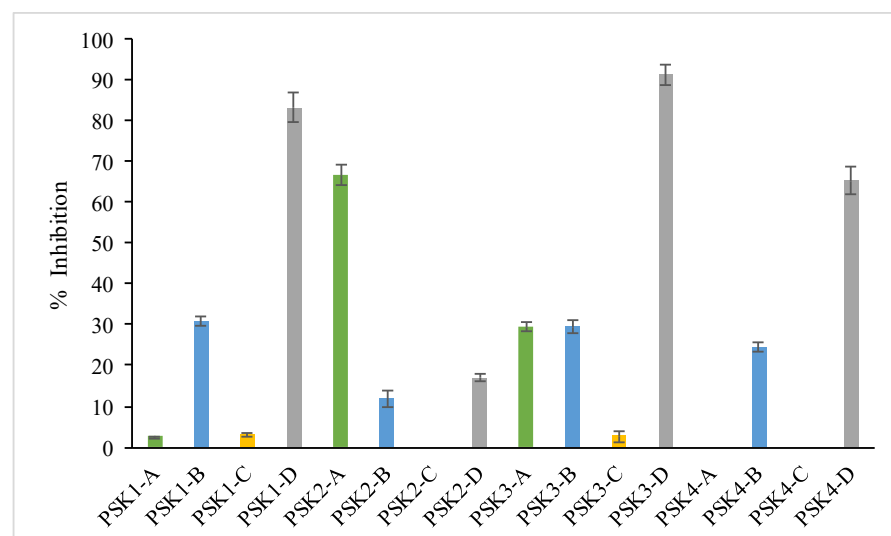


Figure 7. Percentage (%) inhibition of linoleic acid peroxidation (mean $\pm$ Standard Error; $n = 3$) of the extracts (PSK1-PSK2: harvested in December 2019; PSK3-PSK4: harvested in June 2020) of *Petromarula pinnata*.

3.4.6. Inhibition of Soybean Lipoxygenase

This in vitro experiment showed the anti-inflammatory activity of the extract against the soybean LOX (Table 3 and Figure 8). *P. pinnata*'s extracts showed low inhibition of LOX. PSK4-A was the strongest inhibitor, but its activity was half of that of the reference drug Trolox (95.00%). There was a statistically significant three-way interaction between the collection, the processing method, and the extract, i.e., $F(3,32) = 2.259$, $p < 0.001$.

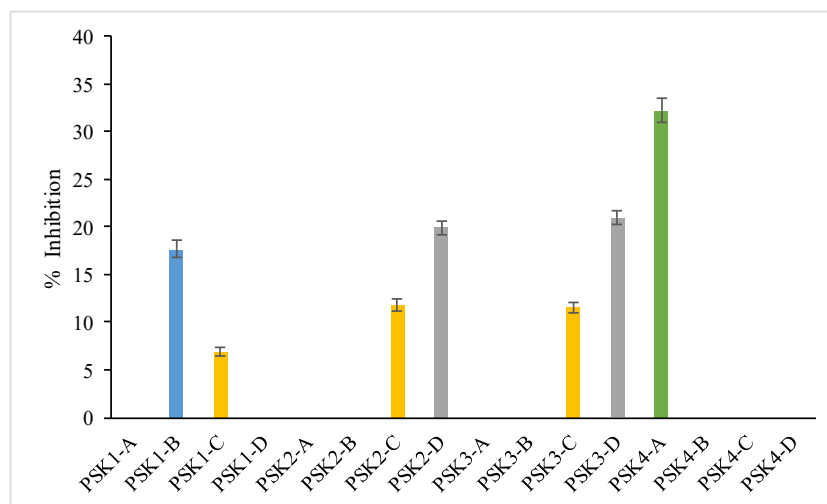


Figure 8. Percentage (%) inhibition of LOX (mean $\pm$ Standard Error; $n = 3$) of the extracts (PSK1-PSK2: harvested in December 2019; PSK3-PSK4: harvested in June 2020) of *Petromarula pinnata*.

3.4.7. Antimicrobial Activity

According to the results presented in Table 4, all extracts (winter and summer collection) exhibited antimicrobial activity against gram-negative and gram-positive bacteria. For the winter collection, the methanol extracts (PSK1-1 and PSK2-C) demonstrated a moderate bactericidal effect for *S. aureus* (MIC: 1.25 mg/mL). The summer collection showed stronger inhibition and bactericidal activity for all bacteria tested as compared to the winter collection. Specifically, the methanol extracts (PSK3-C and PSK4-C) exhibited the strongest bacterial inhibition against *S. aureus* (MIC: 0.63 mg/mL and 0.31 mg/mL) and *E. faecalis* (MIC: 0.63 mg/mL). Differences of significance between sample means were determined with one-way interaction. The significance level was set to 0.05 and the confidence intervals were at $\pm 95\%$ CI. The data were presented as mean $\pm$ standard deviation (SD).

4. Discussion

The investigation herein presents a comprehensive phytochemical and nutritional profiling of *P. pinnata* for the first time along with tested biological activities. The main differences between the two processing methods (air-dried and liquid nitrogen) examined in *P. pinnata* plant material were the weights of the extracts; the air-dried plants had more yield than the ones treated with liquid nitrogen because of the lower water content of the first ones. However, according to the ^1H NMR spectra, there were no significant differences regarding the phytochemical composition between air-dried plants of *P. pinnata* and the ones treated with liquid nitrogen.

According to published data, the phytochemistry of the family Campanulaceae employs alkaloids, flavonoids, terpenoids, polyacetylenes, lignans, fatty acids, and other phenolic compounds [26–28]. In phylogenetic terms, *P. pinnata* is a close relative to *Melanocalyx uniflora* (L.) Morin and members of the genus *Phyteuma* [29]. From members of the genus *Phyteuma*, triterpenoid saponins, phenolic acids, and flavonoids have been isolated and identified [30–32]. In the case of the *P. pinnata* investigated herein, the ^1H NMR spectra of the hexane and dichloromethane extracts showed peaks of fatty acids and terpenes, while the ^1H NMR spectra of the methanol and methanol/water extracts showed peaks

indicating the presence of triterpenoid saponins and phenolic compounds. This profile was in agreement with published data.

From a nutritional viewpoint, there are not many studies on the nutritional value of Campanulaceae plants. Only recently, Tsiftoglou et al. [28] have studied the dietary value of the aerial parts of *C. pelviformis* Lam., another local endemic and edible plant of Crete like the herein studied *P. pinnata*. The latter has been found to be rich in microminerals, ash (7.92%), crude protein (12.90%), and carbohydrates (64.19%), and low in fat (2.46%) [28]. The aerial parts of wild-growing *P. pinnata* examined herein showed similar nutritional value to *C. pelviformis* with ash (9.02%), crude protein (13.27%), carbohydrate (64.54%), and low fat (2.98%). All measurements indicated that the herein focal plant species can be considered a good food source of wild origin in a similar fashion to *C. pelviformis*.

To the best of our knowledge, *P. pinnata* has not been thoroughly studied. There is only one study of Kalpoutzakis et al. [17] where the total phenolic content and the interaction with the DPPH free radical of the methanol extract of several endemic plants of Crete were examined in a comparative way. The latter study reports that the methanol extract of wild-growing *P. pinnata* has low total phenolic content (51.4 ± 1.7 mg GAE/g of extract) and low interaction with the DPPH free radical (<50%). The TPC results of the above-mentioned study were in line with the results of the current study examining ex situ cultivated material of wild origin which showed that the methanol extracts had low total phenolic content (39.25–304.7 mg of GAE/100 g of extract). However, our results suggested that the methanol extracts as well as the rest of the extracts had high interaction with the DPPH free radical (86.81–97.8%). Such differences may be attributed to the fact that we used the aerial parts of the ex situ cultivated *P. pinnata* while Kalpoutzakis et al. [17] used aerial parts of wild-growing *P. pinnata* material; however, the effect of different naturally occurring genotypes cannot be overruled.

In the absence of studies regarding the antioxidant or anti-inflammatory activity of *P. pinnata* or of its close relatives such as *M. uniflora* and members of the genus *Phyteuma*, only published studies regarding plants of the family Campanulaceae can be exploited to contrast the findings of the current investigation. For example, the 50% aqueous methanol extract of frozen leaves and flowers of *Phyteuma orbiculare* has been studied by Abbet et al. [31,32] and the extract is reported to show high antioxidant ability (Oxygen radical absorbance capacity = $26,768 \pm 113$ and $19,933 \pm 1722$ μ mol of Trolox equivalents/100 g FW, respectively). Furthermore, Jaradat et al. [33] have examined the antioxidant activity (DPPH) of the methanol extract of 15 Palestinian species of the genus *Campanula*. The reported extracts of the plants *C. cymbalaria* Sm., *C. erinus* L., *C. kotschyana* A.DC., *C. rapunculoides* L., *C. sidoniensis* Boiss. & C.I. Blanche, and *C. sulphurea* Boiss. have shown high activity, similar to the standard (Trolox) [33]. In the study of Dumlu et al. [34], the methanol extract of *C. alliariifolia* Willd. has shown high antioxidant activity with the DPPH method at a concentration of $500 \text{ mg} \cdot \text{L}^{-1}$. In another study, Korkmaz et al. [35] examined four different extracts of *C. latifolia* L. subsp. *latifolia*, concluding that the acetonitrile extract has the most potent antioxidant activity with both the DPPH and Phosphomolybdate method ($\text{IC}_{50} = 266.16 \text{ } \mu\text{g/mL}$ and $84.77 \text{ } \mu\text{M}$ of quercetin equivalents), followed by the methanol one ($\text{IC}_{50} = 410.67 \text{ } \mu\text{g/mL}$ and $55.30 \text{ } \mu\text{M}$ of quercetin equivalents), the aqueous ($\text{IC}_{50} = 883.72 \text{ } \mu\text{g/mL}$ and $23.93 \text{ } \mu\text{M}$ of quercetin equivalents), and the hexane extract ($\text{IC}_{50} = 1098.17 \text{ } \mu\text{g/mL}$ and $11.93 \text{ } \mu\text{M}$ of quercetin equivalents). In another study, Sarikurkcu et al. [36] have found that the methanol extract of *C. macrostachya* Waldst. & Kit. ex Willd. has shown the highest antioxidant activities in different tests (DPPH: 4.15 mg/mL , ABTS: 2.05 mg/mL , CUPRAC: 1.80 mg/mL , FRAP: 0.83 mg/mL , phosphomolybdenum: 1.69 mg/mL) compared to other extracts. This short overview of literature findings may indicate that the methanol extracts of several Campanulaceae plants show high antioxidant activity such as those examined herein for the ex situ cultivated *P. pinnata*.

This is the first time that the antimicrobial activity of *P. pinnata* has been examined, and unfortunately, there are not many studies regarding the antimicrobial activity of other plants of the family Campanulaceae. According to Mohammed et al. [37], methanol and ethanol

plant extracts of *C. strigosa* Banks & Sol. have been found to possess antimicrobial activity. Specifically, the activity of *C. strigosa* ethanol extracts is reported to be greater than that of the methanol extracts, with concentrations ranging between 100 and 400 µg/mL [37]. It has also been reported that the volatile oil and the aqueous extracts of *C. portenschlagiana* Schult. are effective against several bacterial strains [38]. According to other research, *S. aureus*, *E. coli*, and *E. faecalis* are all shown to be inhibited by the methanol and water extracts of *C. retrorsa* Labill. at varying doses [39]. Therefore, the methanol extracts of the Campanulaceae plants may demonstrate antimicrobial activity. Being a member of Campanulaceae, *P. pinnata* as investigated herein also revealed a moderate bacterial inhibition regarding the plant material collected in the winter and a strong bactericidal effect regarding the plant material collected in the summer from ex situ cultivation. This trend is aligned with the total phenolic content and the antioxidant activity assessed in the in vitro experiments of the present study. Specifically, the methanol extracts showed the lowest MIC value and the highest antioxidant and reducing DPPH activity.

The phytochemical and nutritional investigation of *P. pinnata* conducted in this study for the first time offers new knowledge for this species, thus outdating its previous assessment in terms of potentialities for the medicinal sector [40]. Therefore, in the light of the findings herein, we have reassessed the potential of *P. pinnata* in the medicinal sector (Table 5).

Table 5. Updated scores per examined attribute and overall evaluation regarding the medicinal potential of *Petromarula pinnata* in the medicinal sector based on the findings of the present investigation. Arrows indicate the cases of updated scores assigned to individual attributes (for the scoring system, see [40]).

Superfood (Nutraceutical) Potential	Identified Ethnobotanical Uses	Poisonousness-Toxicity	Identified Phytochemical Compounds	European Medicines Agency (EMA) Monograph Status	Approved Indications	Medicinal Potential	Distinct Ethnobotanical Uses	Distinct Medicinal Properties	Overall Medicinal Potential
0 → 6	6	6	0 → 6	0	0	6	1 → 3	1 → 3	Average (37.04%) → High (66.67%)

The updated evaluation of *P. pinnata* in the medicinal sector compared to the previous assessment [40] is due to the detection of specific major classes of bioactive chemical compounds (terpenoids, phenolics, flavonoids, and fatty acids) in *P. pinnata*, providing a score 6 from 0; due to the enriched profile of associated ethnobotanical properties (antioxidant, anti-inflammatory, and antimicrobial activities), providing a score 3 from 1; as well as due to the strong nutraceutical potential revealed in the present study, resulting in a score 6 from 0 (high nutritional value combined with major bioactive compounds with beneficial effects). This kind of upgrading in the evaluation of *P. pinnata* generates higher individual scores per attribute and higher percentages of the overall score regarding the potential of *P. pinnata* in the medicinal sector, resulting in upgrading from ‘Below average’ (37.04%) to ‘High’ (66.67%) medicinal potential. *Petromarula pinnata* has been cultivated experimentally for research purposes during the last five years in Thessaloniki, northern Greece, both in outdoor conditions and in a non-heated nursery [13–15,17,28,40–42]. As there is great potential for the investigated species to be cultivated as a medicinal or ornamental or edible new crop [13–15,17,28,40–42], the feasibility and readiness timescale for its sustainable exploitation has been recently updated and is designated to date as achievable in the short term [13–15,17,28,40–42]. Therefore, in the frame of the project entitled “Indigenous edible plants of Crete as alternative new crops contributing to biodiversity preservation,

protection from soil degradation and mitigation of climate change impacts” (acronym: Cretan Greens 4 Clima Pro; M16ΣYN-01106), a pilot field cultivation of *P. pinnata* has been recently established in Crete (Heraklion, Messara) to determine several agronomic features related to the productivity of *P. pinnata* and its responses to different fertilization schemes. Given that *P. pinnata* is a range-restricted species and is exclusively found in Crete, Greece (local endemic; monotypic genus), there are unique geographic, phylogenetic, and evolutionary relations to this insular area of Greece. Consequently, its future commercial cultivation should preferably be restricted only to Crete, avoiding establishment in other agro-ecological areas. In this way, its future commercial cultivation can potentially be granted a ‘geographical indication’ (GI) EU quality scheme [43], given that its original germplasm can naturally be sourced only from Crete. In addition, should any designed agro-alimentary end-product from this unique species be produced in the island of Crete or Greece, a Protected Designation of Origin (PDO) and/or Protected Geographical Indication (PGI) label should be granted [43], thus offering opportunities of exclusive branding which can be facilitated by DNA-barcoding and genetic fingerprinting. From a legal viewpoint, the cultivation of *P. pinnata* for commercial purposes is ultimately related to authorized access to wild-growing genetic resources in a direct way (authorization to access Cretan genetic resources in this case) and benefit sharing mechanisms should be in place regarding the utilization of such germplasm for commercial purposes across scales (local, regional, national, global); such issues are governed to date by the EU Directive 511/2014 enforcing the Nagoya protocol. As national sovereign rights are previewed and should be imposed especially on the local endemic genetic resources of any country according to the provisions of the Nagoya Protocol, these legal issues are currently enforced across levels prior to any commercialization of unique locally endemic genetic resources such as the herein studied *P. pinnata*.

5. Conclusions

P. pinnata is a unique (monotypic), wild edible but vulnerable, local endemic plant of Crete with promising potential both as an ornamental or nutraceutical new crop originating from the rich Cretan biodiversity. The plant is low in fat and is a source of protein carbohydrates and fiber and has noteworthy nutritional value for the Cretan diet while also being rich in terpenoid compounds and poor in phenolic compounds. No significant differences were observed in the phytochemical composition between the collection time or the processing method. The total phenolic and total flavonoid assays showed that *P. pinnata* is poor in phenolic and flavonoid compounds. The TAC and DPPH assays showed that the extracts have high antioxidant activity, which is higher in the summer plant material (PSK3 and PSK4), and the activity was in some extracts higher than the standard compounds (Ascorbic acid for TAC and NGDA for DPPH assay). The methanol/water extracts were the strongest inhibitors of the lipid peroxidation while the methanol extracts were not active. The plant extracts were found to be weak inhibitors of 5-LOX, with the strongest inhibitor having 32.19% activity, which is lower than that of the reference compound NGDA (95%). All extracts showed bacterial inhibition for all bacteria tested (Gram-negative and Gram-positive) with a stronger effect observed in the plant material collected in the summer. Therefore, it can be postulated that *P. pinnata* has strong antioxidant activity and moderate to strong bactericidal activity, and does not have anti-inflammatory activity against soybean LOX. Undoubtedly, further investigation is needed to research the species’ phytochemical profile, to isolate the main and active compounds and to assess excessively the biological activities of the extracts with different in vitro and in vivo methods. Nonetheless, this pivotal study consolidated herein for the first time the nutraceutical value of *P. pinnata*, thus upgrading the assessment of its potential for sustainable exploitation in the medicinal and agro-alimentary sectors.

Author Contributions: Conceptualization, D.L. and N.K.; methodology, D.L., K.M.D., O.S.T., S.K. and D.C.; software, D.L. and K.M.D.; validation, N.K., D.L., K.P., E.K., D.H.-L. and O.S.T.; formal analysis, D.L., K.M.D., O.S.T., S.K. and D.C.; investigation, N.K., K.M.D., S.K. and O.S.T.; resources,

N.K., K.P. and D.L.; data curation, K.M.D., S.K., O.S.T. and D.L.; writing—original draft preparation, D.L., O.S.T. and K.M.D.; writing—review and editing, N.K., O.S.T. and D.L.; visualization, K.M.D., N.K., O.S.T. and D.L.; supervision, N.K., O.S.T., D.H.-L. and D.L. All authors have read and agreed to the published version of the manuscript.

Funding: This investigation was conducted in co-operation with the project entitled ‘Indigenous edible plants of Crete as alternative new crops contributing to biodiversity preservation, protection from soil degradation and mitigation of climate change impacts’ (acronym: Cretan Greens 4 Clima Pro; M16ΣΥΝ-01106) which has been co-funded under Measure 16—Cooperation (16.1–16.5) by Greece and the European Union (European Agricultural Fund for Rural Development 2014–2020) and the scientific work of NK and KP has been partially supported by the referred project.

Data Availability Statement: All data supporting the results of this study are included in the manuscript and the datasets are available upon request.

Acknowledgments: The researchers would like to thank Aikaterini-Angeliki Kotoula for valuable help with the statistical analysis of the data. Special thanks to Ilias Giannenas (Laboratory of Nutrition, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki) for permitting the use of PERTEN for the chemical-nutrient analysis of the examined wild edible green.

Conflicts of Interest: The authors declare no conflicts of interest.

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