

Article

Extraction Processing Technologies and Their Effects on Antioxidant Activity in *Cinnamomum camphora* (L.) J. Presl Leaves

Myat Pwint Phyu ¹, Yuchen Cheng ¹, Yuri Kang ¹, Hyunjae Jang ², Seungwoong Lee ³ and Woonjung Kim ^{1,*}

¹ Department of Chemistry, Hannam University, Daejeon 34430, Republic of Korea; myat.mpp@gmail.com (M.P.P.); 000cheng@naver.com (Y.C.); yuri_1005@nate.com (Y.K.)

² Natural Product Research Center & Natural Product Central Bank, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Department of Applied Biotechnology, University of Science and Technology (UST), Jeongeup-si 56212, Republic of Korea; water815@kribb.re.kr

³ Functional Biomaterials Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, Republic of Korea; lswdoc@kribb.re.kr

* Correspondence: wjkim@hnu.kr; Tel.: +82-42-629-8822

Abstract

This study investigated the bioactive potential of *Cinnamomum camphora* (L.) J. Presl (*C. camphora*) leaf extracts obtained using hydrothermal extraction (HE) and ultrasound-assisted extraction (UAE) with 30%, 50%, and 70% ethanol (*v/v*). Extracts were analyzed for their phytochemical composition and biological activities. UAE extracts, particularly with 70% ethanol, exhibited the highest total polyphenol (363.0 ± 1.40 mg GAE/g) and flavonoid (174.5 ± 0.42 mg QE/g) contents. This extract also demonstrated strong antioxidant activities (IC_{50} : 0.024 ± 0.001 mg/mL for DPPH; IC_{50} : 0.363 ± 0.002 mg/mL for ABTS; 3.080 ± 0.044 M Fe^{2+} /g for FRAP) and potent enzyme inhibition ($49.3 \pm 0.35\%$ for tyrosinase; $24.8 \pm 0.34\%$ for elastase; $94.5 \pm 0.12\%$ for α -glucosidase and $77.5 \pm 1.11\%$ for lipase). Antimicrobial activity was most effective against Gram-positive bacteria, notably against *Cutibacterium acnes*, showing the largest inhibition zone (23.0 mm at 10 mg/disc). Overall, antioxidant, enzyme inhibition and antimicrobial activities increased significantly with increasing ethanol concentration, particularly at 70% ethanol. GC–MSD analysis revealed that both HE and UAE extracts contained phenolic acids, terpenes, triterpenes, and sesquiterpenes. Collectively, these findings indicate that the extraction method (UAE) and solvent composition (70% ethanol) influence the bioactivity profile of *C. camphora* leaf extracts, supporting further investigation of their relevance for cosmeceutical and functional applications.

Keywords: *Cinnamomum camphora* (L.) J. Presl; extraction; phytochemical; bioactivity; antioxidant



Academic Editor: Alessandra Napolitano

Received: 13 January 2026

Revised: 28 January 2026

Accepted: 6 February 2026

Published: 9 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Driven by the rapid expansion of the pharmaceutical and cosmeceutical sectors, medicinal plants enriched with multifunctional phytochemicals have become strategic resources for the discovery of sustainable natural bioactive ingredients [1]. *Cinnamomum camphora* (L.) J. Presl, an evergreen species of the Lauraceae family, has emerged as a promising natural resource owing to its chemically diverse secondary metabolite profile [2]. Previous studies have demonstrated that *C. camphora* leaves are abundant in phenolic compounds,

terpenoids, and phenylpropanoids, which have been associated with antioxidant, anti-inflammatory, and antimicrobial activities [3,4]. These biological activities are of particular importance because oxidative stress, inflammation, and microbial infection represent key pathological mechanisms underlying chronic diseases and skin disorders [5,6]. Notably, major constituents such as eugenol, linalool, and cineole have been reported to exhibit free radical scavenging, anti-inflammatory, and antibacterial effects, supporting continued interest in *C. camphora* leaf extracts [7–10].

Despite *C. camphora* leaves being widely reported as a source of bioactive constituents, existing studies have largely emphasized essential oils [11,12], providing limited insight into how extraction parameters govern broader bioactivity outcomes. In particular, the role of ethanol–water solvent polarity in shaping coordinated antioxidant responses, enzyme inhibition activities relevant to skin and metabolic health, and antimicrobial performance has not been systematically evaluated using a unified experimental framework. Consequently, the extent to which polarity-driven differences in extract composition translate into consistent or divergent bioactivity patterns across multiple functional assays remains unclear.

Accordingly, this study aims to assess the influence of ethanol–water solvent polarity on the multi-endpoint bioactivity profile of *C. camphora* leaf extracts through a comparative evaluation across solvent compositions. By examining polarity-dependent trends across complementary antioxidant, enzyme inhibition, and antimicrobial assays, this work seeks to examine how extraction conditions modulate functional performance, thereby providing an evidence-based basis for rational solvent selection in the development of plant-derived bioactive materials.

2. Materials and Methods

2.1. Plant Material and Chemicals

Dried *C. camphora* leaves were purchased from an herbal medicine supplier in Gangwon-do, Republic of Korea, in September 2024. Botanical identification of the plant material was carried out by Prof. Woonjung Kim of the Department of Chemistry, Hannam University, Republic of Korea. The corresponding voucher specimen has been archived in the Herbarium of Hannam University, Daejeon, Republic of Korea, under accession number HNU-CC-2024-0924.

Chemicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Folin and Ciocalteu's phenol reagent, 3,4-dihydroxy-L-phenylalanine (L-DOPA), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Trizma[®] base, tyrosinase from mushroom, α -glucosidase from *Saccharomyces cerevisiae*, elastase from porcine pancreas, lipase from porcine pancreas, 3-(N-morpholino)propanesulfonic acid (MOPS), *p*-Nitrophenyl α -D-glucopyranoside, *N*-succinyl-(Ala)₃-*p*-nitroanilide, gallic acid, kojic acid, L-ascorbic acid, quercetin, acarbose and orlistat were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium nitrite, hydrochloric acid solution and iron (III) chloride hexahydrate were procured from Samchum Chemical Co., Ltd. (Seoul, Republic of Korea). Sodium acetate anhydrous, iron (II) sulfate heptahydrate and ethylenediaminetetraacetic acid (EDTA) were from Duskan Pure Chemicals Co., Ltd. (Ansan-si, Gyeonggi-do, Republic of Korea). Potassium phosphate dibasic anhydrous and calcium chloride anhydrous were purchased from Daejung Chemicals & Metals Co., Ltd. (Siheung-si, Gyeonggi-do, Republic of Korea). For the antimicrobial activity, dehydrated culture media such as Nutrient Agar (NA), Gifu Anaerobic Medium Agar (GAM-A) and Gifu Anaerobic Medium Broth (GAM-B) were obtained from MB cell (SeoCho-Gu, Seoul, Republic of Korea), and Difco[™] Nutrient Broth (NB) was acquired from Becton, Dickinson and Company (Sparks, MD, USA). Only analytical-grade, HPLC or extra-pure-grade chemicals and reagents were used throughout the study.

2.2. Preparation of *C. camphora* Leaves

The dried leaves of *C. camphora* were ground into powder using a laboratory grinder and sieved through an 80-mesh sieve to obtain uniform particle size. The resulting powdered samples were sealed in air-tight zipper bags and stored at $-20\text{ }^{\circ}\text{C}$ until further extraction.

2.3. Hydrothermal and Ultrasound-Assisted Extraction of *C. camphora* Leaves

Dried powder of *C. camphora* leaves was extracted with 30%, 50% and 70% ethanol (v/v) in a solvent-to-sample ratio of 20:1 (v/w). Extractions were conducted separately using a hydrothermal extractor (A-11096 KSP-240L 25L; Kyungseo E&P, Incheon, Republic of Korea) at $60\text{ }^{\circ}\text{C}$ for 3 h or an ultrasonic homogenizer (NE-500Z, All in LabTech, Jeonju-Si, Republic of Korea) at ultrasonic power with a frequency of 50%, 25–26 kHz in pulse mode (1 s on/1 s off) for 30 min. The resulting solutions were filtered through Whatman No. 2 filter paper (Global Life Sciences Solutions Operations, Little Chalfont, UK). The filtrate was concentrated under reduced pressure at $50\text{ }^{\circ}\text{C}$ using a rotatory evaporator (Eyela N-1300, Sunil Instrument Co., Ltd., Gyeonggi-do, Republic of Korea) until ethanol was removed. After that, concentration under reduced pressure using a rotary evaporator (Eyela N-1300, Sunil Instrument Co., Ltd., Gyeonggi-do, Republic of Korea) at $50\text{ }^{\circ}\text{C}$ was performed until the ethanol removal was achieved. The concentrated extract solutions were reconstituted in distilled water and used for all subsequent analyses. The extracts were designated as HE 30%, HE 50%, and HE 70% for HE extracts and UAE 30%, UAE 50%, and UAE 70% for UAE extracts. After preparation, the concentrated liquid extracts were stored at $4\text{ }^{\circ}\text{C}$ in sealed containers and protected from light.

2.4. Phytochemical Content

Total Polyphenol and Flavonoid Contents of HE and UAE Extracts of *C. camphora* Leaves

The total polyphenol content (TPC) was determined using the Folin–Denis method with slight modifications [13]. Briefly, 12 μL of each extract solution was mixed with 12 μL of Folin–Ciocalteu’s phenol reagent and incubated at room temperature for 3 min. Subsequently, 180 μL of a 10% (w/v) sodium carbonate solution was added, and the reaction mixture was incubated in the dark for 1 h. The absorbance was measured at 765 nm using a SpectraMax ABS Plus microplate reader (Molecular Devices, LLC, San Jose, CA, USA). A standard calibration curve constructed with gallic acid was used to determine total polyphenol content. The results were expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g).

The total flavonoid content (TFC) was performed according to the aluminum chloride (AlCl_3) colorimetric method [14] with minor modifications. Each extract solution (25 μL) was mixed with 100 μL of distilled water and 7.5 μL of 5% (w/v) sodium nitrite (NaNO_2). The reaction mixture was incubated in the dark for 5 min, after which 15 μL of 10% (w/v) aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) was added, and the mixture was further incubated in the dark for 6 min. The reaction was completed by adding 50 μL of 1 M sodium hydroxide (NaOH) and allowed to react for 11 min. The absorbance was measured at 510 nm. A standard calibration curve constructed with quercetin was used to determine total flavonoid content. The results were expressed as milligrams of quercetin equivalents per gram of sample (mg QE/g).

2.5. Antioxidant Analysis (DPPH, ABTS Radical Scavenging Activities, and Ferric Reducing Antioxidant Power (FRAP) Assay) of HE and UAE Extracts of *C. camphora* Leaves

The DPPH radical scavenging activity was performed following the previous protocol [15] with slight modifications. Each extract solution (100 μL) was mixed with

100 µL of 0.2 mM DPPH solution. The mixture was incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. L-ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control. The percentage of DPPH radical scavenging activity was calculated using the following formula, and the 50% inhibition activity (IC₅₀) of each extract solution was evaluated.

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A/B)] \times 100$$

where A = absorbance of the extract solution and B = absorbance of the solvent (blank) without sample.

The ABTS radical scavenging activity was assessed following the previous method [16] with minor modifications. The ABTS radical solution was prepared by dissolving two tablets of ABTS diammonium salt in 5 mL of distilled water, followed by the addition of 88 µL of 140 mM potassium persulfate. The mixture was incubated in the dark for 14–16 h then diluted with absolute ethanol at a ratio of 1:88 (v/v) to achieve an absorbance of 0.70 ± 0.02 at 734 nm. For the scavenging activity, 10 µL of each extract solution was mixed with 200 µL of the ABTS radical solution and incubated in the dark for 2.5 min, and the absorbance was recorded at 734 nm. L-ascorbic acid was used as a positive control. The percentage of ABTS radical scavenging activity was calculated using the following formula, and the 50% inhibition activity (IC₅₀) of each extract solution was evaluated.

$$\text{ABTS radical scavenging activity (\%)} = [1 - (A/B)] \times 100$$

where A = absorbance of the extract solution and B = absorbance of the solvent (blank) without sample.

The FRAP assay was conducted following the previous protocol [17]. A fresh FRAP working solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine dissolved in 40 mM hydrochloric acid, and 20 mM iron (III) chloride hexahydrate in a 10:1:1 (v/v/v) ratio. A total of 180 µL of the preheated FRAP working solution (37 °C for 10 min) was added to a reaction mixture consisting of 6 µL of the extract solution and 18 µL of distilled water. The reaction was incubated at 37 °C for 10 min, and the absorbance was measured at 593 nm. The FRAP values were calculated from a standard calibration curve constructed with iron (II) sulfate heptahydrate. The results were expressed as M Fe²⁺ equivalents per gram of sample (M Fe²⁺/g).

2.6. Enzyme Activity Analysis

2.6.1. Tyrosinase and Elastase Inhibition Activities of HE and UAE Extracts of *C. camphora* Leaves

The tyrosinase inhibition activity was evaluated by the previous methods [18,19] with slight modifications. A 200 µL aliquot of 0.1 M potassium phosphate buffer (pH 6.8) was mixed with 20 µL of the extract solution and 20 µL of 10 mM 3,4-dihydroxy-L-phenylalanine (L-DOPA). Then, 10 µL of mushroom tyrosinase (100 unit/mL) was added, and the mixture was incubated at 37 °C for 15 min. The absorbance was measured at 475 nm. Kojic acid was used as a positive control. The percentage of tyrosinase inhibition was calculated using the following formula.

$$\text{Tyrosinase inhibition activity (\%)} = [1 - (A - B) / C] \times 100$$

where A = absorbance of the complete mixture containing the buffer, extract and enzyme solutions, B = absorbance of the mixture without the enzyme solution and C = absorbance of the mixture without the extract solution.

The elastase inhibition activity was evaluated by the method of Lee et al. [20] with slight modifications. A 140 µL aliquot of 0.2 M Tris-HCl buffer (pH 8.0) was mixed with 50 µL of 3.2 mM N-succinyl-(Ala)3-p-nitroanilide. Then, 50 µL of each extract solution was added to the mixture, followed by the addition of 8 µL of elastase (Pancreatin from porcine pancreas, PPE, 1 unit/mL). The reaction mixture was incubated at 37 °C for 20 min, and the absorbance was measured at 405 nm. L-ascorbic acid was used as a positive control. The percentage of elastase inhibition was calculated using the following formula.

$$\text{Elastase inhibition activity (\%)} = [1 - (A - B) / C] \times 100$$

where A = absorbance of the complete mixture containing the buffer, extract and enzyme solutions, B = absorbance of the mixture without the enzyme solution and C = absorbance of the mixture without the extract solution.

2.6.2. α-Glucosidase and Lipase Inhibition Activities of HE and UAE Extracts of *C. camphora* Leaves

The α-glucosidase inhibition activity was evaluated by the previous method [21] with slight modifications. A 90 µL aliquot of α-glucosidase (0.1 U/ mL) was mixed with 50 µL of the extract solution and incubated at 37 °C for 15 min. Following incubation, 1 mM p-nitrophenyl-α-glucopyranoside (p-NPG; 100 µL) was added and incubated again for about 5 min, and the absorbance was measured at 405 nm. Acarbose was used as a positive control, and the percentage of α-glucosidase inhibition was calculated using the following formula.

$$\alpha\text{-Glucosidase inhibition activity (\%)} = [1 - (A/B)] \times 100$$

where A = absorbance of the extract solution and B = absorbance of the solvent (blank) without the sample.

The lipase inhibition activity was evaluated using the method described by Kim et al. [22] with slight modifications. The lipase enzyme solution was prepared by dissolving porcine pancreatic lipase (60 mg) in a mixture containing 6 mL of 10 mM MOPS buffer (3-(N-morpholino) propanesulfonic acid) and 6 mL of 1 mM EDTA (pH 6.8). For the reaction procedure, each extract solution (20 µL) was mixed with the prepared lipase enzyme solution (6 µL) and 100 mM Tris-HCl buffer (170 µL; pH 7.0) containing 5 mM CaCl₂, and the mixture was incubated at 37 °C for 15 min. Subsequently, 4 µL of 10 mM 4-nitrophenyl palmitate (p-NPP) was added, and incubation continued at 37 °C for an additional 15 min. The absorbance was measured at 400 nm. Orlistat was used as a positive control, and the inhibition percentage was calculated using the following formula.

$$\text{Lipase inhibition activity (\%)} = [1 - (B - b) / A] \times 100$$

where B = absorbance of the complete mixture containing the buffer, extract and enzyme solutions, b = absorbance of the mixture without the enzyme solution and A = absorbance of the mixture without the extract solution.

2.7. Antimicrobial Activity

Four bacterial strains from the Korean Collection for type culture (KCTC) were selected to evaluate the antimicrobial activity of *C. camphora* extracts. *Staphylococcus aureus* (*S. aureus*) (KCTC 1621) and *Cutibacterium acnes* (*C. acnes*) KCTC 3314 were selected as Gram-positive bacteria, whereas *Escherichia coli* (*E. coli*) KCTC 1112 and *Pseudomonas aeruginosa* (*P. aeruginosa*) KCTC 2450 were selected to represent Gram-negative bacteria. Each bacterial strain was inoculated in Nutrient Broth (NB) and incubated at 30 °C, except for

C. acnes, which was cultured in Gifu Anaerobic Medium Broth (GAM-B) and incubated at 37 °C. All inoculated bacterial strains were subcultured three times at 24 h intervals to ensure optimal growth conditions. After three subculturings, the optical density of each bacterial suspension was measured at 600 nm using a UV–Vis spectrophotometer (Optizen Pop-s, KLab Inc., Seoul, Republic of Korea) and adjusted to an absorbance range of 0.2–0.4 corresponding to approximately 1×10^5 CFU/mL.

The antimicrobial activity of the HE and UAE extracts of *C. camphora* leaves were assessed using the paper-disc diffusion method [23]. A 200 µL aliquot of each bacterial suspension was evenly spread onto the surface of sterilized agar media: Nutrient Agar (NA) for most strains and Gifu Anaerobic Medium Agar (GAM-A) for *C. acnes*. Sterile paper discs (8 mm diameter) were impregnated with 100 µL of each extract solution at concentrations of 10 mg/disc and 5 mg/disc and allowed to air-dry. The treated paper discs were then placed onto the inoculated agar plates and incubated at 30 °C for 24 h. However, *C. acnes* plates were anaerobically incubated at 37 °C for 48 h. Following incubation, the diameters of inhibition zones were recorded.

2.8. Gas Chromatography–Mass Spectroscopy Detection (GC-MSD) Analysis of HE and UAE Extracts of *C. camphora* Leaves

GC-MSD analyses of HE and UAE extracts of *C. camphora* leaves were assessed using an Agilent 5977B GC–MSD system (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was performed on an HP-5ms capillary column (30 m $\times$ 0.25 µm $\times$ 0.25 µm; Agilent 19091S-433). Helium was employed as the carrier gas at a constant flow rate of 1.0 mL/min, and the injection volume was 1 µL. The front inlet was operated in splitless mode with the injector temperature set at 230 °C. The oven temperature was initially maintained at 60 °C for 3 min, then increased to 300 °C at a rate of 10 °C/min, and maintained at 300 °C for an additional 3 min, resulting in a total run time of 30 min. The MSD was operated in Electron Ionization (EI) mode at 70 eV and scan mode (m/z 10–600) after a 2 min solvent delay. The MS source, MS quadrupole, and transfer line temperatures were maintained at 230 °C, 150 °C, and 230 °C, respectively.

2.9. Statistical Analysis

All experiments, except antimicrobial activity, were conducted in triplicate, and the results are presented as mean $\pm$ standard deviation ($n = 3$). The assumptions of normality and homogeneity of variances were evaluated using the Shapiro–Wilk and Levene’s tests, respectively. Subsequently, analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) with IBM SPSS Statistics (Statistical Package for the Social Sciences) software (version 27.0., 2021 SPSS Inc., Chicago, IL, USA) were performed to assess the statistical significance ($p < 0.05$).

3. Results

3.1. Yields of HE and UAE Extracts of *C. camphora* Leaves

The yield was calculated on a volume basis by comparing the volume of the concentrated extract obtained after rotary evaporation with the volume of the extract after vacuum filtration and is reported as a volume-based yield (%), as shown in Table 1. The yield decreased with increasing ethanol concentration, with 30% ethanol producing the highest yields, followed by 50% and 70%. Compared to the UAE method, the HE method consistently resulted in higher extraction yields, reaching 41.5%, 20.0%, and 5.74% at 30%, 50%, and 70% ethanol, respectively, whereas the UAE yields were 29.5%, 10.7%, and 4.71%.

Table 1. Volume-based yields of HE and UAE extracts of *C. camphora* leaves using 30%, 50% and 70% ethanol.

Extracts	After Vacuum Filtration (mL)	After Rotatory Evaporation (mL)	Yield (% <i>v/v</i>) ¹
HE 30%	328	136	41.5
HE 50%	350	70	20.0
HE 70%	470	27	5.74
UAE 30%	220	65	29.5
UAE 50%	290	31	10.7
UAE 70%	340	16	4.71

¹ Yield (%) calculated against the volume of the concentrated extract obtained after rotary evaporation with the volume of the extract after vacuum filtration.

3.2. Total Polyphenol and Flavonoid Contents of HE and UAE Extracts of *C. camphora* Leaves

Polyphenolic compounds are plant secondary metabolites that possess a wide range of biological effects, which are largely attributed to radical scavenging and antioxidant activities [24]. The results of the total polyphenol and flavonoid contents of HE and UAE extracts of *C. camphora* leaves using 30%, 50% and 70% ethanol are expressed in Table 2.

Table 2. Total polyphenol and flavonoid content of HE and UAE extracts of *C. camphora* leaves using 30%, 50% and 70% ethanol.

Extracts	Total Polyphenol Content (mg GAE ¹ /g)	Total Flavonoid Content (mg QE ² /g)
HE 30%	6.46 ± 0.35 ^f	5.87 ± 0.00 ^{f3}
HE 50%	28.72 ± 0.95 ^d	27.66 ± 0.42 ^d
HE 70%	163.7 ± 1.40 ^b	93.74 ± 0.42 ^b
UAE 30%	14.35 ± 0.35 ^e	13.22 ± 0.00 ^e
UAE 50%	90.84 ± 0.93 ^c	79.54 ± 0.86 ^c
UAE 70%	363.0 ± 1.40 ^a	174.5 ± 0.42 ^a

¹ GAE: gallic acid equivalents. ² QE: quercetin equivalents. ³ Mean ± Standard Deviation (*n* = 3). ^{a–f} Values with different letters are significantly different (*p* < 0.05) by Duncan's multiple range test.

As shown in Table 2, for HE extracts, the highest TPC was observed in the 70% ethanol extract (163.7 ± 1.40 mg GAE/g), followed by the 50% (28.72 ± 0.95 mg GAE/g) and the 30% (6.46 ± 0.35 mg GAE/g). A similar trend was observed for UAE extracts, with the 70% ethanol extract exhibiting the highest TPC (363.0 ± 1.40 mg GAE/g), followed by the 50% (90.84 ± 0.93 mg GAE/g) and the 30% (14.35 ± 0.35 mg GAE/g). For the total flavonoid content, the highest TFC for HE extracts was observed in the 70% ethanol extract (93.74 ± 0.42 mg QE/g), followed by the 50% (27.66 ± 0.42 mg QE/g) and the 30% (5.87 ± 0.00 mg QE/g). Similarly, the UAE extracts showed the highest TFC in the 70% ethanol extract (174.5 ± 0.42 mg QE/g), also followed by the 50% (79.54 ± 0.86 mg QE/g) and the 30% (13.22 ± 0.00 mg QE/g). For both extraction methods, total polyphenol and flavonoid content showed a statistically significant decreasing trend (*p* < 0.05). Overall, 70% ethanol proved to be the most effective solvent for extracting polyphenolic and flavonoid compounds from *C. camphora* leaves.

3.3. Antioxidant Activities (DPPH, ABTS Radical Scavenging Activities and FRAP Assay) of HE and UAE Extracts of *C. camphora* Leaves

The DPPH radical scavenging activity is a widely used spectrophotometric method to evaluate antioxidant activity based on the reduction of the stable DPPH radical by a hydrogen atom or electron transfer [25]. The ABTS radical scavenging activity assesses antioxidant activity by detecting the radical scavenging effects of hydrogen-donating

antioxidants in aqueous phases as well as chain-breaking antioxidants targeting lipid peroxyl radicals [26]. The FRAP assay evaluates antioxidant reducing power by measuring the ability of antioxidants to reduce the ferric–tripyridyltriazine (Fe^{3+} –TPTZ) complex to its ferrous form (Fe^{2+} –TPTZ) under acidic conditions, resulting in the formation of a blue-colored complex [27]. The antioxidant potential of the HE and UAE extracts was evaluated using DPPH, ABTS, and FRAP assays, and the results are presented in Table 3.

Table 3. DPPH and ABTS radical scavenging activities and FRAP assay of HE and UAE extracts using *C. camphora* leaves and using 30%, 50% and 70% ethanol.

Extracts	DPPH Radical Scavenging Activity IC_{50}^1 (mg/mL)	ABTS Radical Scavenging Activity IC_{50}^1 (mg/mL)	FRAP Assay ($\text{M Fe}^{2+}/\text{g}$)
HE 30%	0.214 ± 0.008^a	2.508 ± 0.008^a	0.521 ± 0.029^{e2}
HE 50%	0.176 ± 0.011^b	1.195 ± 0.018^c	0.886 ± 0.037^d
HE 70%	0.081 ± 0.004^d	0.632 ± 0.014^e	2.178 ± 0.167^b
UAE 30%	0.146 ± 0.016^c	1.289 ± 0.007^b	0.609 ± 0.002^e
UAE 50%	0.048 ± 0.002^e	0.778 ± 0.006^d	1.187 ± 0.139^c
UAE 70%	0.024 ± 0.001^f	0.363 ± 0.002^f	3.080 ± 0.044^a
Positive Control	0.004 ± 0.002^{g3}	0.116 ± 0.009^{g4}	-

¹ The concentration of the extracts and standard to scavenge 50% of DPPH and ABTS radicals. ² Mean $\pm$ Standard Deviation ($n = 3$). ^{3,4} L-ascorbic acid. ^{a–g} Values with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

In the DPPH radical scavenging activity, IC_{50} values ranged from 0.081 ± 0.004 to 0.214 ± 0.008 mg/mL for HE extracts and from 0.024 ± 0.001 to 0.146 ± 0.016 mg/mL for UAE extracts. In the ABTS radical scavenging activity, IC_{50} values varied between 0.632 ± 0.014 and 2.508 ± 0.008 mg/mL for HE extracts and between 0.363 ± 0.002 and 1.289 ± 0.007 mg/mL for UAE extracts. In the FRAP assay, reducing power increased proportionally with ethanol concentration, ranging from 0.521 ± 0.029 to 2.178 ± 0.167 M Fe^{2+}/g for HE extracts and from 0.609 ± 0.002 to 3.080 ± 0.044 M Fe^{2+}/g for UAE extracts. The 70% ethanol extract consistently showed the highest antioxidant activities, with a statistically significant difference ($p < 0.05$).

3.4. Tyrosinase and Elastase Inhibition Activities of HE and UAE Extracts of *C. camphora* Leaves

Tyrosinase plays a key role in melanin biosynthesis, and its inhibition is a fundamental strategy for controlling skin hyperpigmentation, making tyrosinase inhibitors important targets in cosmetic applications [28]. Elastase inhibition activity was evaluated to assess the anti-aging potential of the extracts, as elastase plays a crucial role in the degradation of elastin in the dermal extracellular matrix, leading to a loss of skin elasticity [29]. In this study, the tyrosinase and elastase inhibition activities were evaluated at a concentration of 0.5 mg/mL for both the extracts and the corresponding positive controls, and the results are presented in Table 4.

As shown in Table 4, tyrosinase inhibition activity in HE extracts increased with ethanol concentration, with the highest inhibition observed for the 70% ethanol extract ($42.7 \pm 0.09\%$), followed by the 50% ($40.9 \pm 0.23\%$) and the 30% ($35.6 \pm 0.23\%$). A similar trend was observed for UAE extracts, where the 70% ethanol extract exhibited the highest inhibition ($49.3 \pm 0.35\%$), followed by the 50% ($44.8 \pm 0.14\%$) and the 30% ($41.8 \pm 0.21\%$). Kojic acid, used as a positive control, showed significantly higher inhibition ($90.7 \pm 0.32\%$) than all extracts. For elastase inhibition activity, the 70% ethanol extract exhibited the highest inhibition in HE extracts ($22.7 \pm 0.41\%$), followed by the 50% ($14.7 \pm 0.35\%$) and the 30% ($8.61 \pm 0.17\%$). Similarly, UAE extracts showed the highest elastase inhibition at the 70% ethanol extract ($24.8 \pm 0.40\%$), followed by the 50% ($22.2 \pm 0.40\%$) and the 30% ($18.0 \pm 0.05\%$). The elastase inhibition activities of all extracts were significantly lower than

those of the positive control, L-ascorbic acid ($75.3 \pm 0.87\%$). Overall, extracts obtained using higher ethanol concentrations exhibited higher tyrosinase and elastase inhibition activities across both extraction methods ($p < 0.05$), with the UAE extracts showing consistently higher inhibition activities than the corresponding HE extracts.

Table 4. Tyrosinase and elastase inhibition activities of HE and UAE extracts using *C. camphora* leaves and using 30%, 50% and 70% ethanol.

Extracts	Tyrosinase Inhibition Activity (%) (0.5 mg/mL)	Elastase Inhibition Activity (%) (0.5 mg/mL)
HE 30%	35.6 ± 0.23^g	8.61 ± 0.17^{f1}
HE 50%	40.9 ± 0.23^f	14.7 ± 0.35^e
HE 70%	42.7 ± 0.09^d	22.7 ± 0.41^c
UAE 30%	41.8 ± 0.21^e	18.0 ± 0.05^d
UAE 50%	44.8 ± 0.14^c	22.2 ± 0.40^c
UAE 70%	49.3 ± 0.35^b	24.8 ± 0.34^b
Positive Control	90.7 ± 0.32^{a2}	75.3 ± 0.87^{a3}

¹ Mean $\pm$ Standard Deviation ($n = 3$). ² Kojic acid. ³ L-ascorbic acid. ^{a–g} Values with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

3.5. α -Glucosidase and Lipase Inhibition Activities of HE and UAE Extracts of *C. camphora* Leaves

α -Glucosidase is responsible for the hydrolysis of oligosaccharides into absorbable glucose, and inhibition of this enzyme represents an effective strategy for controlling post-prandial blood glucose levels; however, synthetic inhibitors including acarbose, miglitol, and voglibose are associated with adverse gastrointestinal effects [30]. Pancreatic lipase plays a central role in dietary fat digestion, and its inhibition is an effective strategy for reducing fat absorption and managing obesity; consequently, plant extracts rich in polyphenols and flavonoids have been widely investigated as natural lipase inhibitors [31]. The α -glucosidase and lipase inhibition activities of *C. camphora* extracts and the corresponding positive controls were evaluated at 0.5 mg/mL in this study, and the results are summarized in Table 5.

Table 5. α -Glucosidase and lipase inhibition activities of HE and UAE extracts using *C. camphora* leaves and using 30%, 50% and 70% ethanol.

Extracts	α -Glucosidase Inhibition Activity (%) (0.5 mg/mL)	Lipase Inhibition Activity (%) (0.5 mg/mL)
HE 30%	56.4 ± 0.89^e	82.5 ± 0.75^{c1}
HE 50%	63.8 ± 0.58^d	89.1 ± 1.58^b
HE 70%	92.4 ± 0.49^b	98.0 ± 1.69^a
UAE 30%	80.2 ± 0.18^c	54.2 ± 0.39^f
UAE 50%	91.9 ± 0.14^b	69.2 ± 0.60^e
UAE 70%	94.5 ± 0.12^a	77.5 ± 1.11^d
Positive Control	51.9 ± 0.08^{f2}	82.4 ± 0.61^{c3}

¹ Mean $\pm$ Standard Deviation ($n = 3$). ² Acarbose. ³ Orlistat. ^{a–f} Values with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

As shown in Table 5, α -glucosidase inhibition activity ranged from $56.4 \pm 0.89\%$ to $92.4 \pm 0.49\%$ in HE extracts, while UAE extracts exhibited inhibition activities ranging from $80.2 \pm 0.18\%$ to $94.5 \pm 0.12\%$. For both extraction methods, all extracts showed higher inhibition activity than that of the positive control acarbose ($51.9 \pm 0.08\%$). The lipase inhibition activity ranged from $82.5 \pm 0.75\%$ to $98.0 \pm 1.69\%$ in HE extracts, whereas UAE showed lower inhibition activity ranging from $54.2 \pm 0.39\%$ to $77.5 \pm 1.11\%$. HE extracts exhibited statistically significant higher lipase inhibition than the corresponding

UAE extracts ($p < 0.05$), with the 70% ethanol extract showing the highest inhibition activity among all samples. Notably, the lipase inhibition activity of HE extracts was comparable to or exceeded that of the positive control orlistat ($82.4 \pm 0.61\%$) under the same experimental conditions. Overall, extracts obtained using higher ethanol concentrations exhibited higher α -glucosidase and lipase inhibition activities across both extraction methods ($p < 0.05$).

3.6. Antimicrobial Activity of HE and UAE Extracts of *C. camphora* Leaves

Plant-derived antimicrobial agents have attracted growing interest as alternative or complementary therapeutic options, as plants are rich sources of bioactive compounds with established antibacterial and antifungal properties [32]. The antimicrobial activity of *C. camphora* extracts are described in Table 6.

Table 6. Antimicrobial activity of HE and UAE extracts of *C. camphora* leaves using 30%, 50% and 70% ethanol against selected microorganisms.

Microorganisms	Extracts	Diameter of Inhibition Zone (mm)	
		5 mg/disc	10 mg/disc
Gram-Positive	<i>C. acnes</i>	HE 30%	¹
		HE 50%	12.0
		HE 70%	15.0
		UAE 30%	-
		UAE 50%	15.0
		UAE 70%	17.0
	<i>S. aureus</i>	HE 30%	-
		HE 50%	-
		HE 70%	18.0
		UAE 30%	-
		UAE 50%	10.0
		UAE 70%	19.0
Gram-Negative	<i>E. coli</i>	HE 30%	-
		HE 50%	-
		HE 70%	-
		UAE 30%	-
		UAE 50%	-
		UAE 70%	11.0
	<i>P. aeruginosa</i>	HE 30%	-
		HE 50%	-
		HE 70%	11.0
		UAE 30%	-
		UAE 50%	11.0
		UAE 70%	12.0

¹ Not detected.

As shown in Table 6, the strongest antimicrobial activity was observed against *C. acnes*, for which all extracts exhibited clear inhibition zones at 10 mg/disc. Notably, the 70% ethanol UAE extract showed the greatest activity, with an inhibition zone of 23.0 mm. Against *S. aureus*, all extracts except the 30% ethanol HE extract produced measurable inhibition at 10 mg/disc, with the highest activity again observed for the 70% ethanol UAE extract (22.0 mm). In contrast, moderate antimicrobial activities were detected against the Gram-negative strains, *E. coli* and *P. aeruginosa*, with the largest inhibition zones recorded for the 70% ethanol UAE extract at 10 mg/disc (14.0 and 15.0 mm, respectively). Overall, antimicrobial activity increased with ethanol concentration and was generally more pronounced in the UAE extracts, particularly against Gram-positive bacteria.

3.7. Gas Chromatography–Mass Spectrometry Detection (GC-MSD) of HE and UAE Extracts of *C. camphora* Leaves

The GC-MSD chromatograms of the HE and UAE extracts of *C. camphora* leaves are presented in Figure 1, with the corresponding chemical constituents with their retention times, classifications, major ions [m/z], and their CAS Nos. are summarized in Table 7.

The GC-MSD analysis identified a variety of predominant bioactive compounds, including phenolic derivatives, monoterpenes, triterpenes, and sesquiterpenes, several of which have been reported in the literature to be associated with antioxidant, antimicrobial, antidiabetic, anti-lipase and anti-inflammatory activities. For HE extracts, no detectable peaks were observed in the 30% ethanol extract yielded, possibly due to low extraction efficiency or limitations of the GC-MSD conditions for detecting these compounds. The 50% ethanol extract was dominated by 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-(6S-cis) (29.5%), a compound previously reported to exhibit antioxidant activity [33]. The 70% ethanol extract exhibited greater chemical diversity, containing nine major compounds, with Pluchidiol (8.40%) as the most abundant, contributing to its antioxidant activity [34]. For UAE extracts, alpha-Benzamido-2-hydroxycinnamic acid (43.7%) and (trans)-Isoeugenol-Methyl Ether (26.5%) were the predominant compounds in the 30% ethanol extract, both of which have been reported to have antioxidant, antimicrobial, and anti-inflammatory properties [35]. In the 50% and 70% ethanol extracts, 2(4H)-Benzofuranone derivatives (6S-cis) and alpha-Benzamido-2-hydroxycinnamic acid were the predominant compounds, consistent with their reported antioxidant-related properties [36].

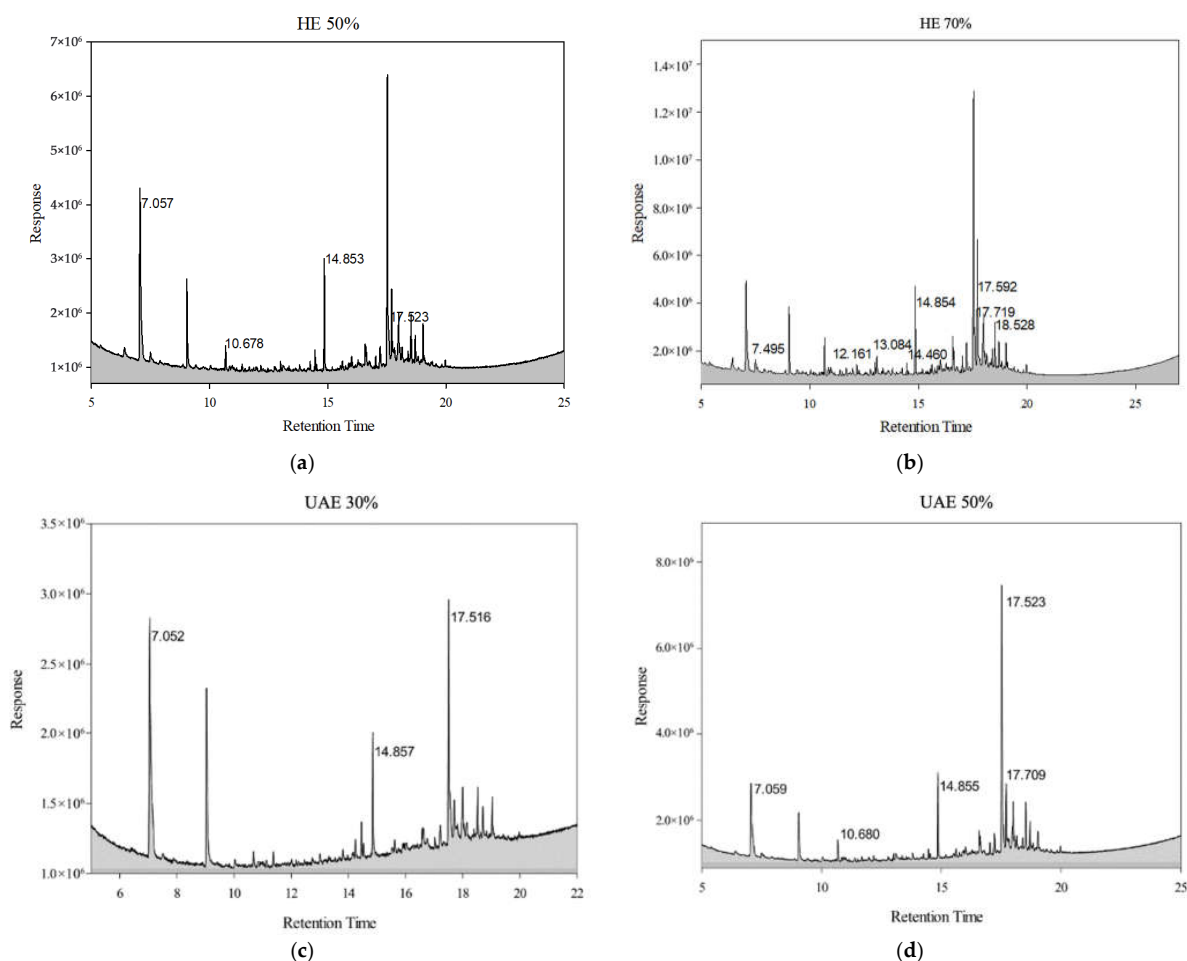
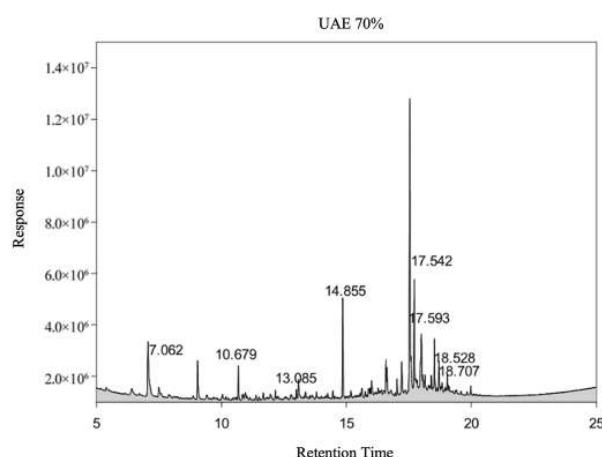


Figure 1. Cont.



(e)

Figure 1. GC-MSD chromatograms of HE and UAE extracts of *C. camphora* leaves. (a) HE 50%; (b) HE 70%; (c) UAE 30%; (d) UAE 50%; (e) UAE 70%.

Table 7. Chemical constituents and peak areas of bioactive compounds detected in GC-MSD analysis of HE and UAE extracts of *C. camphora* leaves using 30%, 50% and 70% ethanol.

Extracts	Rt ¹ (min)	Compounds	Classification	Major Ions m/z	CAS No.	Relative Peak Area (%)
HE 50%	7.057	alpha-Benzamido-2-hydroxycinnamic acid	Phenolic derivative	148.10, 105.00, 77.00	2000418-22-6	27.5%
	10.68	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	Maleimide	139.10, 96.10, 67.10	020189-42-8	2.11%
	14.85	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	Triterpene	180.10, 137.10, 111.00	015356-74-8	8.64%
	17.52	5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6S-cis)- (Loliolide)	Terpene	178.10, 140.00, 111.10	005989-02-6	29.5%
HE 70%	7.495	Benzenemethanol	Aromatic alcohol	108.10, 79.10, 51.10	000100-51-6	1.14%
	12.16	2-Oxabicyclo [2.2.2] octane, 1,3,3-trimethyl-(Eucalyptol)	Cyclic monoterpene oxide	142.10, 83.10, 43.00	000470-82-6	0.70%
	13.08	Benzaldehyde, 4-hydroxy-3-methoxy (Vanillin)	Aromatic aldehyde	137.10, 111.10, 84.00	000121-33-5	2.09%
	14.46	Phenol, 2,4-bis(1,1-dimethylethyl)	Aromatic hydrocarbon	191.20, 163.10, 84.00	000096-76-4 9	0.75%
	14.86	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Triterpene	180.10, 137.10, 111.00	017092-92-1	5.77%
	17.59	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-(Spathulenol)	Sesquiterpene	163.10, 123.10, 93.10	006750-60-3	3.46%
	17.72	Pluchidiol	Phenolics	166.10, 124.10, 79.10	2000224-58-5	8.40%
	18.53	Platambin	Sesquiterpene	159.10, 133.10, 81.10	058556-80-29	3.41%
UAE 30%	7.052	alpha-Benzamido-2-hydroxycinnamic acid	Phenolic derivative	148.10, 105.00, 77.00	2000463-15-2	43.7%
	14.86	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	Triterpene	180.10, 137.10, 67.10	015356-74-8	10.5%
	17.52	(trans)-Isoeugenol-Methyl Ether	Phenylpropene	178.10, 140.00, 111.10	2000138-02-1	26.5%

Table 7. Cont.

Extracts	Rt ¹ (min)	Compounds	Classification	Major Ions m/z	CAS No.	Relative Peak Area (%)
UAE 50%	7.059	alpha-Benzamido-2-hydroxycinnamic acid	Phenolic derivative	148.10, 105.00, 77.00	2000463-16-2	16.7%
	10.68	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl	Maleimide	139.10, 96.00, 67.10	020189-42-8	2.51%
	14.86	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	Triterpene	180.10, 137.10, 111.10	017092-92-1	8.56%
	17.52	5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6S-cis) (Loliolide)	Terpene	178.10, 140.00, 111.10	005989-02-6	33.7%
	17.71	2-Cylcohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)	Cyclic α , β -unsaturated ketone	166.00, 124.10, 95.00	077846-84-5	8.39%
UAE 70%	7.062	alpha-Benzamido-2-hydroxycinnamic acid	Phenolic derivative	148.10, 127.00, 105.00	2000463-15-2	10.3%
	10.68	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl	Maleimide	139.10, 96.00, 67.10	020189-42-8	2.95%
	13.09	Benzaldehyde, 4-hydroxy,3-methoxy (Vanillin)	Aromatic aldehyde	151.00, 123.10, 100.9	000121-33-5	2.22%
	14.86	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	Triterpene	180.00, 137.10, 111.00	015356-74-8	7.71%
	17.54	5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6S-cis)	Triterpene	178.10, 140.00, 111.1	005989-02-6	29.1%
	17.59	Terephthalic acid dihydrazide	Fatty hydrazide derivative	162.10, 135.10, 107.10	000136-64-1	3.19%
	18.53	Platambin	Sesquiterpene	159.10, 133.10, 107.10	058556-80-2	4.14%
	18.71	4,4,8-Trimethyltricyclo [6.3.1.0(1,5)] dodecane-2,9-diol	Sesquiterpene alcohol	164.10, 135.10, 107.10	2000319-49-5	2.84%

¹ Retention time.

4. Discussion

This study demonstrates that solvent composition and the extraction technique are decisive factors governing the phytochemical profiles and bioactivities of *C. camphora* leaf extracts. Hydrothermal extraction produced higher volume-based yields than ultrasound-assisted extraction, reflecting differences in extract concentration after solvent removal rather than an absolute recovery of chemical constituents. Such behavior is consistent with moderate thermal conditions in enhancing solvent penetration and mass transfer. Similar observations have been reported in previous studies, where elevated extraction yields under thermal conditions did not necessarily correspond to increased phenolic enrichment [37,38]. In contrast, extracts prepared with 70% ethanol consistently exhibited the highest total polyphenol and flavonoid contents, confirming that solvent polarity, rather than total yield, is the primary determinant of phenolic recovery in this species. Intermediate ethanol–water mixtures have been widely reported to provide optimal solvation for phenolic compounds due to balanced polarity and hydrogen-bonding capacity [39]. Furthermore, the UAE extracts contained significantly higher polyphenol and flavonoid contents than HE, suggesting improved extraction selectivity under UAE conditions. This effect is commonly attributed to ultrasonic cavitation, which enhances mass transfer and facilitates intracellular release while minimizing prolonged thermal exposure [40,41].

The enhanced phenolic enrichment was reflected in antioxidant performance. UAE extracts consistently exhibited stronger DPPH, ABTS, and FRAP activities, demonstrating a close association between phenolic content and radical scavenging capacity. This relationship is mechanistically supported by the redox properties of polyphenols and flavonoids, which act as electron and hydrogen donors and chelate pro-oxidant metal ions [42]. Similar positive relationships between total phenolic content and antioxidant capacity have

been reported for other medicinal plant extracts, particularly when ultrasound-assisted or hydro-alcoholic extraction methods were employed [43,44]. Collectively, these findings indicate that phenolic compounds are major contributors to the antioxidant activity of *C. camphora* leaf extracts in this study.

Enzyme inhibition activities relative to cosmetic and functional applications exhibited a comparable trend. Tyrosinase, elastase, and α -glucosidase inhibition increased with ethanol concentration and was consistently higher in UAE extracts, indicating that extraction conditions favoring phenolic enrichment also enhance enzyme inhibitory potential. Polyphenol- and flavonoid-rich extracts have been reported to inhibit tyrosinase and elastase through hydrogen bonding and hydrophobic interactions at enzyme active sites, supporting their relevance to skin-whitening and anti-aging applications [45–49]. Similarly, flavonoids and related compounds contribute to α -glucosidase inhibition through hydrogen bonding and π - π stacking interactions [50,51]. These observations suggest that enhanced phenolic and flavonoid compounds in the extracts is associated with increased enzyme inhibition potential. In contrast, lipase inhibition was higher in HE extracts, which may be associated with heat-induced differences in phytochemical composition under hydrothermal conditions, resulting in an extract profile distinct from that obtained by ultrasound-assisted extraction [52,53]. Previous studies have shown that lipase inhibition is mediated by hydrophobic and π - π interactions involving moderately polar flavonoids and related phytochemicals [54], which are not necessarily the primary contributors to antioxidant activity. Therefore, the observed lipase inhibition likely reflects differences in the overall phytochemical composition rather than the action of individual constituents.

Antimicrobial activity assessed using the paper-disc diffusion method revealed greater activity against Gram-positive bacteria (*C. acnes* and *S. aureus*) than against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). A similar pattern has been widely reported for *C. camphora* species and other plant extracts, where Gram-positive bacteria generally exhibit higher susceptibility than Gram-negative strains [55]. The antimicrobial activity observed is likely attributable to the combined action of multiple constituents, including phenolic acids and oxygenated monoterpenes. These compounds can simultaneously target different bacterial structures—such as cell wall synthesis and membrane permeability—enhancing the overall efficacy of the extracts for anti-acne cosmetic applications [56–58].

The GC-MSD analysis, performed solely to identify the volatile and semi-volatile constituents, provided a descriptive chemical profile present in the extracts. The results indicate that both solvent composition and the extraction technique influenced the qualitative composition and relative abundance of volatile compounds, providing an overview of the volatile phytochemical composition of the extracts and characterizing how extraction conditions affect their chemical profiles.

Overall, the enhanced antioxidant, enzyme inhibition, and antimicrobial activities observed in the extracts obtained with higher ethanol compositions and ultrasound-assisted extraction were closely associated with increased polyphenol and flavonoid enrichment. These results support the study's objective by demonstrating that extraction strategy and solvent polarity critically influence the phytochemical composition and, consequently, the functional bioactivity profile of *C. camphora* leaf extracts.

5. Conclusions

In conclusion, phytochemical composition in *C. camphora* leaves was significantly influenced by the solvent polarity and extraction methods. The 70% ethanol extract obtained from the UAE method exhibited the highest antioxidant, antimicrobial, and enzyme inhibition properties. Conversely, the HE method was particularly effective in extracting bioactive compounds with lipase inhibition activity. The GC-MSD analysis effectively

characterized major volatile compounds, including phenolics, terpenes, triterpenes, and sesquiterpenes, known for their reported diverse bioactivities. These findings highlight the potential applications of hydrothermal and ultrasound-assisted extracts of *C. camphora* leaves as antioxidants, as well as enzyme inhibitors and antimicrobial agents in the food, cosmetic and pharmaceutical industries.

Author Contributions: Conceptualization, M.P.P., Y.K. and W.K.; methodology, M.P.P. and Y.C.; software, M.P.P. and Y.C.; validation, M.P.P., Y.C. and Y.K.; formal analysis, M.P.P. and Y.C.; investigation, M.P.P. and Y.C.; resources, Y.K. and W.K.; data curation, M.P.P.; writing—original draft preparation, M.P.P.; writing—review and editing, M.P.P., Y.K., H.J., S.L. and W.K.; visualization, M.P.P.; supervision, W.K.; project administration, W.K.; funding acquisition, W.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Korea Institute for Advancement of Technology (KIAT) grant funded by the Korean government (MOTIE) (RS-2024-00409639, HRD Program for Industrial Innovation) and the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (RS-2023-00281517).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding authors.

Acknowledgments: I would like to express my heartfelt gratitude to all the authors who supported the completion of this research, especially to the Regional Innovation System & Education (RISE) program through the Daejeon RISE Center, funded by the Ministry of Education (MOE) and the Daejeon Metropolitan City, Republic of Korea (2025-RISE-06-013).

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

References

- Ooi, S.L.; Pak, S.C. Natural products with pharmaceutical activities. *Molecules* **2025**, *30*, 4557. [CrossRef] [PubMed]
- Zhang, T.; Zheng, Y.; Fu, C.; Yang, H.; Liu, X.; Qiu, F.; Wang, X.; Wang, Z. Chemical variation and environmental influence on essential oil of *Cinnamomum camphora*. *Molecules* **2023**, *28*, 973. [CrossRef] [PubMed]
- Lee, S.H.; Kim, D.S.; Park, S.H.; Park, H. Phytochemistry and applications of *Cinnamomum camphora* essential oils. *Molecules* **2022**, *27*, 2695. [CrossRef]
- Sobhy, S.; Al-Askar, A.A.; Bakhiet, E.K.; Elsharkawy, M.M.; Arishi, A.A.; Behiry, S.I.; Abdelkhalek, A. Phytochemical characterization and antifungal efficacy of Camphor (*Cinnamomum camphora* L.) extract against phytopathogenic fungi. *Separations* **2023**, *10*, 189. [CrossRef]
- Zhu, W.; Hu, C.; Ren, Y.; Lu, Y.; Song, Y.; Ji, Y.; Han, C.; He, J. Green synthesis of zinc oxide nanoparticles using *Cinnamomum camphora* (L.) Presl leaf extracts and its antifungal activity. *J. Environ. Chem. Eng.* **2021**, *9*, 106659. [CrossRef]
- Jiang, H.; Wang, J.; Song, L.; Cao, X.; Yao, X.; Tang, F.; Yue, Y. GC×GC-TOFMS analysis of essential oils composition from leaves, wigs and seeds of *Cinnamomum camphora* L. Presl and their insecticidal and repellent activities. *Molecules* **2016**, *21*, 423. [CrossRef]
- Poudel, D.K.; Rokaya, A.; Ojha, P.K.; Timsina, S.; Satyal, R.; Dosoky, N.S.; Satyal, P.; Setzer, W.N. The chemical profiling of essential oils from different tissues of *Cinnamomum camphora* L. and their antimicrobial activities. *Molecules* **2021**, *26*, 5132. [CrossRef]
- Nisar, M.F.; Khadim, M.; Rafiq, M.; Chen, J.; Yang, Y.; Wan, C.C. Pharmacological properties and health benefits of eugenol: A comprehensive review. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 2497354. [CrossRef]
- Min, Z.; Zheng, B.; Yan, D. Distinction and pharmacological activity of monoterpenes and sesquiterpenes in different chemotypes of *Cinnamomum camphora* (L.) Presl. *Appl. Sci.* **2025**, *15*, 8922. [CrossRef]
- Chen, Q.; Zhao, X.; Lu, T.; Yang, Y.; Hong, Y.; Tian, M.; Zhou, Y. Chemical composition, antibacterial, and anti-inflammatory activities of essential oils from flower, leaf, and stem of *Rhynchanthus beesianus*. *BioMed Res. Int.* **2021**, *2021*, 5562461. [CrossRef]
- Cherif, M.C.; Boughendjioua, H.; Caputo, L.; Camele, I.; Tahraoui, T.; De Feo, V.; Elshafie, H.S. FTIR characterization and bioactivity assessment of *Cinnamomum camphora* essential oil: Antioxidant, anti-enzymatic, and antifungal properties against phytopathogens. *Chem. Biodivers.* **2025**, *22*, e00720. [CrossRef]

12. Nguyen, T.; Mai, K.; Doan, D.; Phan, T.P. Optimization of extraction of volatile compounds from *Cinnamomum camphora* (L.) J. S. Presl leaves using microwave-assisted hydro-distillation method. *Trop. J. Nat. Prod. Res.* **2025**, *9*, 2214. [[CrossRef](#)]
13. Junsathian, P.; Nakamura, S.; Katayama, S.; Rawdkuen, S. Antioxidant and antimicrobial activities of Thai edible plant extracts prepared using different extraction techniques. *Molecules* **2022**, *27*, 6489. [[CrossRef](#)] [[PubMed](#)]
14. Boubker, A.; El Ouardi, A.; El Kamli, T.; El Hamidi, A.; Kaicer, M.; Kichou, F.; Ameer, N.; Errafii, K.; Ben Aakame, R.; Sifou, A. Phytochemical analysis, antioxidant and antibacterial activities, minerals element profiling, and identification of bioactive compounds by UPLC-HRMS Orbitrap in four aromatic and medicinal plants. *Molecules* **2025**, *30*, 1279. [[CrossRef](#)] [[PubMed](#)]
15. Baliyan, S.; Mukherjee, R.; Priyadarshini, A.; Vibhuti, A.; Gupta, A.; Pandey, R.P.; Chang, C.M. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules* **2022**, *27*, 1326. [[CrossRef](#)]
16. Uwineza, P.A.; Gramza-Michałowska, A.; Bryła, M.; Waśkiewicz, A. Antioxidant activity and bioactive compounds of *Lamium album* flower extracts obtained by supercritical fluid extraction. *Appl. Sci.* **2021**, *11*, 7419. [[CrossRef](#)]
17. Heckmann, M.; Stadlbauer, V.; Drotarova, I.; Gramatte, T.; Feichtinger, M.; Arnaut, V.; Atzmüller, S.; Schwarzing, B.; Röhl, C.; Blank-Landeshammer, B.; et al. Identification of Oxidative-stress-reducing plant extracts from a novel extract library—Comparative analysis of cell-free and cell-based in vitro assays to quantitate antioxidant activity. *Antioxidants* **2024**, *13*, 297. [[CrossRef](#)]
18. Li, J.J.; Kim, K.H.; Yook, H.S. Evaluation of the fermented products of *Fructus Ligustri Lucidi* for use as raw materials in functional health foods and cosmetics. *J. Korean Soc. Food Sci. Nutr.* **2023**, *52*, 691–700. [[CrossRef](#)]
19. Choi, M.H.; Kim, K.H.; Yook, H.S. Antioxidant activity of fermented *Kaempferia parviflora* and inhibitory action against tyrosinase and elastase. *J. Korean Soc. Food Sci. Nutr.* **2018**, *47*, 1076–1084. [[CrossRef](#)]
20. Lee, D.S.; Kim, K.H.; Yook, H.S. Antioxidant effects of fractional extracts from strawberry (*Fragaria ananassa* var. ‘Seolhyang’) leaves. *J. Korean Soc. Food Sci. Nutr.* **2018**, *47*, 263–270. [[CrossRef](#)]
21. Cheng, Y.; Kang, Y.; Kim, W. Solvent fractionation of *Polygonum cuspidatum* Sieb. Et Zucc. for antioxidant, biological activity, and chromatographic characterization. *Int. J. Mol. Sci.* **2025**, *26*, 7011. [[CrossRef](#)] [[PubMed](#)]
22. Huan, T.T.; Trang, H.T.T.; Nhung, C.T.C.; Nhan, H.H.; Son, T.T. In vitro study of effective factors for the inhibitory assay on pancreatic lipase. *J. Med. Pharm.* **2023**, *4*, 7–12. [[CrossRef](#)]
23. Hulankova, R. Methods for determination of antimicrobial activity of essential oils In vitro—A review. *Plants* **2024**, *13*, 2784. [[CrossRef](#)]
24. Ahmad, Z.; Rauf, A.; Orhan, I.E.; Mubarak, M.S.; Akram, Z.; Islam, M.R.; Imran, M.; Edis, Z.; Kondapavuluri, B.K.; Thangavelu, L.; et al. Antioxidant potential of polyphenolic compounds, sources, extraction, purification and characterization techniques: A Focused Review. *Food Sci. Nutr.* **2025**, *13*, e71259. [[CrossRef](#)]
25. Gulcin, I.; Alwasel, S.H. DPPH radical scavenging assay. *Processes* **2023**, *11*, 2248. [[CrossRef](#)]
26. Loganayagi, N.; Siddhuraju, P.; Manian, S. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. *J. Food Sci. Technol.* **2013**, *50*, 687–695. [[CrossRef](#)] [[PubMed](#)]
27. Rajurkar, N.; Hande, S. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian J. Pharm. Sci.* **2011**, *73*, 146. [[CrossRef](#)] [[PubMed](#)]
28. Namiecińska, E.; Jaszczak, J.; Hikisz, P.; Daško, M.; Woźniczka, M.; Budzisz, E. Evaluation of tyrosinase inhibitory activity of carbathioamidopyrazoles and their potential application in cosmetic products and melanoma treatment. *Int. J. Mol. Sci.* **2025**, *26*, 3882. [[CrossRef](#)]
29. Eun, C.-H.; Kang, M.-S.; Kim, I.-J. Elastase/collagenase inhibition compositions of *Citrus unshiu* and its association with phenolic content and antioxidant activity. *Appl. Sci.* **2020**, *10*, 4838. [[CrossRef](#)]
30. Li, Z.; Tu, Z.; Wang, H.; Zhang, L. Ultrasound-assisted extraction optimization of α -glucosidase inhibitors from *Ceratophyllum demersum* L. and identification of phytochemical profiling by HPLC-QTOF-MS/MS. *Molecules* **2020**, *25*, 4507. [[CrossRef](#)] [[PubMed](#)]
31. Seyedan, A.; Alshawsh, M.A.; Alshagga, M.A.; Koosha, S.; Mohamed, Z. Medicinal plants and their inhibitory activities against pancreatic lipase: A review. *Evid. Based Complement. Altern. Med.* **2015**, *2015*, 973143. [[CrossRef](#)]
32. Angelini, P. Plant-derived antimicrobials and their crucial role in combating antimicrobial resistance. *Antibiotics* **2024**, *13*, 746. [[CrossRef](#)] [[PubMed](#)]
33. Singaravadiel, C.; Joseph Santhanaraj, K. Gas chromatography and mass spectroscopic determination of phytocompounds in *Cissus vitifolia* leaf. *Der Pharmacia Lettre* **2016**, *8*, 292–297.
34. Ravitchandirane, H.; Singh, V.; Rajavel, A.; Sella, R.N. Characterization and metabolomic analysis of Plant-derived Extracellular Vesicles (PdEVs) isolated from indigenous medicinal plants. *J. Appl. Biol. Biotechnol.* **2024**, *12*, 251–260. [[CrossRef](#)]
35. Obafemi, A.S.; Felicia, C.A.; Bukola, C.A.; Abiodun, S.A. Gas chromatography / mass spectrometry (GC-MS) analysis of *Jatropha curcas* latex and its antimicrobial activity on clinical isolates. *World J. Adv. Res. Rev.* **2020**, *8*, 012–018. [[CrossRef](#)]
36. Sutarman; Miftahurrohmat, A.; Prihatiningrum, A.E.; Arifin, S. Biomass extract of *Ipomea carnea* and its inhibition against *Trichoderma asperellum*. *E3S Web Conf.* **2021**, *316*, 03011. [[CrossRef](#)]

37. Lohvina, H.; Sandor, M.; Wink, M. Effect of ethanol solvents on total phenolic content and antioxidant properties of seed extracts of fenugreek (*Trigonella foenum-graecum* L.) varieties and determination of phenolic composition by HPLC-ESI MS. *Diversity* **2021**, *14*, 7. [\[CrossRef\]](#)
38. Agregan, R.; Munekata, P.E.S.; Franco, D.; Dominguez, R.; Carballo, J.; Muchenje, V.; Barba, F.J.; Lorenzo, J.M. Phenolic content and antioxidant activity of extracts from *Bifurcaria bifurcata* alga obtained by diverse extraction conditions using three different techniques (hydrothermal, ultrasounds and supercritical CO₂). *Environ. Eng. Manag. J.* **2019**, *18*, 1535–1542. [\[CrossRef\]](#)
39. Kurniasari, R.; Suzery, M.; Cahyono, B. Analysis of total phenolics, flavonoids, and antioxidant activity of cashew leaf extract (*Anacardium occidentale* L.) with varying ethanol concentrations. *J. Ris. Kim.* **2024**, *15*, 116–130. [\[CrossRef\]](#)
40. Liu, Z.; Liao, H.; Dai, Y.; Qi, Y.; Zou, Z. Characterization and anti-ultraviolet radiation activity of proanthocyanidin-rich extracts from *Cinnamomum camphora* by ultrasonic-assisted method. *Molecules* **2024**, *29*, 796. [\[CrossRef\]](#)
41. Shirsath, S.R.; Sonawane, S.H.; Gogate, P.R. Intensification of extraction of natural products using ultrasonic irradiations—A review of current status. *Chem. Eng. Process. Process Intensif.* **2012**, *53*, 10–23. [\[CrossRef\]](#)
42. Hassanpour, S.H.; Doroudi, A. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. *Avicenna J. Phytomed.* **2023**, *13*, 354–376.
43. Ghazzawi, H.A.; Al-Sayyed, H.F.; Al-Kurd, R.A.; Mwalla, M.M.; Arafat, T.A.; AbdelQader, S.M. Effect of different extraction solvents on the antioxidant content and capacity of nine seasonal fruits. *Clin. Nutr. Open Sci.* **2021**, *38*, 33–42. [\[CrossRef\]](#)
44. Khorasani Esmaeili, A.; Mat Taha, R.; Mohajer, S.; Banisalam, B. Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from in vivo and in vitro grown *Trifolium pratense* L. (red clover). *BioMed Res. Int.* **2015**, *2015*, 643285. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Tung, X.Y.; Yip, J.Q.; Gew, L.T. Searching for natural plants with antimelanogenesis and antityrosinase properties for cosmeceutical or nutricosmetics applications: A systematic review. *ACS Omega* **2023**, *8*, 33115–33201. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Liu, C.M.; Perng, M.H.; Chen, C.Y. Antioxidant activities of crude extracts from peel and seed of *Cinnamomum camphora*. *Biomed. Res.* **2018**, *29*, 2854–2858.
47. Gouvinhas, I.; Saavedra, M.J.; Alves, M.J.; Garcia, J. Exploring the Impact of Ultrasound-Assisted Extraction on the Phytochemical Composition and Bioactivity of *Tamus communis* L. Fruits. *Pharmaceuticals* **2025**, *18*, 1342. [\[CrossRef\]](#)
48. Brás, N.F.; Gonçalves, R.; Mateus, N.; Fernandes, P.A.; Ramos, M.J.; De Freitas, V. Inhibition of Pancreatic Elastase by Polyphenolic Compounds. *J. Agric. Food Chem.* **2010**, *58*, 10668–10676. [\[CrossRef\]](#)
49. Abdulazeez, A.T.; Musa, M.; Abubakar, F.A.; Saliu, O.A.; Tijjani, H.; Idowu, O.A. Inhibitory Effect of Major Terpenoids of Essential Oil from *Agathosma betulina* Leaves on Collagenase and Elastase Enzymes Involved in Skin Aging. *Pharm. Fronts* **2025**, *07*, e105–e113. [\[CrossRef\]](#)
50. Chen, J.; Wu, S.; Zhang, Q.; Yin, Z.; Zhang, L. α -Glucosidase inhibitory effect of anthocyanins from *Cinnamomum camphora* fruit: Inhibition kinetics and mechanistic insights through in vitro and in silico studies. *Int. J. Biol. Macromol.* **2020**, *143*, 696–703. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Huang, W.; Huang, G.; Zhang, L.; Da Yu, E.; Yang, W.; Ye, M.; Zou, S.; Ni, L.; He, H.Q. Lignan-rich extract from *Cinnamomum camphora* leaf attenuates metabolic syndrome by modulating glycolipid metabolism and gut microbiota in T2DM mice. *Phytomedicine* **2024**, *135*, 156118. [\[CrossRef\]](#)
52. Cheng, Y.; Xue, F.; Yu, S.; Du, S.; Yang, Y. Subcritical Water Extraction of Natural Products. *Molecules* **2021**, *26*, 4004. [\[CrossRef\]](#)
53. Fernando, I.T.; Perera, K.I.; Athauda, S.B.P.; Sivakanesan, R.; Kumar, N.S.; Jayasinghe, L. Heat stability of the in vitro inhibitory effect of spices on lipase, amylase, and glucosidase enzymes. *Food Sci. Nutr.* **2019**, *7*, 425–432. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Li, M.M.; Chen, Y.T.; Ruan, J.C.; Wang, W.J.; Chen, J.G.; Zhang, Q.F. Structure–activity relationship of dietary flavonoids on pancreatic lipase. *Curr. Res. Food Sci.* **2023**, *6*, 100424. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Chen, L.; Fan, X.; Qi, H.; Chen, S.G.; Li, R.; Liu, Y.J. Study on chemical diversity, antioxidant and antibacterial activities, and HaCaT cytotoxicity of *Camphora tenuipilis* (a traditional aromatic plant from Xishuangbanna). *Plants* **2025**, *14*, 3409. [\[CrossRef\]](#) [\[PubMed\]](#)
56. He, J.; Fan, Z.; Jiang, Q.; Yang, X.; Sun, J.; Pang, Y.; Fang, X.; Zhang, D.; Li, Y.; Liu, Y.; et al. Sustainable natural bio-antimicrobial: Composition, bacteriostatic activity, and antibacterial strategy of *Cinnamomum camphora* essential oil. *Ind. Crops Prod.* **2025**, *233*, 121493. [\[CrossRef\]](#)
57. Taofiq, O.; González-Paramás, A.; Barreiro, M.; Ferreira, I. Hydroxycinnamic acids and their derivatives: Cosmeceutical significance, challenges and future perspectives, a review. *Molecules* **2017**, *22*, 281. [\[CrossRef\]](#)
58. Zengin, H.; Baysal, A. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure–activity relationships evaluated by SEM microscopy. *Molecules* **2014**, *19*, 17773–17798. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.