

Article

Chemical Composition and Brine Shrimp Toxicity of Twigs Essential Oil from Azorean *Cryptomeria japonica* (Thunb. ex L.f.) D. Don

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Abstract

The growing worldwide demand for essential oils (EOs) brings new opportunities for Azorean *Cryptomeria japonica* aerial parts waste valorization. Therefore, the phytochemical and bioactivity investigation of EOs from different Azorean *C. japonica* tissues, such as twigs (Az-CJT), remains imperative to add more value to *C. japonica*'s EO industry, alongside the contribution to the local sustainable circular bioeconomy. This study provides, for the first time, GC-MS analysis and brine shrimp toxicity of the EO hydrodistilled from Az-CJT and aims to compare these parameters with those determined for a commercial Azorean *C. japonica* (branches and foliage) EO obtained through steam distillation. The main Az-CJT EO components were α -eudesmol (19.53%), phyllocladene (14.80%), elemol (12.43%), nezukol (11.34%), and γ -eudesmol (5.32%), while α -pinene (28.62%), sabinene (24.30%), phyllocladene (5.10%), β -myrcene (5.09%), and limonene (4.93%) dominated in the commercial EO. Thus, Az-CJT EO exhibited the highest sesquiterpenoids (43.52%), diterpenes (20.85%), and diterpenoids (13.21%) content, while the commercial EO was dominated by monoterpenes (74.61%). The Az-CJT EO displayed significantly higher toxicity than the commercial EO, with mortality rates of 87.7% and 16.9%, respectively, at 100 μ g/mL. This result is likely related to the substantially higher terpenoid content of Az-CJT EO (61.20% vs. 9.44%), largely attributed to the sesquiterpenoid fraction. Due to its distinct chemical profile, Az-CJT EO may have differential commercial applications, warranting further investigation into its bioactive value and safe use. In conclusion, this study adds knowledge on the potentialities of *C. japonica* aerial parts' EOs from the Azorean region.

Keywords: Japanese cedar; forest biomass residues valorization; twigs; essential oil; chemical composition; sesquiterpenoids; diterpenes; diterpenoids; brine shrimp lethality; sustainable forest management



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1. Introduction

In nature, plants, being sessile organisms, are unable to escape from unfavorable biotic and abiotic conditions. As a major evolutionary strategy to facilitate their survival and reproductive success, higher plants produce various secondary metabolites like terpenes and

terpenoids—the most extensive and varied class of such compounds—to adapt, communicate, and defend themselves in their environment. In conifers specifically, the biosynthesis of defense-related terpenoids is a highly dynamic process intimately linked to specialized anatomical structures, playing a crucial role in their protection from wounding and attacks by pests and pathogens. Both constitutive and inducible oleoresin terpenoids involved in conifer defense accumulate within existing cortical resin ducts and in resin ducts associated with xylem trauma. The *de novo* formation of these resin ducts in the cambium zone and developing xylem, in response to injury, is a distinctive characteristic of the induced defense mechanisms found in long-lived conifer trees [1,2].

One of the long-living conifer trees is the Japanese cedar (*Cryptomeria japonica*), widely known as *sugi*, a large evergreen species belonging to the Cupressaceae family. It features a straight trunk supporting wide-spreading branches with drooping branchlets. The awl-shaped leaves are spirally arranged and point forward, with their bases tightly clasping the twigs [3], and this is the plant part used in this study.

While native to Japan, *C. japonica* has also been widely introduced into other temperate areas. In particular, it was purportedly introduced to the Azores archipelago (Portugal) during the mid-19th century, where it is commonly known as *criptoméria* [4]. Currently, in this archipelago, forests account for nearly a third of the land area, of which a large part is dominated by introduced tree species, with *C. japonica* as the most prominent and commercially valuable. It occupies around 60% of the Azores' total wood-producing forest area, with the largest plantations found on São Miguel Island [5]. Thus, *C. japonica*, a plant that has a long history of utilization for both ornamental and construction purposes, stands as the foremost national timber species in both the Japanese and Azorean archipelagos [4,6,7]. In fact, the wood (or heartwood) of this species possesses an excellent durability for exterior construction, a pleasant aroma [8], and a significant resistance against both fungal decay [8,9] and wood-feeding insects (in particular, termites [10]). These characteristics are primarily due to the presence of minor components (generally less than 10%), such as terpene and polyphenolic metabolites, that make *C. japonica* of great interest to researchers who intend to determine its bioactive potential for several purposes. For instance, (i) to prove the positive health effects associated with the applications of *C. japonica* in Asian traditional medicine [11] and (ii) to search for novel, leading metabolite compounds with potential as active principles in future drugs and green pesticide applications.

In fact, numerous investigations into the chemical composition and biological activities of extractives obtained from *C. japonica* have been conducted by various research groups over recent decades [12,13], revealing a wide range of valuable bioactivities. Therefore, *C. japonica* extractives from different geographical origins have broad application prospects, including uses in green plant protection, the sanitary industry, and as natural health-promoting agents within the pharmaceutical, food, and cosmetic industries [12,13]. In addition, *C. japonica*'s volatile organic compounds have been demonstrated to provide relaxation and stress relief properties in humans [13]. Thus, the essential oil (EO) obtained from this plant may serve as a valuable tool in mental health management, particularly when administered through aromatherapy.

As a result of *C. japonica*'s importance for landscaping and timber production, large amounts of diverse biomass residues (i.e., wastes and by-products) are generated from both forest operations and the wood industry, including aerial plant parts, bark, wood chips, and sawdust, which, if left unattended, can represent an environmental risk. Nevertheless, these biomass residues are a source of value-added bioproducts, such as EOs and/or their individual components (EOCs) [13], with no competition for land areas. Moreover, biomass residue valorization is a desirable goal from a circular bioeconomy and sustainable forest management perspective. Furthermore, there has been an increasing demand for EOs in the

global market [14], driven by renewed interest in bioactive natural products, because they are generally less hazardous and more efficacious than their synthetic equivalent. Indeed, it is projected that the global market demand for EOs will grow from approximately USD 20 billion in 2025 to USD 37.3 billion by 2029 [14].

The Azorean *C. japonica* aerial parts (Az-CJAP), i.e., branches and foliage, are the primary biomass residues from local *C. japonica* wood logging, also representing, currently, the main source used by local wood producers to obtain EOs through steam distillation (SD), with use mainly as household aromatherapy diffusers. However, the huge applications for EOs, alongside the interest of the various industrial sectors in greener technologies, could bring new challenges to local *C. japonica* EO industry development.

In this context, an investigation into the chemical composition, yield, and bioactivity of EOs obtained from different Az-CJAP tissues remains imperative. In fact, besides plant species and distillation methods, the plant part used for EO extraction is among the major factors that significantly affect the chemical composition and yield of an EO. In turn, as is well established, the bioactivity effectiveness of each EO mainly depends on its chemical profile [15,16]. Typically, major EOCs primarily determine the biological properties of an EO; however, minor EOCs can also play an important role by contributing through synergistic, additive, or antagonistic interactions to its overall bioactivity. Therefore, investigating the viability of a specific application of an EO requires knowledge of its complete chemical composition. Additionally, to ensure the safe use of EOs, they must satisfy several criteria, including assessment of their potential toxicity to eukaryotic cells. In this context, the brine shrimp lethality activity (BSLA) assay is considered a useful preliminary tool for assessing the toxicity of extracts or isolated compounds toward humans by employing brine shrimp (*Artemia salina* Leach) nauplii as an alternative in vivo model in place of laboratory animals. This test also serves as a tool to assess the feasibility of using highly toxic EOs in pest management [17].

As part of our ongoing strategy to valorize Azorean *C. japonica* biomass residues and support the local *C. japonica* EO industry in meeting diverse market demands, we have recently shown that the EOs hydrodistilled from various residues—foliage, needle-shaped leaves, cones, bark, and sawdust—exhibited diverse bioactivities of varying effectiveness, including antimicrobial, antioxidant, anticholinesterase, and anti-inflammatory properties [18–20].

In the present study, the focus was to produce EO from the Azorean *C. japonica* twigs (Az-CJT) tissue via hydrodistillation (HD), with the following purposes: (i) to determine its chemical composition, BSLA, and other parameters (yield, density, color, and odor); (ii) to compare the Az-CJT EO chemical profile, BSLA, and yield with those reported [18] for EOs obtained via HD from other Az-CJAP tissues (foliage, leaves, and cones) of the same tree population as the studied twigs sample; and (iii) to compare the Az-CJT EO chemical profile and BSLA with those assessed for a commercial Azorean *C. japonica* EO obtained through SD. The results will aim to strengthen knowledge of the chemical basis and general toxicity of Az-CJAP EOs for the effective multipurpose utilization of relatively underutilized Azorean *C. japonica* biomass residues. This, in turn, will enhance the value of the Azorean *C. japonica*'s EO sector, thereby promoting the local circular bioeconomy in a sustainable way.

To the authors' knowledge, this is the first report on the Az-CJT EO's chemical composition. Additionally, to date, no information has been reported on the toxicity potential of CJT EO. Furthermore, data dealing with the chemical composition of CJT EO from other geographical origins remains scarce.

2. Materials and Methods

2.1. Chemicals and Biological Material

A commercially supplied C7–C33 *n*-alkanes mixture was obtained from Restek (Bellefonte, PA, USA). Methylene chloride (purity of 99.8%), anhydrous sodium sulfate, Tween 20, and 96% ethanol were acquired from Sigma-Aldrich (St. Louis, MO, USA). The commercial Az–CJAP (branches and foliage) EO, obtained through SD, was purchased from Essentia Azorica (São Miguel, Azores, Portugal) website at <https://essentiaazorica.com/en/product/japanese-cedar-cryptomeria-japonica/> (accessed on 12 September 2024). The brine shrimp cysts were bought from JBL GmbH & Co. (Neuhofen, Germany).

2.2. Plant Material and Study Area Characterization

The aerial parts of *C. japonica* (Figure 1A) at dormant stage, provided by Marques, S.A., were collected in January 2022 (winter season) from a wood production forest (60-year-old planted tree population, average height of 20 m, and andosol-type soil) growing in “Achada” (latitude 37°48′51.1″ N, longitude 25°14′31.6″ W, and an altitude of 733 m), located in the Northeast Region of São Miguel Island, in the Azores archipelago, Portugal. This archipelago, situated in the North Atlantic Ocean, approximately 1500 km from mainland Portugal, consists of nine major islands, with São Miguel being the largest. The climate across the Azores is predominantly temperate (Type C in the Köppen–Geiger classification), specifically Cfb, characterized by mild summers and no dry season [21].



Figure 1. (A) Azorean *Cryptomeria japonica* aerial parts. (B) Foliage. (C) Twig. (D) Cross-section of the twig.

The aerial parts were randomly collected early in the morning from at least 20 healthy trees to ensure a representative sample. The freshly picked plant material was taken straight to a laboratory at the University of the Azores, where it was dried in a shaded, well-

ventilated area at room temperature (20 °C) until its weight became constant. The dried material was then separated into several samples, including foliage (Figure 1B), awl-shaped leaves, reproductive organs (i.e., female cones and male cones), and twigs (Figure 1C,D), with the latter plant part being used in the present study. Prior to distillation, the twigs sample was sectioned into chips approximately 2–3 cm in length.

2.3. EO Obtention via the HD Process

The EO was obtained by subjecting the dried Az-CJT to HD, using a Clevenger-type apparatus, according to the European Pharmacopoeia guidelines [22]. The sample-to-water ratio was 1:10 g/mL, and the distillation time was approximately 3 h, starting after the first distillate drop fell into the collecting unit of the apparatus, since, after this period, no significant volume increase was observed. The isolated EO was dried through the addition of anhydrous sodium sulfate. After decantation, the EO was stored in a sealed amber vial at 4 °C for no longer than six months prior to chemical and bioassay analyses. The EO yield is expressed in mL per 100 g of dry weight (d.w.) matter. Each HD was performed in triplicate.

2.4. EO Physical Properties (Density and Color) Determination

The density (ρ) of the Az-CJT EO at room temperature was calculated through the following equation: ρ (g/mL) = EO_m/EO_v , in which EO_m and EO_v represent the weight and volume of the EO, respectively. The subjective visual color of the Az-CJT EO was evaluated. Additionally, the color measurement was performed through the online tool available at the PINETOOL website at <https://pinetools.com/> (accessed on 2 October 2022). A photograph of the EO, taken against a white background, was uploaded to retrieve the hexadecimal (HEX) color code and the Red Green Blue (RGB) color values.

2.5. EO Chemical Composition Analysis

Three replicates of the Az-CJT EO and commercial Azorean *C. japonica* EO samples were analyzed for their chemical profile through gas chromatography–mass spectrometry (GC–MS) in electron ionization (EI) mode at 70 eV. The analysis was performed using a Shimadzu GCMS–QP2010 Ultra gas chromatograph–mass spectrometer (Shimadzu Corp., Tokyo, Japan), equipped with a ZB–5MSPlus (5% phenyl, 95% methyl siloxane) fused-silica capillary column (60 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness) from Phenomenex Inc. (Torrance, CA, USA). The experimental conditions were the following: a volume of 0.1 μ L of EO sample dissolved in methylene chloride (0.1 g/mL), injected in the split mode at a ratio of 24.4:1; carrier gas (helium) adjusted to a linear velocity at 36.3 cm/s; oven temperature programmed from 50 °C to 260 °C at 2 °C/min, and then held isothermally at 260 °C for 5 min; injector and detector temperature both at 280 °C; transfer line and ion source temperatures at 300 °C and 260 °C, respectively; mass detector scan range of 40–400 amu; and a scan time of 0.3 s. The retention indices (RIs) of the EOCs were determined with a homologous series of *n*-alkanes (C7–C33), and their relative percentages were calculated by integrating the total ion current (TIC) chromatogram peaks.

The identification of the EOCs was based on comparisons of their RI and EI–mass spectra with those obtained from a custom-made library, based upon the analyses of available standards and reference EOs [18,23], as well as from commercial spectra libraries (FFNSC4.0, NIST11, and Wiley10) and literature data [24,25]. The identification of the EOCs was confirmed via co-injections with available standards.

2.6. EO Toxicity Determination Using the In Vivo BSLA Assay

The potential cytotoxic activity of the Az-CJT EO and commercial Azorean *C. japonica* EO samples was determined through BSLA assays, according to the Meyer et al. [26] method, with modifications.

The brine shrimp encysted embryos (cysts) product was stored in a dark and dry place, protected from humidity, until use. This product already included salt, so the suspension was prepared in artificial seawater, with 16 g of the cysts (with the salt) per 500 mL of deionized water, according to the manufacturer's specifications. The cysts were then incubated at 25 ± 1 °C for 48 h under constant light and aeration. After 48 h, the cysts hatched, and the first nauplii stage (instar I) was employed in the assay.

A stock solution of each EO was prepared at 300 mg/mL in ethanol (a drop of tween 20 was used as a surfactant), and then diluted to 1 mg/mL in deionized water. This diluted solution was made homogeneous via sonication (3 cycles at 80% power). The assay was then performed in a 96-well microplate. Each well received 100 µL of the suspension containing 15 nauplii. Subsequently, 20 µL of each diluted EO solution was added. The volume in each well was then adjusted to 200 µL by adding water filtered from the incubation tray. After 24 h of contact time, the percentage of nauplii mortality was calculated (non-motile nauplii were considered dead). The negative control, prepared with artificial seawater, ethanol, and the surfactant, was used to correct the assay results for the natural mortality rate. All experiments were conducted in triplicate.

In addition, the lethal concentration 50 and 90 (LC₅₀ and LC₉₀) values of the Az-CJT EO were calculated (using stock EO solution dilutions at 40, 70, 80, and 100 µg/mL concentrations), being defined as the EO sample concentration able to kill 50% and 90% of the brine shrimp nauplii population, respectively, after 24 h of exposure.

2.7. Statistical Analysis

Data are expressed as mean $\pm$ standard deviation (SD) of at least three independent experiments. The statistical significance of differences among mean values was established at $p < 0.05$. The LC₅₀ and LC₉₀ with a 95% confidence interval (CI) were determined by Probit Analysis using IBM SPSS Statistics version 29.0.2.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

Progressive climate change and shifting pest ranges threaten the protective mechanisms that conifers have evolved to thrive. Therefore, research on conifer phytochemicals can help develop better conservation strategies to safeguard forest ecosystems. One of the most typical defense mechanisms in conifers is related to oleoresin, which is produced through the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways [1,2]. The volatile profiles of species are primarily determined by the terpene synthases (TPS) family. However, these biosynthetic pathways, as well as the catalytic efficiencies of the TPS, can exhibit spatial heterogeneity across distinct plant tissues (e.g., twigs vs. leaves) and temporal variation within identical tissues at different ontogenetic stages, reflecting complex regulatory mechanisms [15,16].

3.1. Az-CJT EO Obtention and Physical Properties

The EO from the dried Az-CJT was produced via HD with a Clevenger-type apparatus, since it is a technique included in the European Pharmacopoeia [22]. In fact, according to the International Organization for Standardization [27], an EO is defined as a product obtained from vegetable raw material through distillation processes (HD, SD, and dry distillation) or from the epicarp of *Citrus* fruit species via cold pressing.

The obtained EO presented a density of 0.90 ± 0.012 g/mL, thus, lower than that of water. The hedonic odor perception evaluation (pleasant/unpleasant profile) of Az-CJT EO revealed a pleasant odor, which can be classified as a terpene-type odor [28]. Concerning its color, the subjective visual evaluation indicates a dark yellow EO. In addition, the color measurement, performed through the online tool available at PINETOOL, revealed a HEX color code of #6d5518 and RGB color values of 109, 85, and 24, respectively.

3.2. Yield of Az-CJT EO

The Az-CJT EO content was $0.33 \pm 0.01\%$ *v/w* (d.w.), revealing a yield value lower than those of EOs hydrodistilled from cones (0.67–0.7%), foliage (2.1%), and leaves (2.63%), obtained from the same Azorean *C. japonica* tree population [18]. In addition, the yield of the EO sample under study was lower than that of resin-rich bark EO (0.8%) but similar to that of sawdust EO (0.27%), both obtained from Azorean *C. japonica* [20].

The obtained results agree with those found by Ho et al. [29] on the EOs yield of different tissues (leaves, twigs, heartwood, sapwood, and bark) from Taiwanese *C. japonica*. In fact, it was observed that Az-CJT EO presented a similar yield value to that of Taiwanese CJT EO ($0.31 \pm 0.02\%$ *v/w*, d.w.), despite the different distillation time used for the HD process performed in each study (3 h vs. 8 h, respectively). Furthermore, the authors [29] also found that leaf tissue had the highest EO yield ($3.93 \pm 0.09\%$ *v/w*, d.w.).

The study of Chang et al. [30] on the EOs yield of different tissues from Japanese *C. japonica* also revealed that leaf EO exhibited the highest value (1.42%), followed, in decreasing order, by wood (0.38%), twigs (0.05%), and bark (0.03%) EOs. However, the Japanese CJT EO, obtained using HD for 6 h, presented a yield value 6.6-fold lower than that of the EO sample in this study.

The above data show that the type of plant tissue significantly affects *C. japonica* EO yield, with twigs producing a lower amount compared to non-woody plant parts, such as leaves. In our research, as well as in the reported studies [29,30], since the samples originated from the same population of trees, the variations observed in EO content can primarily be attributed to anatomical differences between plant parts. Specifically, the disparity in EO yield likely reflects how secretory structures, which store the EOCs, are distributed unevenly and vary in abundance across different plant parts. Further studies are needed to compare the localization of EOs in different *C. japonica* tissues using microscopic histochemical analysis.

On the other hand, variations in the EO yield of a given plant organ within the same species can be attributed to several factors, including exogenous factors, such as growing region, reflecting an adaptive response to local ecological conditions, or endogenous factors (e.g., plant age and the developmental stage of the plant organ).

3.3. Chemical Profile of Az-CJT EO

The chemical composition of the Az-CJT EO and commercial Azorean *C. japonica* EO samples is detailed in Appendix A Table A1, and their total ion current (TIC) chromatograms are shown in Figure 2A,B. For comparative purposes, the chemical composition reported [18] for EOs hydrodistilled from other Az-CJAP samples (foliage, awl-shaped leaves, female cones, and male cones) of the same tree population as the studied twigs is also summarized in Appendix A Table A1. The identified EOCs comprise both terpenes and terpenoids. Terpenes include monoterpene hydrocarbons (MHs), sesquiterpene hydrocarbons (SHs), and diterpene hydrocarbons (DHs), whereas terpenoids include oxygen-containing monoterpenes (OCMs), oxygen-containing sesquiterpenes (OCSs), and oxygen-containing diterpenes (OCDs).

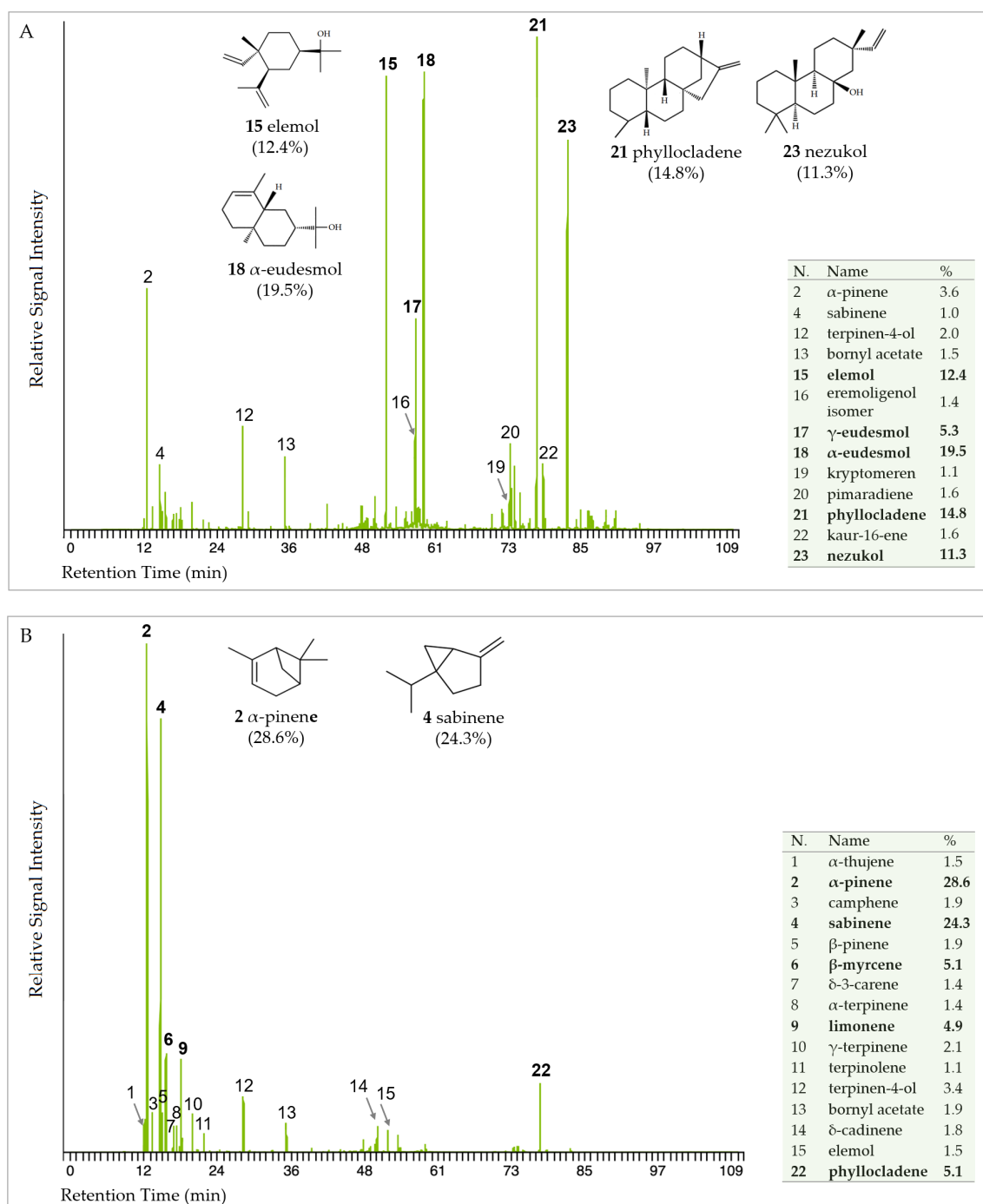


Figure 2. GC–MS total ion current (TIC) chromatograms obtained from a ZB–5MSPlus capillary column relative to the following: (A) essential oil (EO) from Azorean *Cryptomeria japonica* twigs; (B) commercial Azorean *C. japonica* EO. Major components ($\geq 1\%$) are assigned in the chromatograms. Components with content equal to or greater than 5% are highlighted in boldface.

Regarding the chemical composition of the Az–CJT EO and commercial Azorean *C. japonica* EO, a total of 94 vs. 68 EOCs were identified, accounting for 93.57% and 95.50% of the total EO composition, respectively. The identified EOCs were distributed as follows: 14 (7.80%) vs. 14 (74.61%) MH; 16 (4.47%) vs. 13 (6.42%) OCM; 23 (3.72%) vs. 18 (4.76%) SH; 22 (43.52%) vs. 13 (2.73%) OCS; 11 (20.85%) vs. 8 (6.69%) DH; and 8 (13.21%) vs. 2 (0.29%) OCD (Table A1). Thus, for the Az–CJT EO sample, terpene classes content decreased in the order OCS > DH > OCD > MH > OCM $\approx$ SH. In comparison, the commercial EO sample

exhibits a markedly different trend, with the terpene classes content decreasing as follows: MH $\gg$ DH $\approx$ OCM > SH > OCS > OCD.

The results revealed that 94 EOCs were identified in Az-CJT, reflecting a higher complexity compared to the commercial sample, which presented 68 EOCs. Both EOs share these 68 common EOCs, indicating a similar qualitative composition. However, the relative content of certain EOCs varies notably between the two samples (Table A1 and Figure 2A,B). Among the identified EOCs (Table A1), those with content equal to or greater than 1% are assigned in the chromatograms (Figure 2A,B). In the Az-CJT EO, the major EOCs ($\geq 5\%$) were, in decreasing order, α -eudesmol (18), phyllocladene (21), elemol (15), nezukol (23), and γ -eudesmol (17), accounting for 63.42% of the total EO, and, among the remaining 30.15%, eight EOCs presented a content $\geq 1\%$ (2, 4, 12, 13, 16, 20, 21, and 23), accounting for 13.57% of the total EO (Figure 2A). In contrast, the major EOCs ($\geq 5\%$) in the commercial EO were, in decreasing order, α -pinene (2), sabinene (4), phyllocladene (21), β -myrcene (6), and limonene (9), accounting for 68.04% of the total EO, and, among the remaining 27.46%, 11 EOCs presented a content $\geq 1\%$ (1, 3, 5, 7, 8, and 10–15), accounting for 19.93% of the total EO (Figure 2B).

Among the identified EOCs in the EO samples under comparison (Table A1), 11 are reported in Table 1, selected as major EOCs ($\geq 5\%$) of these samples. Table 1 also shows the total grouped EOCs by chemical classes and the terpene-to-terpenoid ratio values. In addition, these data are compared with those of EOs hydrodistilled from CJT from other geographical regions [29,30].

Table 1. Relative content (%) of major components ($\geq 5\%$) and total grouped components of a commercial Azorean *C. japonica* essential oil (C-EO), and of EOs obtained from female cones (FC), male cones (MC), awl-shaped leaves (L), foliage (F), and twigs (Tw) from the same Azorean *Cryptomeria japonica* population.

EO Components (EOCs)		<i>C. japonica</i> Origin Samples							
		Azores						Taiwan	Japan
		C-EO ¹	FC-EO ²	MC-EO ²	L-EO ²	F-EO ²	Tw-EO ¹	Tw-EO ³	Tw-EO ⁴
Class	Name								
MH	α -Pinene	28.62	44.6	37.6	25.8	17.0	3.59	0.2	-
MH	Sabinene	24.30	3.9	6.1	14.1	8.1	1.00	-	-
MH	β -Myrcene	5.09	1.7	4.7	3.4	2.9	0.58	t	-
MH	Limonene	4.93	1.0	3.2	1.1	1.3	0.37	t	-
OCM	Terpinen-4-ol	3.38	3.7	11.8	2.7	3.5	1.98	t	-
OCS	Elemol	1.49	3.9	2.0	11.5	13.6	12.43	8.7	6.80
OCS	γ -Eudesmol	0.11	3.5	1.2	3.5	3.6	5.32	11.8	6.05
OCS	β -Eudesmol	-	7.1	1.0	3.8	5.2	-	NR	NR
OCS	α -Eudesmol	0.70	5.9	1.2	5.1	6.3	19.53	25.2	10.87
DH	Phyllocladene	5.10	2.0	4.5	7.4	11.6	14.80	NR	NR
OCD	Nezukol	0.15	0.6	0.4	1.0	3.3	11.34	NR	NR
Total Identified EOCs (%)		95.50	92.0	97.6	96.6	93.7	93.57	93.1	76.8
Total Grouped EOCs (%)									
MH		74.61	59.6	70.8	54.9	37.9	7.80	0.4	-
OCM		6.42	5.7	14.5	4.1	5.5	4.47	0.5	-
SH		4.76	3.3	0.2	2.7	2.1	3.72	7.3	22.7
OCS		2.73	20.8	6.4	25.1	31.4	43.52	73.7	43.3
DH		6.69	2.0	5.3	8.8	13.5	20.85	0.1	0.6
OCD		0.29	0.6	0.4	1.0	3.3	13.21	11.1	10.2
Total terpenes (%)		86.06	64.9	76.3	66.4	53.5	32.37	7.8	23.3
Total terpenoids (%)		9.44	27.1	21.3	30.2	40.2	61.20	85.3	53.5
Ratio terpenes/terpenoids		9.10	2.4	3.6	2.2	1.3	0.53	0.1	0.4

¹ Data from our study. ^{2,3,4} Data retrieved from Lima et al. [18], Ho et al. [29], and Chang et al. [30], respectively. Legend: t, trace; (-), not detected; NR, not reported; MH, monoterpene hydrocarbons; OCM, oxygen-containing monoterpene; SH, sesquiterpene hydrocarbons; OCS, oxygen-containing sesquiterpenes; DH, diterpene hydrocarbons; OCD, oxygen-containing diterpenes. For each sample, the major EOC and the major grouped EOCs are highlighted in boldface.

It is noteworthy that the EO from Az-CJT is relatively poor in MH (7.80%) or in its major MH (α -pinene, 3.59%), which is in contrast with EOs from other aerial parts of the same plant [18] or with the studied commercial EO sample. In fact, in all these EOs, the major chemical class was MH, though in varying amounts (37.9–74.61% of the total EOs), increasing as follows: foliage EO (37.9%) < leaves EO (54.9%) < female cones EO (59.6%) < male cones EO (70.8%) < commercial EO (74.61%) (Table 1). The major EOC in all these EOs was α -pinene, with 28.62% in the commercial EO and 17.0–44.6% in the other EOs. In these EOs, the relative abundance of α -pinene followed a pattern similar to that reported for the MH content: cones EOs (37.6–44.6%) > leaves EO (25.8%) > foliage EO (17.0%) (Table 1). In addition, the EO hydrodistilled from an Azorean *C. japonica* resin-rich bark sample revealed a significant source of MH (63.97%), mainly due to its α -pinene content (42.7%), whereas this terpene class was not identified in the EO hydrodistilled from an Azorean *C. japonica* sawdust sample, which, in turn, was richest in OCS (66.64%) [20].

Comparing the chemical composition of Az-CJT EO with those of CJT EOs from other geographical regions, the study of Ho et al. [29] reported that Taiwanese CJT EO also exhibits a high prevalence of OCS (73.7%), mainly α -eudesmol (25.2%), γ -eudesmol (11.8%), and elemol (8.7%). However, the DH content was remarkably lower (<0.5%), and the OCD content (11.1%) (Table 1) differed qualitatively, primarily consisting of ferruginol (4.5%) and sandaracopimarinol (3.4%) [29]. A similar pattern was observed in the study of Chang et al. [30] on Japanese CJT EO, i.e., a prevalence of OCS (43.3%), mainly α -eudesmol (10.87%), elemol (6.80%), and γ -eudesmol (6.05%). However, in this CJT EO, the SH were the second major class (22.7%) (Table 1). Moreover, a qualitative difference in the OCD composition, mainly constituted by isopimarol [30], was also detected. The variations in the chemical composition between the three CJT EOs are probably due to genetic differences, as well as environmental and agronomic factors associated with the locations of the distinct tree populations from which they were obtained.

In addition, Ho et al. [29] found that leaf EO exhibited the highest MH content (34.8%), followed, in decreasing order, by bark (27.7%), twigs (0.4%), and sapwood (0.1%) EOs, while there was no presence of this terpene class in heartwood EO. A similar MH content pattern was observed in the Chang et al. [30] study: leaves EO (24.3%) followed by bark EO (3.6%), alongside the absence of MH in twigs and wood EOs.

The above data suggest that *C. japonica* EOs derived from woody tissues, such as twigs, tend to have terpene-to-terpenoid ratio values below 1, mainly due to elevated levels of OCS, while exhibiting lower amounts of MH compared to EOs obtained from non-woody plant parts, such as leaves. This compositional variation reflects distinct ecological functions of terpene classes in different plant tissues [31].

Finally, it should be noted that comparing chemical composition data between EO samples is very difficult, even within the same plant species growing in the same region. This difficulty arises from variations not only in the raw materials but also in the extraction protocols used (e.g., methods, apparatus, and extraction time), among other factors, including the harvest period. For instance, the Az-CJT EO was obtained using HD, whereas the commercial EO (branches and foliage) was produced by SD. Nonetheless, the SD method is often associated with higher MH content, whereas HD can favor higher OCS, DH, and OCD levels. This is likely because the returning distillate water in the HD method facilitates the recovery of water-soluble or less volatile EOCs [23].

3.4. Toxicity of Az-CJT EO Against *A. salina*

The *in vivo* BSLA assay is widely used to evaluate the general toxicity of a product, including EOs, e.g., [32], since *A. salina* is highly sensitive to a wide range of chemical agents. Therefore, it is considered essential as a preliminary assay in the search for bioactive

compounds, where toxicity against *A. salina* may indicate a possible relevant biological activity, for instance, antitumor, anti-acetylcholinesterase, fungicide, and insecticide [17,32–35].

This research evaluated the toxic potential of the studied EOs on *A. salina*, serving as a preliminary indicator of their effect on eukaryotic systems. The results are presented in Figure 3 and Table 2. Figure 3 shows the BSLA of the Az-CJT EO and commercial Azorean *C. japonica* EO samples at a concentration of 100 µg/mL. For comparative purposes, the BSLA values reported for the EOs from other Az-CJAP samples (foliage, leaves, female cones, and male cones), at the same concentration [18], are also presented in Figure 3.

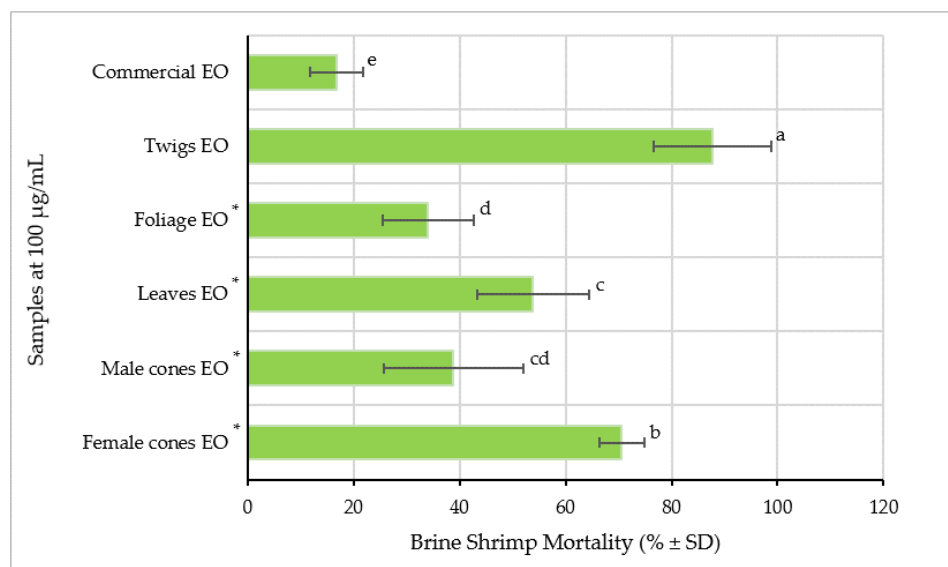


Figure 3. Toxicity of the essential oil (EO) from Azorean *Cryptomeria japonica* twigs against *Artemia salina* nauplii (instar I) at 100 µg/mL (after 24 h exposure), compared with that of EOs from other aerial parts of the same *C. japonica* population and with that of a commercial Azorean *C. japonica* EO. * Data retrieved from Lima et al. [18]. For each bar, means followed by different letters represent significantly different values ($p < 0.05$).

Table 2. Estimated values of lethal concentration 50 and 90 (LC₅₀ and LC₉₀) of essential oil (EO) from Azorean *Cryptomeria japonica* twigs against *Artemia salina* nauplii, after 24 h of exposure.

Sample	Concentration (µg/mL)	LC ₅₀ (95% CI) ¹	LC ₉₀ (95% CI) ¹	Slope ± SEM (95% CI) ¹	H ²
Twigs EO	40, 70, 80, 100	73.99 (68.86–77.75)	104.86 (97.47–119.25)	8.46 ± 1.37 (7.09–9.83)	0.33

Legend: CI, confidence interval; SEM, standard error mean. ¹ Values of LC_{50,90} and 95% CI are expressed in µg/mL of EO required to cause *A. salina* nauplii death. ² H, Heterogeneity factor (χ^2 /df).

The results revealed that the Az-CJT EO exhibited significantly higher toxicity than the commercial EO, with mortality rates of $87.7 \pm 11.0\%$ and $16.9 \pm 5.0\%$, respectively ($p < 0.001$). The remaining Az-CJAP EOs [18] showed intermediate mortality rates, ranging from 34.1% to 70.6%, increasing in the following order: foliage EO (34.1%) < male cones EO (38.8%) < leaves EO (53.9%) < female cones EO (70.6%) (Figure 3). In addition, the BSLA of the Az-CJT EO was determined at 40, 70, and 80 µg/mL sample concentration, revealing mortality rate values of $10.8 \pm 5.5\%$, $64.6 \pm 2.8\%$, and $78.1 \pm 9.1\%$, respectively. Thus, the results demonstrated that the Az-CJT EO displayed a toxic effect in a concentration-dependent manner, with the estimated LC₅₀ and LC₉₀ values being presented in Table 2. Based on Karchesy's toxicity classification [36], BSLA LC₅₀ values fall into four categories: strongly toxic (LC₅₀ < 100 µg/mL), moderately toxic (LC₅₀: 100–500 µg/mL), weakly toxic (LC₅₀: 500–1000 µg/mL), and non-toxic (LC₅₀ > 1000 µg/mL). Based on the adopted toxicity

classification, the studied Az-CJT EO sample is strongly toxic to brine shrimp, presenting an estimated LC_{50} value of 73.99 $\mu\text{g/mL}$.

The available data on the BSLA of the identified EOCs in the Azorean EO samples under analysis (Figure 3) showed that α -pinene, the major MH, and terpinen-4-ol, the major OCM, displayed weak activity (mortality rate values of 36.6% and 22.7%, respectively) [18]. This indicates that they contribute minimally to the overall toxicity of the twigs and female cones EOs, which are the most toxic samples, with mortality rates of 87.7% and 70.6%, respectively ($p = 0.012$). The pronounced toxicity of these EOs may result either from other major EOCs or from the modulatory effects of minor EOCs. Further investigations are needed to clarify this issue.

Interestingly, as shown in Table 1, the BSLA potency of the compared EO samples (Figure 3) decreases in an order that correlates with the increasing terpenes/terpenoids ratio (values indicated in parentheses), as follows: twigs EO (0.53) > foliage, leaves, and cones EOs (1.3–3.6) $\gg$ commercial EO (9.1). Thus, the superior BSLA of the Az-CJT EO appears to be related to its terpenoid-rich profile, mainly due to the OCS content, which decreased in the following order: twigs EO (43.52%) > foliage, leaves, and cones EOs (6.4–31.4%) > commercial EO (2.73%) (Table 1). In fact, data from the literature indicate that the cytotoxic effect of several EOs may be attributable, at least, to the presence of this terpene class, which is capable of inducing apoptotic cell death [37,38]. In particular, it has been found that eudesmol-skeleton EOCs, including α -eudesmol and γ -eudesmol, are cytotoxic agents towards several tumor cell lines, such as mouse melanoma (B16-F10), human chronic myelocytic leukemia (K562), and human hepatocellular carcinoma (HepG2), while also demonstrating the ability to induce caspase-mediated apoptosis in the HepG2 cells. Therefore, these natural apoptosis inductor agents might be of great potential interest for liver cancer chemotherapy [39]. In addition, α -eudesmol is reported to be able to block voltage-gated calcium channels (VGCC) and, thus, can be useful in neurogenic inflammation and brain injury [40–42]. Furthermore, γ -eudesmol is reported as a potent antioxidant agent, as assessed by single electron transfer-based antioxidant methods [43]. Data from Table 1 revealed that the α -eudesmol content of the twigs EO was at least 3.7-fold higher than that of the EOs from other tissues, and 28.0-fold higher than that of the commercial EO. Similarly, the γ -eudesmol content of the twigs EO was at least 1.5-fold higher than that of the EOs from other tissues and 48.4-fold higher than that of the commercial Azorean *C. japonica* EO.

A previous study [20] on the BSLA of EOs from other Azorean *C. japonica* biomass residues, such as sawdust, revealed that this OCS-rich (67%) EO, mainly β + α -eudesmol (13.5%), is also classified as strongly toxic against brine shrimp, presenting an estimated LC_{50} value similar to that of Az-CJT EO (around 73.0 $\mu\text{g/mL}$ for both EOs), but a superior LC_{90} value (136.0 vs. 104.9 $\mu\text{g/mL}$). On the other hand, and also according to our findings, Azorean *C. japonica* bark EO, characterized by an MH-rich (64.0%) profile, mainly α -pinene (42.7%), is considered moderately toxic (LC_{50} : 313.0 $\mu\text{g/mL}$) [20], according to Karchesy's toxicity criterion [36]. Other research has found that EOs rich in SH or OCS exhibit higher toxicity in the BSLA assay than those that are MH-rich [44].

A limitation of this study is that, based on the preliminary cytotoxicity results, the Az-CJT EO may contain highly cytotoxic EOCs. However, without further investigation, such as assays on mammalian cells or non-target organisms, its bioactive potential—including antitumor, antimicrobial, neuroprotective, and pesticidal activities—and toxicity profile remain insufficiently characterized. This lack of comprehensive data restricts our ability to ensure the safety of its potential applications in aromatherapy, air purification, food and crop preservation, anti-aging formulations, or treatment of distinct cancer or neural disorders.

4. Conclusions

Among the EOs of commercial importance, conifer EOs, such as those from *C. japonica*, are noteworthy because they can be obtained in large amounts from diverse forest biomass residues, generated by both forest management and the timber industry. However, due to the heterogeneous composition and bioactivity of *C. japonica* biomass residues, further research is needed for their effective conversion into different value-added EO products. In this context, CJT EO remains among the least studied.

In this study, the HD of Azorean-grown CJT yielded an EO composed of 94 identified constituents (93.57% of the total EO), dominated by OCS (mainly α -eudesmol, γ -eudesmol, and elemol) and DH/OCD (mainly phyllocladane and nezukol), which accounted for 63.42% of the EO. Eight additional major ($\geq 1\%$) compounds included α -pinene, sabinene, terpinen-4-ol, bornyl acetate, eremoligenol isomer, pimaradiene, kaur-16-ene, and kryptomerene (13.57% of the EO). A notable observation is that Az-CJT EO exhibited significantly lower MH content compared to EOs hydrodistilled from non-woody parts of *C. japonica* (such as leaves) and the studied commercial Azorean *C. japonica* EO, all of which are characteristically rich in MH.

The distinct chemical composition of Az-CJT EO, characterized by a sesquiterpenoid-rich profile, likely contributes to its remarkably high cytotoxicity in the brine shrimp lethality assay ($LC_{50} = 73.99 \mu\text{g/mL}$). This cytotoxicity is 5.2-fold greater than that of the commercial EO, suggesting potent bioactivity. Given that its major phytochemicals are known for pesticide, antimicrobial, antioxidant, anti-inflammatory, antitumor, and neuro-protective properties, this EO represents a promising raw material for green plant protection, pharmaceutical, cosmetic, and aromatherapeutic applications. Moreover, HD offers a green and sustainable production route for small and medium-sized enterprises to produce Az-CJT EO.

Further studies should evaluate safety, in vivo efficacy, and formulation strategies, such as microencapsulation, to improve EO stability and bioavailability.

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Institutional Review Board Statement: The aerial parts of *C. japonica* at dormant stage, provided by Marques, S.A., were collected in January 2022 (winter season) from a wood production forest (60-year-old planted tree population, average height of 20 m, and andosol-type soil) growing in “Achada” (latitude 37°48′51.1″ N, longitude 25°14′31.6″ W, and an altitude of 733 m), located in the Northeast Region of São Miguel Island, in the Azores archipelago, Portugal.

Data Availability Statement: Data are contained within the article.

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Abbreviations

Az–CJAP, Azorean *Cryptomeria japonica* aerial parts; Az–CJT, Azorean *Cryptomeria japonica* twigs; BSLA, brine shrimp lethality activity; CJT, *Cryptomeria japonica* twigs; DH, diterpene hydrocarbons; d.w., dry weight; EO, essential oil; EOC, essential oil component; GC–MS, gas chromatography coupled with mass spectrometry; HD, hydrodistillation; LC₅₀, half-maximal lethal concentration; LC₉₀, lethal concentration 90%; MH, monoterpene hydrocarbons; OCD, oxygen-containing diterpenes; OCM, oxygen-containing monoterpenes; OCS, oxygen-containing sesquiterpenes; RI, retention indices; RT, retention time; SD, steam distillation; SH, sesquiterpene hydrocarbons; TPS, terpene synthases.

Appendix A

Table A1. Chemical composition of the essential oil (EO) hydrodistilled from Azorean *Cryptomeria japonica* twigs, compared with that of EOs hydrodistilled from other aerial parts (AP) of the same *C. japonica* population, and with that of a commercial Azorean *C. japonica* EO (com. EO).

Class	Component	RT	RI _C	RI _L	IP ¹	Relative Content (%)		
						Twigs EO ²	Other AP-EOs ³	Com. EO ⁴
MH	Tricyclene	12.028	918	921	a, c	t	0.1–0.3	0.31
MH	α-Thujene	12.180	921	924	b, c	0.15	0.7–2.1	1.53
MH	α-Pinene	12.603	928	932	a, c	3.59	17.0–44.6	28.62
MH	Camphene	13.506	944	946	a, c	0.33	1.0–2.1	1.90
MH	Sabinene	14.788	967	969	a, c	1.00	3.9–14.1	24.30
MH	β-Pinene	15.094	973	974	a, c	0.29	1.1–1.6	1.93
MH	β-Myrcene	15.726	984	988	a, c	0.58	1.7–4.7	5.09
MH	δ-3-Carene	16.951	1005	1008	a, c	0.25	0.8–1.3	1.36
MH	α-Terpinene	17.466	1013	1014	a, c	0.26	0.6–3.4	1.40
MH	p-Cymene	17.940	1020	1020	a, c	0.16	t–0.9	0.32
MH	Limonene	18.244	1024	1024	a, c	0.37	1.0–3.2	4.93
MH	β-Phellandrene	18.346	1026	1025	b, c	0.15	0.4–1.1	0.79
MH	γ-Terpinene	20.132	1053	1054	a, c	0.46	0.8–4.9	2.13
MH	Terpinolene	21.952	1080	1086	a, c	0.17	0.5–1.3	1.06
OCM	Linalool	22.896	1094	1095	a, c	0.11	t	0.10
OCM	cis-p-Menth-2-en-1-ol	24.604	1119	1118	c	t	t–0.3	0.14
OCM	trans-Pinocarveol	25.730	1135	1135	c	t	-	-
OCM	trans-p-Menth-2-en-1-ol	25.835	1137	1136	b, c	t	t–0.2	0.08
OCM	Camphene hydrate	26.722	1150	1145	c	t	-	t
OCM	Borneol	27.880	1166	1165	a, c	0.06	t–0.5	0.07
OCM	Isopinocamphe	28.168	1170	1153	c	t	-	t
OCM	Terpinen-4-ol	28.500	1175	1174	a, c	1.98	2.7–11.8	3.38
OCM	α-Terpineol	29.490	1189	1186	a, c	0.33	t–0.8	0.13
OCM	cis-Piperitol	29.693	1191	1195	c	t	-	-
OCM	trans-Piperitol	30.445	1203	1207	c	t	-	t
OCM	Linalyl acetate	33.225	1244	1253	a, c	0.07	-	0.14
OCM	Bornyl acetate	35.583	1278	1287	a, c	1.45	0.2–1.4	1.93
OCM	Isobornyl acetate	35.734	1280	1283	c	t	-	t
OCM	Methyl myrtenate	36.313	1289	1294	c	0.07	-	-
OCM	α-Terpenyl acetate	39.712	1340	1346	b, c	0.12	t–0.3	0.28
SH	β-Elemene	42.544	1383	1389	b, c	0.50	t–0.4	0.15
SH	β-Ylangene	44.370	1411	1419	c	t	-	t
SH	β-Caryophyllene	44.457	1412	1417	b, c	0.10	t–0.2	0.19
SH	β-Copaene	45.097	1423	1430	c	0.15	t–0.4	0.08
SH	trans-β-Farnesene	46.540	1446	1454	c	t	-	t
SH	α-Humulene	46.703	1448	1452	c	t	t	0.08
SH	β-Acoradiene	47.374	1459	1469	c	t	-	-
SH	4,5-di- <i>epi</i> -Aristolochene	47.599	1463	1471	c	t	-	-
SH	Selina-4,11-diene	47.814	1466	1474	c	0.06	-	-
SH	trans-Cadina-1(6),4-diene	47.912	1468	1475	c	0.13	-	t
SH	cis-4,10-Epoxyamorphene	48.083	1470	1481	c	0.06	-	-
SH	Germacrene D	48.297	1474	1484	a, c	0.49	t–0.9	0.87
SH	β-Selinene	48.789	1481	1489	c	0.19	t	0.08
SH	trans-Muurola-4(14),5-diene	48.974	1484	1493	c	0.06	-	0.12
SH	α-Selinene	49.217	1488	1498	c	0.39	-	0.08
SH	α-Murolene	49.375	1491	1500	b, c	0.14	t–0.3	0.40
SH	β-Bisabolene	49.943	1500	1505	c	0.06	-	t

Table A1. Cont.

Class	Component	RT	RI _C	RI _L	IP ¹	Relative Content (%)		
						Twigs EO ²	Other AP-EOs ³	Com. EO ⁴
SH	γ-Cadinene	50.257	1505	1513	b, c	0.23	t–0.4	0.55
OCS	Cubebol	50.380	1507	1514	c	0.06	-	0.10
SH	δ-Cadinene	50.563	1510	1514	a, c	0.76	0.2–1.3	1.82
SH	Zonarene	50.835	1515	1526	c	t	-	t
SH	<i>trans</i> -Cadina-1(2),4-diene	51.409	1525	1533	c	t	-	-
SH	α-Cadinene	51.666	1529	1537	c	0.06	t	0.11
OCS	Elemol	52.414	1541	1548	a, c	12.43	2.0–13.6	1.49
SH	Germacrene B	52.979	1551	1559	c	0.08	-	t
OCS	(<i>E</i>)-Nerolidol	53.087	1553	1561	c	0.11	-	t
OCS	Germacren D-4-ol	54.021	1568	1574	a, c	0.47	t–0.7	t
OCS	β-Oplopenone	55.585	1595	1607	c	0.35	-	t
OCS	5,7-di- <i>epi</i> -α-Eudesmol	55.790	1598	1607	c	0.10	-	-
OCS	1,10-di- <i>Epi</i> -Cubenol	56.286	1607	1618	c	0.07	-	t
OCS	10- <i>epi</i> -γ-Eudesmol	56.673	1614	1622	c	0.38	t–1.0	t
OCS	1- <i>Epi</i> -Cubenol	56.998	1619	1627	c	0.14	-	-
OCS	Eremoligenol isomer	57.147	1622	-	c	1.39	-	-
OCS	γ-Eudesmol	57.249	1624	1630	a, c	5.32	1.2–3.6	0.11
OCS	Hinesol	57.672	1631	1640	c	0.47	-	-
OCS	τ-Cadinol	57.800	1634	1638	b, c	0.45	t–1.2	0.10
OCS	<i>epi</i> -α-Cadinol	57.922	1636	1638	c	0.48	-	t
OCS	δ-Cadinol	58.081	1639	1644	c	0.23	t	t
OCS	α-Eudesmol	58.636	1648	1652	a, c	19.53	1.2–5.9 ³	0.70
OCS	Intermedeol	58.743	1650	1636	c	0.67	-	-
OCS	7- <i>epi</i> -α-Eudesmol	58.887	1653	1662	c	0.11	-	-
OCS	Bulnesol	59.091	1657	1670	c	0.08	-	-
OCS	Elemyl acetate	59.220	1659	-	c	0.22	-	-
OCS	Hinesol acetate	65.483	1773	1783	c	0.11	-	-
OCS	Oplopanonyl acetate	69.962	1860	1851	c	0.35	-	t
DH	Rimuene	71.441	1888	1896	c	t	t	-
DH	Sclarene	71.559	1891	1974	c	0.10	-	-
DH	Isopimara-9(11),15-diene	71.688	1893	1905	c	0.47	t–0.2	0.10
DH	Rosa-5,15-diene	73.061	1921	1926	c	0.15	-	0.40
DH	Kryptomen	73.206	1924	1933	c	1.06	-	0.34
DH	Pimaradiene	73.751	1935	1948	a, c	1.55	-	0.40
DH	Sandaracopimara-8(14),15-diene	74.652	1954	1968	c	0.82	t–0.7	0.17
DH	Isophyllocladene	74.944	1960	1966	c	t	t	-
OCD	Manool oxide	76.036	1982	1987	c	0.14	-	t
DH	Phyllocladene	77.453	2012	2016	a, c	14.80	2.0–11.6	5.10
DH	Kaur-16-ene	78.476	2034	2042	a, c	1.55	t–0.6	0.15
DH	Abietatriene	78.822	2041	2055	c	0.26	t–0.1	t
OCD	Nezukol	82.516	2121	2132	a, c	11.34	0.4–3.3	0.28
OCD	Sandaracopimarinal	84.679	2170	2184	c	0.47	-	-
OCD	Phyllocladanol	85.981	2199	2209	c	0.44	-	-
OCD	Sandaracopimarinal	88.450	2256	2269	c	0.06	-	-
OCD	Isopimarol	90.157	2296	2310	c	0.06	-	-
OCD	6,7-Dehydroferruginol	90.364	2301	2315	c	0.25	-	-
OCD	<i>trans</i> -Ferruginol	90.507	2305	2297	c	0.45	-	-
Total identified components (%)						93.57	92.0–97.6	95.50

Results are mean of $n = 3$. Standard error (SE) < 0.7% for compounds with a percentage of < 30%. For compounds > 30%, SE < 2%. Legend: RT, retention time (minutes) values on a ZB-5MSPlus capillary column; RI_C, retention indices calculated on the same column, relative to C7–C33 *n*-alkanes; RI_L, retention indices obtained from the literature [24,25]; t, trace (<0.05%); MH, monoterpene hydrocarbons; OCM, oxygen-containing monoterpene; SH, sesquiterpene hydrocarbon; OCS, oxygen-containing sesquiterpene; DH, diterpene hydrocarbon; OCD, oxygen-containing diterpene. ¹ Identification procedure (IP): a = authentic standard compounds, b = reference EOs [15,18,23], and c = MS and RI (data from the literature [24,25] and spectra libraries, as described in Section 2.5). ² Components with content equal to or greater than 1% are highlighted in boldface. ³ Data retrieved from Lima et al. [15]: components range, relative to the chemical composition of EOs obtained from four different aerial parts (foliage, awl-shaped leaves, female cones, and male cones). All these EOs also contain β-eudesmol (1.0–7.1%). ⁴ Commercial EO sample obtained through steam distillation from Azorean *C. japonica* aerial parts, i.e., branches and foliage.

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