Cryptomeria japonica

Subjects: Forestry

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Cryptomeria japonica, a commercially important tree throughout Asia and the Azores Archipelago (Portugal), is currently waste/by-products of wood processing that can be converted into eco-friendly and high added-value products, such as essential oils (EOs), with social, environmental and economic impacts.

Cryptomeria japonica essential oil By-products valorization Terpenes/terpenoids Chemotypes

1. Introduction

Numerous plant-derived essential oils (EOs), due to their valuable odoriferous and bioactivity properties, and GRAS (Generally Recognized as Safe) status, have applications in many fields, such as aromatherapy, cosmetic, cosmeceutical, food, beverage, household, pharmaceutical, phytomedicine and pest control. However, the bioactivity and potential commercial use of EOs depends on their complex mixture of organic compounds, produced through the secondary metabolic pathways of aromatic plants [1][2][3][4], such as the conifer Cupressaceae family [4].

Cryptomeria japonica (Thunb. ex L.f.) D. Don (Cupressaceae), commonly called a Japanese cedar or *sugi*, is a forest tree endemic to Japan, and widely distributed in warm and cool temperate climates. *Cryptomeria* is a monotypic genus that includes only one species with two recognized varieties: *C. japonica* var. *japonica* and *C. japonica* var. *sinensis*, the latter being native to China [5].

C. japonica is a very large, conical, evergreen monoecious tree that can reach up to 70 m (230 ft) in height with a trunk diameter of up to 4 m (13 ft). It is a fast growth tree that prefers moist, deep and well-drained soils [6]. The bark is reddish-brown, fibrous and peels off in vertical strips. The leaves are odorous due to the presence of EO and are 0.5–1 cm (0.20–0.39 in) long and needle-like in structure. The seed cones are globular up to 1–2 cm (0.39–0.79 in) in diameter with about 20–40 scales [7].

C. japonica is one of the main plantation forest tree species in Asian countries (Japan, Korea, Taiwan, India and China) and in the Azores Archipelago (Portugal). In Azores, forests account for 31% of the land area, where *C. japonica* occupies over 12,698 hectares and is the most commercially important tree species. It was introduced in Azores in the mid-19th century, where it developed very well due the similar pedological and climatic conditions to those of its original country. In Azores, *C. japonica* is often planted around farms to create shelter lines, which increase pasture productivity by creating favorable microclimates for crop and livestock [8].

2. Antimicrobial Activity of *C. japonica* EO in Food Industry and Human Diseases

EOs are generally accepted as natural antimicrobials and antioxidants that can be used in the food industry as bio-preservatives to increase shelf life and quality of food products [9]. In addition, infections caused by fungi and bacteria represent a key issue due to the development of resistant species to current fungicides and antibiotics. Therefore, EOs could be an ecological and effective alternative to synthetic antimicrobial agents [9].

Table 1 shows the antimicrobial activity of *C. japonica* EO from different tissues and geographical origins, against several gram-positive and gram-negative bacterial strains, as well as against various fungal species. The minimal inhibitory concentrations (MICs) were determined by the broth dilution method.

Table 1. Antimicrobial activities of *Cryptomeria japonica* essential oil by plant organ and country of origin.

Origin	Plant Organ	Target Species	Efficiency	Ref.
Portugal	Leaves, heartwood, bark	*Mycobacterium tuberculosis, Botrytis cinerea, Fusarium circinatum, Cryphonectria parasitica, Aspergillus niger, Trichoderma harzianum, Cladosporium cladosporioides, Cladosporium sp., Candida albicans, Candida tropicalis, Saccharomyces cerevisiae, Cryptococcus neoformans, Aspergillus fumigatus, Microsporum gypseum, Trichophyton rubrum, Trichophyton mentagrophytes*	Effective against *M. Tuberculosis*, *T. harzianum*, *B. cinerea*, *C. cladosporioides* and *Cladosporium sp.*, MICs range 0.025–0.25 mg/mL	[10]
South Korea	Leaves plus twigs	*Candida albicans, Candida pseudotropicalis, Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis, Cryptococcus neoformans, Aspergillus fumigatus*	Effective, MICs 2.18 mg/mL or higher	[11]

Origin	Plant Organ	Target Species	Efficiency	Ref.
Taiwan	Leaves, heartwood, sapwood, bark	*Trametes versicolor, Lenzites betulina, Laetiporus sulphureus, Gloeophyllum trabeum, Fusarium oxysporum, Rhizoctonia solani, Ganoderma australe, Fusarium solani, Pestalotiopsis funereal, Collectotrichum gloeosporioides*	Highly effective, IC_{50} range 0.039 > 0.500 mg/mL, except bark EO	[12]
Japan	Heartwood	*Staphylococcus epidermis, Trichophyton rubrum*	Effective, MIC = 0.313 mg/mL, except *S. epidermis*	[13]
South Korea	Leaves	*Staphylococcus epidermis, Propionibacterium acne*	Effective, MICs range 0.156–10 µL/mL	[14]
South Korea	Leaves	*Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus pyogenes, Streptococcus mutans, Streptococcus sanguinis, Streptococcus sobrinus, Streptococcus ratti, Streptococcus criceti, Streptococcus anginosus, Streptococcus gordonii, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Prevotella intermedia, Porphylomonas gingivalis*	Effective, MICs range 0.025–12.8 mg/mL, except *E. coli*	[15]
South Korea	Leaves plus twigs	*Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Citrobacter freundii, Acinetobacter calcoaceticus, Staphylococcus aureus, Bacillus subtilis, Klebsiella oxytoca, Klebsiella pneumoniae, Pseudomonas*	Ineffective, MICs > 21.8 mg/mL	[11]

Origin	Plant Organ	Target Species	Efficiency	Ref.
		aeruginosa, Serratia marcescens		
Taiwan	Leaves, heartwood, twigs, bark	*Legionella pneumophila*	Ineffective, MBC > 2 mg/mL	[16]

MIC—minimal inhibitory concentration; MBC—minimal bactericidal concentration; IC_{50}—half maximal inhibitory concentration.

Antimicrobial compounds are usually highly correlated with antioxidant effects [17]. In fact, besides microbial contamination, lipid peroxidation is a real problem related to food deterioration and the addition of antioxidants is an attractive strategy to retard or even stop oxidation processes. *C. japonica* extracts from different tissues are rich in phenolics, which are well-known natural antioxidants and antibacterial agents [18][19]. Contrary to *C. japonica* extracts, EO from various tissues of this plant exhibited weak antioxidant activities (in 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assays), presenting the sapwood EO with the best value (IC_{50} = 113 μg/mL), followed by twigs, heartwood, bark and lastly leaf EOs [20]. The effect of sapwood EO on the free radical scavenging assay is attributed to its hydrogen-donating ability, possibly due to the high content of ferruginol (an oxygenated diterpene). Thus, sapwood EO or ferruginol can also be used as natural food preservatives in food industry. In fact, it has already been reported that diterpenes show higher antioxidant and antimicrobial effects than monoterpenes [21].

Enrichment of *C. japonica* EO fractions with specific compounds can be a useful strategy to pest/microbes management, where deterpenation with vacuum fractional distillation can be effective. Kusumoto and Shibutani [22] had submitted EO from *C. japonica* leaves to an open system mild heat treatment, which decreased the content of monoterpene hydrocarbons and increased the content of oxygenated sesquiterpenes and diterpenes. They found that the evaporation residue had higher antifungal activity than the crude EO. Also, Salha et al. [23] reported the same findings for *Origanum majorana* EO. Further studies should explore this issue.

3. Acaricidal and Insecticidal Activities of *C. japonica* EO

The EOs play a functional role in the plant chemical defense against phytopathogens and pests [2][24]. For that reason, they are a potentially good source of environmentally friendly pesticides or pest-control agents. **Table 2** shows the insecticidal and acaricidal effects of *C. japonica* EO from different tissues.

Spider mites, such as *Tetranychus urticae* and *Tetranychus kanzawai*, are known as world pests of agricultural crops. However, since spider mites easily build up a tolerance to pesticides, EOs have been studied as alternative eco-pesticides. Yamashita et al. [25] found that EO from the leaves of *C. japonica* is a fruitful option to control these mites (**Table 2**). They mainly attributed this acaricidal activity to the ent-kaurene compound, followed by elemol.

Silverfish *(Lepisma saccharina)*, another pest common in libraries and museums where paper books and labels are abundant, are insects that owe their survival to their secretive life in damp, cool places. As observed in a Wang et al. [26] study, this pest is also sensitive to *C. japonica* leaf EO, which highly repelled silverfish (>80% of repellency at 0.01 mg/cm^3 after 4 h) and killed it on contact assay (LD$_{50}$ value of 0.087 mg/cm^3 after 10 h) (**Table 2**). These authors assigned these toxic effects to volatiles (monoterpenes) and non-volatiles compounds (such as ent-kaurene and elemol), respectively.

Biodegradation of wood caused by termites is one of the most serious problems for wood utilization, and extractives from wood tissues can provide natural protection against harmful pests. Therefore, and as shown in **Table 2**, EO from *C. japonica* (namely from leaf and heartwood) can be a natural termicide. In particular, elemol plus ent-kaurene chemotype is associated with high antitermitic activity (by contact assay), which in the Cheng et al. [27] study was correlated with elemol and α-terpineol compounds.

It is interesting to note that EO from *C. japonica* leaves is more effective in larvicidal and repellent activities against *A. aegypti* and *A. albopictus* than their methanolic extracts [28][29]. However, methanolic extracts from other *C. japonica* tissues (such as sapwood) have also been stated as excellent mosquito larvicidal agents [29][30].

The larvicidal effects of *C. japonica* leaf EO on *Anopheles gambiae* (the main malaria vector), and revealed promising results in the laboratory (**Table 2**) and semi-field conditions.

Table 2. Insecticidal and acaricidal activities of *Cryptomeria japonica* essential oil from different tissues.

Plant Organ	Pests	Bioassay	Efficiency	Ref
Leaves	*Lepisma saccharina*	Contact assay (on treated filter papers) against the adult silverfish	Significant toxicity with LD$_{50}$ of 0.087 mg/cm^3 after 10 h	[26]
		Repellent assay	More than 80% of repellency at 10 μg/cm^3 after 4 h	
Leaves	*Aedes aegypti*	Aqueous suspension of essential oil against the fourth-instar mosquito larvae	Significant larvicidal activity with LC$_{50}$ of 37.5 μg/mL after 24 h	[31]
Bark			Significant larvicidal activity with LC$_{50}$ of 48.1 μg/mL after 24 h	
Sapwood			Significant larvicidal activity with LC$_{50}$ of 82.7 μg/mL after 24 h	
Heartwood			Significant larvicidal activity with LC$_{50}$ of 72.0 μg/mL after	

Plant Organ	Pests	Bioassay	Efficiency	Ref
			24 h	
Leaves	*Aedes albopictus*	Aqueous suspension of essential oil against the fourth-instar mosquito larvae	Significant larvicidal activity with LC$_{50}$ range of 51.2–57.9 µg/mL after 24 h	[28]
Leaves	*Aedes aegypti*	Repellent assay	82% of repellency at 1.92 µg/cm^3 after 20 min	[32]
Bark			More than 70% of repellency at 1.92 µg/cm^3 after 20 min	
Twigs			About 70% of repellency at 1.92 µg/cm^3 after 20 min	
Wood			More than 50% of repellency at 1.92 µg/cm^3 after 20 min	
Leaves	*Aedes albopictus*	Repellent assay	71% of repellency at 1.92 µg/cm^3 after 20 min	[32]
Bark			More than 60% of repellency at 1.92 µg/cm^3 after 20 min	
Twigs			More than 60% of repellency at 1.92 µg/cm^3 after 20 min	
Wood			More than 60% of repellency at 1.92 µg/cm^3 after 20 min	
Leaves	*Anopheles gambiae*	Aqueous suspension of essential oil against the third-instar mosquito larvae	Significant larvicidal activity with LC$_{50}$ of 40.9 µg/mL after 24 h	[33]
Leaves	*Coptotermes formosanus*	Contact assay (on treated filter papers) against the adult termite	High mortality, with LD$_{50}$ of 1.57 mg/g after 7 days	[27]
Bark			Inactive	[34]
Sapwood			Mortality of 100% with LC$_{50}$ of 4.7 mg/g after 5 days	
Heartwood			Mortality of 100% with LC$_{50}$ of 2.8 mg/g after 5 days	
Leaves	*Reticulitermes chinensis*	Contact assay (on treated filter papers) against the adult termite	High mortality, with LC$_{50}$ of 0.9 µL/mL after 5 days	[35]
Bark			LC$_{50}$ of 19.6 µL/mL after 5	

Plant Organ	Pests	Bioassay	Efficiency	Ref
			days	
Sapwood			LC_{50} of 158.3 µL/mL after 5 days	
Heartwood			High mortality, with LC_{50} of 1.8 µL/mL after 5 days	
Leaves	*Tetranychus kanzawai*	Contact assay (on treated leaf discs) against spider mites	Significant mortality, with LC_{50} of 1109 µg/mL after 96 h	[25]
Leaves	*Tetranychus urticae*	Contact assay (on treated leaf discs) against spider mites	Significant mortality, with LC_{50} of 1150 µg/mL after 96 h	[25]
Leaves	*Reticulitermes chinensis*	Contact assay (on treated filter papers) against the adult termite	High mortality, with LC_{50} of 0.9 µL/mL after 5 days	[35]
Bark			LC_{50} of 19.6 µL/mL after 5 days	
Sapwood			LC_{50} of 158.3 µL/mL after 5 days	
Heartwood			High mortality, with LC_{50} of 1.8 µL/mL after 5 days	
Leaves	*Tetranychus kanzawai*	Contact assay (on treated leaf discs) against spider mites	Significant mortality, with LC_{50} of 1109 µg/mL after 96 h	[25]
Leaves	*Tetranychus urticae*	Contact assay (on treated leaf discs) against spider mites	Significant mortality, with LC_{50} of 1150 µg/mL after 96 h	[25]

LD_{50}—lethal dose 50; LC_{50}—lethal concentration 50.

4. Other Biocidal Activities of *C. japonica* EO

EO from leaves of *C. japonica* from Azores (α-pinene chemotype) has recently been reported to have high molluscicidal activity against *Radix peregra* (Lymnaeidae), a freshwater snail that hosts a number of significant parasites, such as *Fasciola hepatica* [36]. This parasite is the causing agent of fascioliasis, a well-known veterinary problem of vertebrate domestic livestock, which causes animal production losses and consequent economic costs [37]. The lethal concentrations (LC_{50}) of the EO varied between 33.3 and 61.8 ppm on an aqueous suspension assay [36]. Although the author has not assessed individual EO components for molluscicidal activity, it is worth pointing out that this EO has α-pinene as the major component. Moreover, this same EO chemotype exhibited inhibitory activities against *Pseudaletia unipuncta* (Lepidoptera: Noctuidae), an important pest of agricultural crops.

Particularly, this leaf EO inhibited eggs hatching and thereafter exhibited lethal and sub-lethal effects. Additionally, it showed high repellency properties against adults [38].

More recently, some other *C. japonica* bioactivities have been described. For instance, Tanaka et al. [39] reported that chemical components of *C. japonica* leaves suppress the growth of invasive plants (*Robinia pseudoacacia*) and weeds (*Trifolium repens*), which are the largest competitor of agricultural crops, with negative impact on productivity.

In addition, there are some studies on the growth inhibition activities of *C. japonica* chemical components (mainly from bark) against harmful marine [40] and freshwater [18] algae. The strong growth-inhibitory activity of the *C. japonica* bark EO against the bacillariophyceae *Skeletonema costatum* (commonly known as red tide plankton) was correlated with the presence of ferruginol [40], which has a strong antioxidant activity, as already mentioned. The potential of a new use for components of *C. japonica* bark EO to control red tide plankton growth, a serious environmental problem in the world's oceans, which can have an adverse impact on aquaculture.

5. Pharmacological Properties of *C. japonica* EO

Aromatic plants, such as *C. japonica*, synthesized and emitted complex mixtures of volatile organic compounds (VOCs) in order to facilitate their growth and survival [41][42].

The VOCs from *C. japonica* have already been reported to provide relaxing and stress-relieving effects on mice [43] and on humans [44]. In this way, *C. japonica* EO could be a useful tool in mental health management.

In addition, the most abundant VOCs emitted from *C. japonica* wood during the drying process, in the wood industry, are sesquiterpenes, such as δ-cadinene, α-muurolene and β-cadinene. These VOCs were assessed as having soothing effects in a human sensory evaluation [45]. Moreover, the main constituent of *C. japonica* heartwood EO is δ-cadinene [12][41]. It is believed that this compound and other VOCs of *C. japonica* suppress the sympathetic nervous system activity in humans, especially in women, stimulating relaxing and pleasurable emotions [46]. Furthermore, VOCs from *C. japonica* leaves display antitussive effects in guinea pigs [47] and increases the output of fluid in the respiratory tract [48].

On the other hand, different tissues of *C. japonica* also have bioactive compounds with benefits to the skin, which can have several skin health applications (inhibiting melanin production, skin ageing, antiseptic, etc.) [21][49][50]. In particular, phytocompounds from this heartwood have been reported to possess antifungal properties in vitro against *Trichophyton rubrum*, a major cause of *Tinea pedis* [13]. Moreover, EO from *C. japonica* leaves also showed excellent anti-inflammatory and antibacterial activities in vitro, being an attractive acne-mitigating candidate [14]. Another recent study [51] also stated that phytochemicals compounds from *C. japonica* by-products, namely diterpenoids, can be useful in skin health.

In addition, the EO from *C. japonica* leaves, namely its diterpenes, is a strong inhibitor of acetylcholinesterase activity, which could be an effective therapeutic agent for Alzheimer's disease [52]. Alternatively, this EO can be explored in the preventive dentistry field, as an effective inhibitor of oral bacteria [15][53], or as an anticancer chemopreventive [54] and antiulcer [55] agent. The results of this last study, suggested that 4-terpineol could have gastroprotective activity.

Overall and according to the literature [54], various parts of *C. japonica* have been used in Asian folk medicine for a variety of indications, including liver ailments, and an antitussive, and for its antiulcer activities. Hence, more studies are warranted with respect to these health benefits, namely, the toxic effects.

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