

Unraveling the biotechnological potential of Azorean *Cryptomeria japonica* by-products

Tese de Doutoramento

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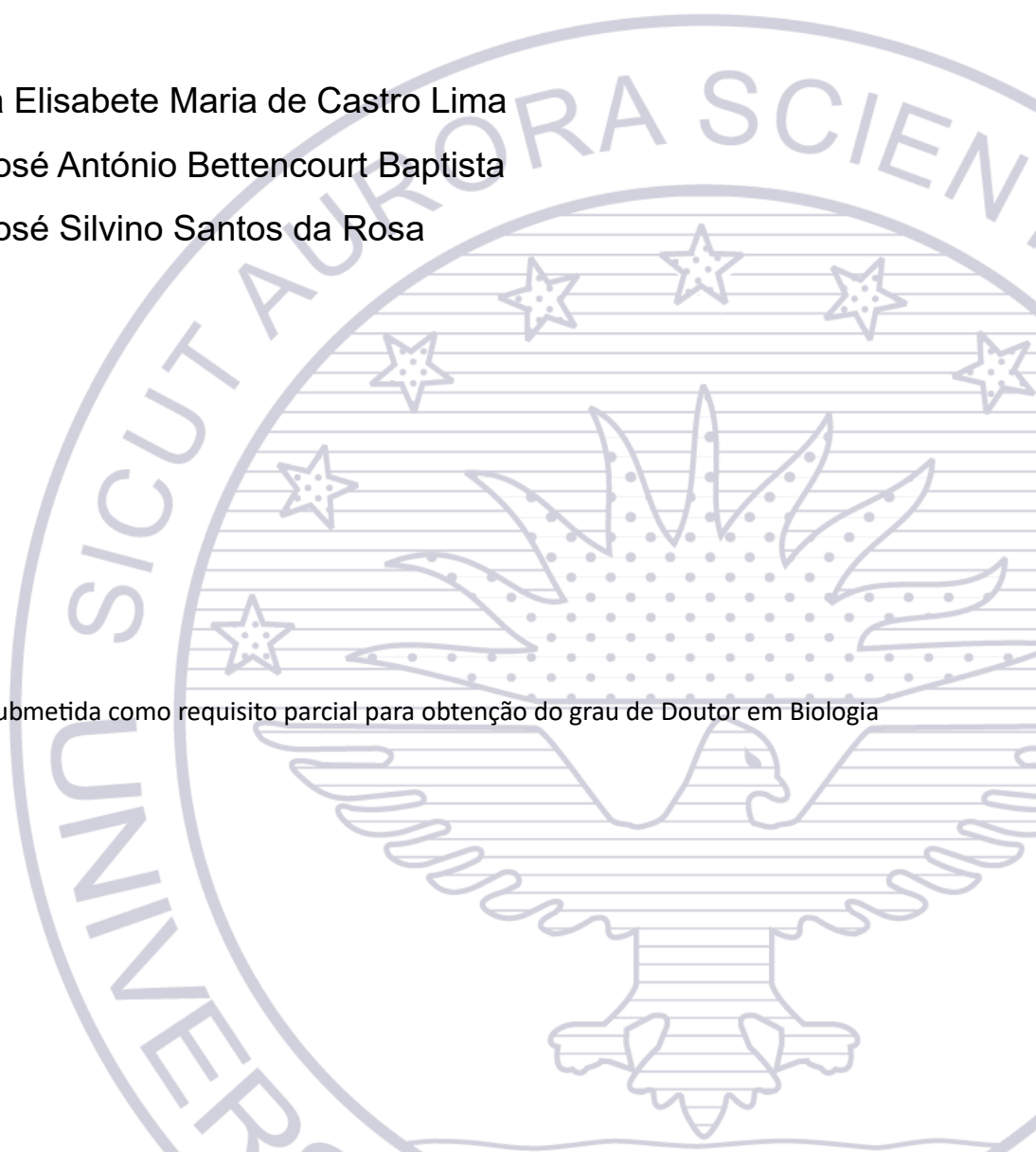
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Statement of authorship

I hereby declare that this thesis is the result of my own work and research. Where the work of others has been used, this has been fully acknowledged and referenced. All scientific data obtained throughout this PhD project and herein presented was submitted, peer-reviewed and published in indexed scientific journals as the following research articles:

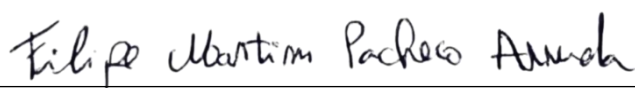
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As these studies were carried out in collaboration with other authors, I also declare that I was the main responsible for designing and carrying out the experimental and field work, as well as interpreting the results, composing the manuscripts and submitting the research papers for publication as the first author.



Dedicado aos meus pais

Pela confiança, pelo apoio incondicional e pelo exemplo que sempre me guiou.

Abstract

Forestry biomass residues represent a global challenge in waste management but also a significant opportunity for the development of a circular bioeconomy. This potential remains insufficiently explored for *Cryptomeria japonica* biomass residues (CJBR) which can be upcycled into high-value products, such as essential oils (EOs). Owing to their remarkable chemical diversity, EOs constitute a promising source of multifunctional bioactive compounds with antioxidant, antibacterial, and antifungal properties, among others. Harnessing this potential not only supports sustainable forest residue valorization but also aligns with the growing global demand for plant-derived products. Nonetheless, realizing this opportunity requires the establishment of robust quality-assurance measures to ensure consistency and reliability in product performance.

Cryptomeria japonica (Japanese cedar or “sugi”), a long-living aromatic conifer native to Japan, is a monoecious evergreen species within the Cupressaceae family and belongs to a monotypic genus. Valued for centuries, its uses range from ornamental and construction purposes to a component in Asian folk medicine. *C. japonica* was introduced to the Azores islands in the mid-19th century and has since become a dominant feature of the archipelago’s landscape. Currently, *C. japonica* is also the principal timber species in the Azores, occupying approximately 60% of the region’s total wood-producing forest area. Consequently, large quantities of diverse CJBR, such as foliage, cones, branches, bark, sapwood, heartwood, wood chips, and sawdust, are generated annually from forestry operations and wood industry. Among these, CJBR aerial parts (i.e. branches and foliage) are, nowadays, the main source used by local wood producers to obtain EO through steam distillation (SD). These EOs are mainly employed for household aromatherapy; however, their heterogeneous, complex composition and variable bioactivity limit the development of standardized and higher-value applications.

Nevertheless, given the chemical complexity of *C. japonica* EOs it is hypothesized that a controlled fractionation during the hydrodistillation (HD) process enables the selective recovery of fractions exhibiting distinct chemical compositions and thus differential biological activities. Based on this rationale, the present thesis aims to optimize and characterize, for the first time, the HD fractionation of Azorean *C. japonica* foliage (Az-CJF) and female cones (Az-CJFC) EOs to obtain targeted fractions with enhanced specific bioactivities for potential industrial applications.

The Az–CJF and Az–CJFC EOs were fractionated into six EO fractions (Frs. 1–6) collected during HD at sequential timeframes (HDTs: 0–2, 2–10, 10–30, 30–60, 60–120, and 120–240 min). In addition, Az–CJF and Az–CJFC EOs were also obtained by a typical HD over 4 h, for comparative purposes. The obtained EO samples (crude EOs and fractions) were evaluated for their chemical composition (GC–FID/GC–MS analyses), yield, physical properties (density and colour) and biological activities (antibacterial, antifungal, antioxidant, and cytotoxicity on HaCaT cells and brine shrimp nauplii). Furthermore, regression models were developed to predict EO yield, HD rate, and composition (major components) for a given HDT.

Results indicated that early fractions (Frs. 1–3) are richer in monoterpenes, while later fractions (Frs. 4–6) are richer in sesquiterpenoids and diterpenes. This compositional shift was reflected in their bioactive profiles, as initially hypothesized. Overall, later fractions displayed higher antioxidant, antimicrobial and cytotoxicity effects than earlier fractions. In practice, the results of this research can help reduce the distillation time and operating costs associated with the HD process, as well as produce EO fractions with unique compositions with different commercial applications, which increases the value of the *C. japonica* EO industry.

In conclusion, this study shows that manipulating the HDT effectively controls the chemical composition of *C. japonica* EO fractions. This allows EO producers to create higher-value products by controlling HDT and can also serve as an incentive for the local *C. japonica* EO industry to optimize steam distillation (SD) timeframes. Ultimately, this approach enhances the valorisation of Azorean CJBR – a by-product of the local timber industry – and strengthens the local circular economy.

Keywords: Azores; *Cryptomeria japonica*; biomass residues valorization; hydrodistillation; essential oil fractionation; antibacterial; antifungal; antioxidant; HaCaT cytotoxicity; *Artemia salina* cytotoxicity.

Resumo

Os resíduos de biomassa florestal representam um desafio global na gestão de resíduos, mas também uma oportunidade significativa para o desenvolvimento de uma bioeconomia circular. Este potencial permanece insuficientemente explorado no caso dos resíduos de biomassa de *Cryptomeria japonica* (CJBR), que podem ser valorizados através da produção de produtos de elevado valor acrescentado, como os óleos essenciais (OEs). Devido à sua notável diversidade química, os OEs constituem uma fonte promissora de compostos bioativos multifuncionais com propriedades antioxidantes, antibacterianas e antifúngicas, entre outras. O aproveitamento deste potencial não só promove a valorização sustentável dos resíduos florestais, como também está em consonância com a crescente procura global por produtos de origem vegetal. No entanto, para concretizar esta oportunidade, é indispensável estabelecer medidas rigorosas de controlo de qualidade que assegurem a consistência e fiabilidade do desempenho dos produtos.

Cryptomeria japonica (cedro-do-Japão), uma conífera aromática de vida longa, é uma espécie monóica e perenifolia pertencente à família Cupressaceae e a um género monotípico. Originária do Japão, a *C. japonica* foi amplamente introduzida noutras regiões temperadas. Em particular, foi introduzida nos Açores em meados do século XIX, tornando-se desde então num elemento característico da paisagem insular. Atualmente, esta planta é também a principal espécie florestal produtora de madeira nos Açores, ocupando aproximadamente 60% da área total de floresta produtiva da região. Consequentemente, grandes quantidades de CJBR, como folhagem, cones, ramos, casca, cerne, alburno, aparas e serradura, são geradas anualmente pelas operações florestais e pela indústria madeireira. Entre estes, as partes aéreas (ramos e folhagem) constituem, atualmente, a principal fonte utilizada pelos produtores locais de madeira para a obtenção de OE através de destilação por arraste de vapor (SD). Estes OEs são principalmente utilizados em aromaterapia doméstica; contudo, a sua composição heterogénea e complexa, bem como a variabilidade da sua bioatividade, limitam o desenvolvimento de aplicações normalizadas e de maior valor acrescentado.

Dada a complexidade química dos OEs de *C. japonica*, coloca-se a hipótese de que um fracionamento controlado durante o processo de hidrodestilação (HD) permita a recuperação seletiva de frações com composições químicas distintas e, consequentemente, com diferentes perfis biológicos. Com base nesta premissa, a presente tese teve como objetivo otimizar e caracterizar, pela primeira vez, a HD fracionada de folhagem (Az-CJF) e cones femininos (Az-

CJFC) de *C. japonica* dos Açores, de modo a obter frações direcionadas e potenciar bioatividades específicas com potenciais aplicações industriais.

Os OEs de Az-CJF e Az-CJFC foram fracionados em seis frações (Frs. 1–6), recolhidas durante a HD em intervalos de tempo sequenciais (HDTs: 0–2, 2–10, 10–30, 30–60, 60–120 e 120–240 min). Adicionalmente, OEs de Az-CJF e Az-CJFC foram também obtidos por HD convencional durante 4 h, para fins comparativos. As amostras obtidas (OEs brutos e frações) foram avaliadas quanto à sua composição química (análises por GC–FID/GC–MS) rendimento, propriedades físicas (densidade e cor) e atividades biológicas (antibacteriana, antifúngica, antioxidante e citotoxicidade em células HaCaT e náuplios de *Artemia salina*). Além disso, foram desenvolvidos modelos de regressão para prever o rendimento, a taxa de HD e a composição química (principais constituintes) para determinados HDTs.

Os resultados indicaram que as frações iniciais (Frs. 1–3) são mais ricas em monoterpenos, enquanto as frações posteriores (Frs. 4–6) destacam-se pelos teores superiores de sesquiterpenóides e diterpenos. Esta variação composicional refletiu-se nos perfis bioativos, conforme inicialmente hipotetizado. Globalmente, as últimas frações exibiram maior atividade antioxidante, antimicrobiana e citotóxica do que as iniciais. Na prática, os resultados desta investigação podem contribuir para reduzir o tempo de destilação e os custos operacionais associados ao processo de HD, bem como permitir a obtenção de frações de OE com composições únicas e diferentes aplicações comerciais, aumentando assim o valor da indústria de OEs de *C. japonica*.

Em conclusão, este estudo demonstra que a manipulação dos HDTs permite controlar eficazmente a composição química das frações de OE de *C. japonica*. Tal possibilita aos produtores de OEs criar produtos de maior valor acrescentado através do controlo da HDT e pode também servir de incentivo para a otimização dos tempos de destilação por vapor (SD) na indústria local de OE de *C. japonica*. Em última análise, esta abordagem contribui para a valorização dos CJBR açorianos – subprodutos da indústria madeireira local – e para o fortalecimento da economia circular regional.

Palavras-chave: Açores; *Cryptomeria japonica*; valorização de resíduos de biomassa; hidrodestilação; fracionamento de óleo essencial; atividade antibacteriana; atividade antifúngica; atividade antioxidante; citotoxicidade em células HaCaT; citotoxicidade em *Artemia salina*.

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