

Essential Oil Characterization of Two Azorean *Cryptomeria japonica* Populations and Their Biological Evaluations

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Essential oils from foliage, bark and heartwood of *Cryptomeria japonica* D. Don from Azores Archipelago (Portugal) were analyzed by GC and GC-MS. Two populations, of black and reddish heartwood color, were studied. The main compounds found in the foliage of both populations were α -pinene (9.6-29.5%), (+)-phyllocladene (3.5-26.5%), *ent*-kaur-16-ene (0.2-20.6%), sabinene (0.5-19.9%) and limonene (1.4-11.5%), with a large variation in individual compounds from each population. Heartwood oils were characterized by a high content of cubebol (2.8-39.9%) and *epi*-cubebol (4.1-26.9%) isomers, which were absent in the foliage. Elemol and eudesmol isomers were found in the foliage and heartwood oils, while (+)-phyllocladene was absent in heartwood. Black and reddish bark oils were composed of the diterpenes dehydroferruginol (1.9-5.1%) and ferruginol (2.6-11.5%), along with the sesquiterpenes δ -cadinene (10.4-15.9%), α -muurolene (3.3-5.4%), *epi*-zonarene (4.0-5.0%), cubenol (9.3-14.0%), τ -muurolol (4.8-10.7%), β -eudesmol (3.0-9.9%), γ -eudesmol (1.9-7.0%) and hedychariol (1.4-6.2%). Azorean *C. japonica* oils exhibited significant chemical differences compared with native plants from Asia.

The essential oils showed moderate antimicrobial activity against the pathogenic fungus *Cryptococcus neoformans* and human pathogenic bacteria (especially against multidrug-resistant *Mycobacterium tuberculosis*). The antimicrobial activity of the essential oils may be attributed to compounds such as *ent*-kaur-16-ene, (+)-phyllocladene, ferruginol and elemol, which are present in different proportions within the complex oil mixture. These results suggest a potential use for *C. japonica* oils obtained from wood industry leftovers.

Keywords: *Cryptomeria japonica*, Essential oils, GC-MS, Pathogenic fungi, *Mycobacterium tuberculosis*.

Cryptomeria japonica D. Don. (Cupressaceae), also known as Sugi or Japanese cedar, is an Asian conifer that was introduced into the Azores Islands in the mid-19th century. *C. japonica* progressively overcame endemic tree species, and nowadays is widely distributed in the islands, representing 57% of the total wood producing forest area, and with sawmills generating wastes having only minor and non-commercial applications [1]. Typically, *C. japonica* heartwood has a red color, but, in some cases, a black/dark color can also occur, reducing its commercial value [2,3]. During the last years, the incidence of reddish heartwood color of *C. japonica* trees in the Azores islands has been declining, leading to a rise of economically lower valued black heartwood specimens. The regional Azores government, in 1998, initiated a Forest Breeding Program in order to evaluate the genetic basis of the Azores sugi population. This program intended to improve the genetic species of *Cryptomeria* and to increase the productivity and quality of wood [4].

In recent years, particular interest has been placed on the study of *C. japonica* essential oils (EOs) from Japan, Korea, Taiwan and China, with some significant activities being reported, such as antiulcer [5], antifungal [6,7], antimicrobial [8-10], cancer chemopreventive [11], neuropharmacological [12], antimosquito [13-15], antitermite [16,17] and insecticidal [18,19].

In a strategy of valorization of an unrecognized natural resource in the Azorean islands, the main purpose of this study was to find a promising potential use for the unutilized bark, foliage and wood leftovers of *C. japonica*, by obtaining and chemically identifying their EOs, as well as evaluating their antimicrobial activities against wood deteriorating fungi, human pathogenic fungi and bacteria. This is the first study reporting the composition of the EOs of the two different *C. japonica* populations, black and reddish heartwood trees, of Azores, Portugal (Faial island).

For these purposes, two tree sampling sites were chosen, taking into account sugi heartwood color, a physical property that could influence the composition of the EOs. The black heartwood population was assigned as A, and the reddish heartwood population as B. Table 1 shows the yields of obtained EOs from the different tissues of *C. japonica* analyzed.

Comparing oil yields, taking into account the heartwood color of each *C. japonica* specimen, the variation in the foliage of black heartwood trees was higher than in the oils of reddish heartwood foliage, based on fresh weight (A- 1.1-1.9% and B- 0.5-0.8%). For heartwood oils of both populations, the yields obtained showed little differences (A- 0.6-2.0% and B- 0.5-2.1%), with the same being