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In vitro DNA protective potential of *Chamaecyparis lawsoniana* (A.Murray bis) Parl. and *Thuja plicata* Donn ex D.Don essential oils

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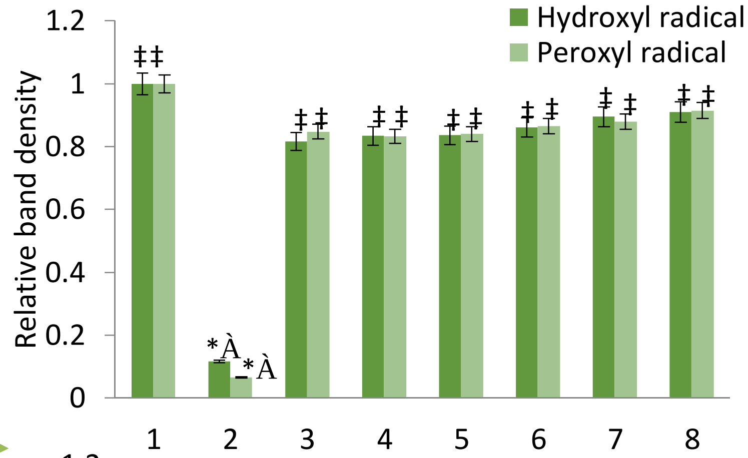
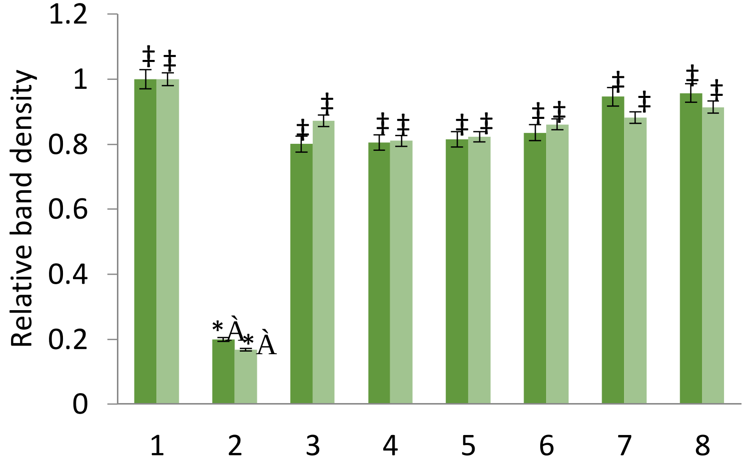
Chamaecyparis lawsoniana

Thuja plicata

Essential oils





Abstract:
Chamaecyparis lawsoniana (A.Murray bis) Parl., known as Port Orford cedar or Lawson cypress and *Thuja plicata* Donn ex D.Don, also called giant arborvitae, are a species of the Cupressaceae family. Multiple significant biological activities of *C. lawsoniana* and *T. plicata* essential oils have been widely investigated but their genotoxic and/or antigenotoxic properties are not well established. In the present study, two *in vitro* antioxidant assays were performed to assess the possible DNA protective activity of five different concentrations (0.19, 0.38, 0.75, 1.5, and 3%) of essential oils against oxidative damage induced by hydroxyl and peroxyl radicals. According to the obtained results, both essential oils have a remarkable DNA protective activity against hydroxyl and peroxyl radicals induced DNA damage. These findings suggest that *C. lawsoniana* and *T. plicata* essential oils in tested concentrations could be valuable candidates for medicinal and pharmaceutical applications.

Keywords: Antigenotoxicity; *Chamaecyparis lawsoniana*; essential oils; hydroxyl radical; peroxyl radical;*Thuja plicata.*

Introduction

Medicinal potential of essential oils is usually associated with antimicrobial, antioxidative and antiviral effects [1].

Although many studies have been conducted on the various biological activity of *C. lawsoniana* (Figure 1) and *T. plicata* (Figure 2) essential oils, there are no studies on their genotoxic and antigenotoxic effects to our knowledge. According to these data, essential oils from *C. lawsoniana* and *T. plicata* were tested for ability to protect DNA from oxidative damage induced by hydroxyl and peroxyl radicals.

Figure 1. *Chamaecyparis lawsoniana*
(https://plantmaterials.wordpress.com/portfolio/chamaecyparis-lawsoniana/)

Figure 2. *Thuja plicata*
(https://www.treesandshrubsonline.org/articles/thuja/thuja-plicata/)

[1] J. Reichling, P. Schnitzler, U. Suschke, R. Saller, Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties--an overview, Forsch Komplementmed, 16 (2009) 79-90.

Materials and methods

The protective effect of *C. lawsoniana* and *T. plicata* essential oils in concentrations of 3, 1.5, 0.75, 0.38, and 0.19% against hydroxyl and peroxyl radicals induced DNA damage was investigated *in vitro* using the salmon sperm DNA [2,3] and quercetin (0.01%) as a standard drug [4].

DNA bands on the agarose gels were visualized under UV light (UV transilluminator, Vilber Lourmat, France) at 365 nm, photographed, and analyzed using ImageJ software (version 1.48 for Windows, Softonic International, Barcelona, Spain).

Results were expressed as mean ± SD and statistical evaluation of data was analyzed with one-way analysis of variance (ANOVA) using SPSS statistical software package, version 13.0 for Windows. The significance level was set at $p < 0.05$.

[2] Y.W. Lin, Y.T. Wang, H.M. Chang, J.S.B. Wu, DNA protection and antitumor effect of water extract from residue of jelly fig (*Ficus awkeotsang* Makino) achenes, Journal of Food and Drug Analysis, 16 (2008) 63-69.

[3] L.L. Zhang, L.F. Zhang, J.G. Xu, Q.P. Hu, Comparison study on antioxidant, DNA damage protective and antibacterial activities of eugenol and isoeugenol against several foodborne pathogens, Food and Nutrition Research, 61 (2017), 1353356.

[4] C.A. Poorna, M. Resmi, E. Soniya, *In vitro* antioxidant analysis and the DNA damage protective activity of leaf extract of the *Excoecaria agallocha* linn Mangrove plant. In: Stoytcheva, M. (Ed.), Agricultural Chemistry. Intech, UK, 2013.

Results and discussion

The DNA protective activity of several concentrations of *C. lawsoniana* essential oil against the damage induced by hydroxyl and peroxyl radicals is shown in Figure 3.

There was no statistically significant difference in the relative electrophoretic band densities of the *C. lawsoniana* essential oil and intact DNA from salmon sperm as negative control. DNA protective activity was similar or stronger than quercetin, a well-known protective compound.

C. lawsoniana essential oil, at the concentration range from 0.19 to 3%, demonstrated a strong protective effect against hydroxyl and peroxyl radicals induced DNA damage.

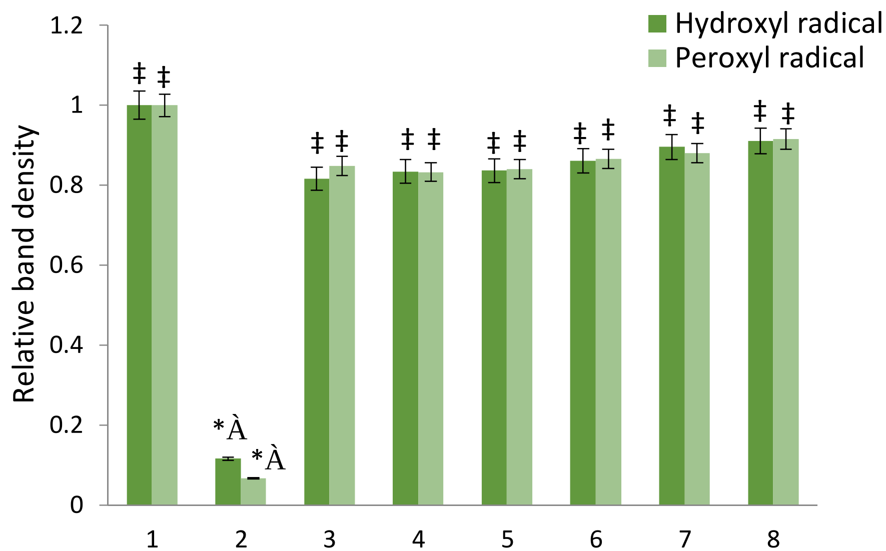


Figure 3. Protective potential of essential oil of *Chamaecyparis lawsoniana* against hydroxyl and peroxyl radicals-induced DNA damage. DNA from salmon sperm (1, negative control), DNA damage control (2, positive control), quercetin (3, 0.01%, standard), essential oil in concentrations of 3, 1.5, 0.75, 0.38, and 0.19% (4, 5, 6, 7, and 8).
$^{*}p < 0.05$ when compared with the negative control, $^{\dagger}p < 0.05$ when compared with the standard, $^{\ddagger}p < 0.05$ when compared with the positive control

Results and discussion

No statistically significant differences in the relative electrophoretic band densities were observed between different concentrations of *T. plicata* essential oil and the respective negative control.

In lower concentration *T. plicata* essential oil almost completely protected DNA against hydroxyl radicals induced DNA damage (Figure 4).

T. plicata essential oil reduced peroxyl radicals induced DNA damage in a concentration dependent manner compared to the negative and positive controls.

The observed activity was comparable to that of the well known antioxidant quercetin.

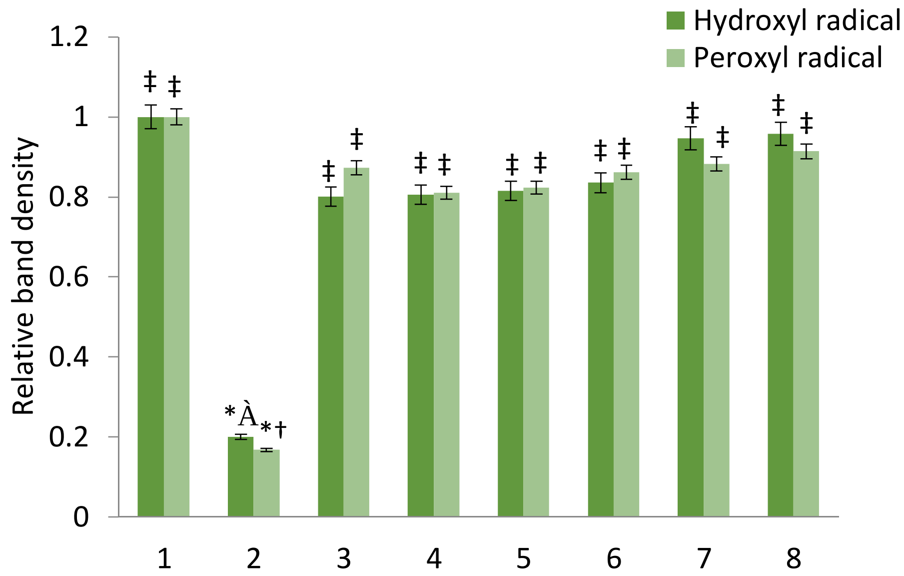


Figure 4. Protective potential of essential oil of *Thuja plicata* against hydroxyl and peroxyl radicals-induced DNA damage.
DNA from salmon sperm (1, negative control), DNA damage control (2, positive control), quercetin (3, 0.01%, standard), essential oil in concentrations of 3, 1.5, 0.75, 0.38, and 0.19% (4, 5, 6, 7, and 8).
$^{*}p < 0.05$ when compared with the negative control, $^{\dagger}p < 0.05$ when compared with the standard, $^{\ddagger}p < 0.05$ when compared with the positive control

Results and discussion

Observed DNA protective ability of *C. lawsoniana* and *T. plicata* essential oils can be attributed to the dominant compounds in both oils.

According to the previous study by Nikolić et al. [5] the most dominant compound in essential oil of *C. lawsoniana* was limonene (Figure 5), while the major compound of *T. plicata* essential oil was *α*-thujone (Figure 6).

[5] B.M. Nikolić, S.D. Milanović, I.Lj. Milenković, M.M. Todosijević, I.Ž. Đorđević, N.Z. Brkić, Z.S. Mitić, P.D. Marin, V.V. Tešević, Bioactivity of *Chamaecyparis lawsoniana* (A. Murray) Parl. and *Thuja plicata* Donn ex D. Don essential oils on *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae) larvae and Phytophthora de Bary 1876 root pathogens, Industrial Crops and Products, 178 (2022) 114550.

Results and discussion

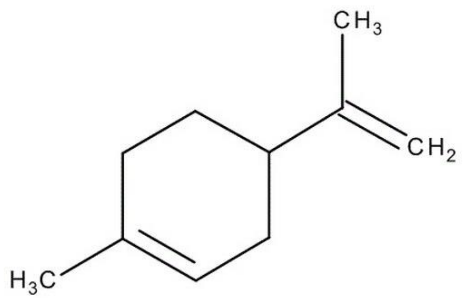


Figure 5. Structure of limonene, the principal component of *C. Lawsoniana* essential oil

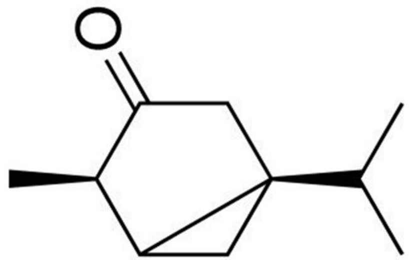


Figure 6. Structure of *α*-thujone, the principal component of *T. plicata* essential oil

Bearing in mind the previously published data [6, 7], on the absence of genotoxic potential of limonene and thujone in the lowest concentrations, presence of these compounds in tested essential oils may be responsible, at least in part, for their more significant DNA protective activity in lower concentrations than in the higher concentrations.

[6] M. Bacanli, A.A. Başaran, N. Başaran, The antioxidant and antigenotoxic properties of citrus phenolics limonene and naringin, Food and Chemical Toxicology, 81 (2015) 160-170.
[7] O. Pelkonen, K. Abass, J. Wiesner, Thujone and thujone-containing herbal medicinal and botanical products: toxicological assessment, Regulatory Toxicology and Pharmacology, 65 (2013) 100-107.

Conclusions

- The results of our study show that both oils have a remarkable DNA protective activity against hydroxyl and peroxyl radicals induced damage.
- This is the first study showing DNA protective effect of *C. lawsoniana* and *T. plicata* essential oils.
- Further studies with *in vivo* biological models should provide a better understanding of the mechanisms underlying DNA protective ability of these two essential oils.

Acknowledgments

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