28-29 September 2023,
Kragujevac, Serbia

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# 2nd International Conference on Chemo and Bioinformatics
# ICCBIKG_2023

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Sponsored by

2nd International Conference on Chemo and BioInformatics, Kragujevac, September 28-29, 2023, Serbia.

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**Publisher:**

Institute for Information Technologies, University of Kragujevac, Serbia, Jovana Cvijića bb, 2023

**Press:**

„Grafo Ink“, Kragujevac

**Impression:**

120 copies

CIP - Каталогизација у публикацији - Народна библиотека Србије, Београд

54:004(048)(0.034.2)
57+61]:004(082)(0.034.2)

INTERNATIONAL Conference on Chemo and BioInformatics (2 ; 2023 ;
Kragujevac) Book of Proceedings [Elektronski izvor] / 2nd International Conference on
Chemo and BioInformatics, ICCBIKG 2023, September 28-29, 2023 Kragujevac,
Serbia ; [editors Zoran Marković, Nenad Filipović]. - Kragujevac : University,
Institute for Information Technologies, 2023 (Kragujevac : Grafo Ink). - 1 USB
fleš memorija ; 1 x 2 x 6 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž
120. - Bibliografija uz svaki rad.

ISBN 978-86-82172-02-4

a) Хемија -- Информациона технологија -- Зборници b) Биомедицина --
Информациона технологија -- Зборници

COBISS.SR-ID 125908489

# Oxidative DNA damage preventive activity of essential oils of three *Pinus* species*: P. mugo, P. sibirica,* and *P. silvestre*

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DOI: 10.46793/ICCBI23.321M

**Abstract**: Pharmacological properties of essential oils of *Pinus* species are mainly associated with antimicrobial, antioxidant, anticancer, anti-aging, and anti-inflammatory potencies. However, only limited scientific information has been gathered regarding the genotoxic and antigenotoxic activities of *Pinus* essential oils. Therefore, the aim of the present study was to investigate the *in vitro* DNA protective activities of three commercial essential oils of *Pinus* species: *P. mugo*, *P. sibirica*, and *P. silvestre,* against the oxidative damage induced by hydroxyl and peroxyl radicals. The tested essential oils significantly reduced the DNA damage caused by the free radicals. Due to its capacity to decrease DNA damage, essential oils of *Pinus* species can be of great importance in clinical applications.

**Keywords**: essential oils, DNA damage, free radicals, antigenotoxicity, *Pinus*

## 1. Introduction

Essential oils (EOs) obtained from medicinal plants have been known to possess a number of beneficial pharmacological properties and are widely used in traditional medicine for the treatment of different disorders [1]. Also, EOs are considered to be less toxic and with fewer side-effects compared to synthetic chemicals [2], which makes them safe to be used as medicinal ingredients [3]. Reports indicate the beneficial properties of *Pinus* species such as anti-inflammatory, antioxidant [4], antineoplastic and immuno-

modulatory [5]. The *Pinus* species EOs have been used in the food and pharmaceutical industries [6] and for medicinal purposes in aromatherapy worldwide.

*P. mugo* Turra, *P. sibirica* Du Tour, and *P. silvestre* L. are a species of the genus *Pinus* of the Pinaceae family from the Gymnospermae class. *P. mugo,* also known as dwarf mountain pine, is used in traditional medicine for treating various illnesses such as respiratory diseases [7], coughs and throat inflammation [8]. Several studies reported anti-inflammatory, cytotoxic [9], analgesic, cardioprotective, and neuroprotective activities of *P. mugo* EO [10]. On the other hand, *P. sibirica*, known as cedar nuts, is traditionally used in folk medicine for the prevention and treatment of various diseases such as infections, wounds, rheumatism and arthritis, and for liver and kidney dysfunction [11]. Finally, *P. sylvestre,* also known as scots pine, is a traditional remedy with antibacterial, antifungal, anti-inflammatory, antioxidant, antiseptic, anti-parasitic, anti-viral, anti-allergenic, antispasmodic, and anti-hyperglycemic activities [12]. Besides, there have been studies on the potential therapeutic value of *P. mugo*, *P. sibirica*, and *P. silvestre* EOs, but not sufficient literature data has been published regarding their genotoxic and/or antigenotoxic properties. Therefore, this study aims to evaluate their *in vitro* antigenotoxic effects using two antioxidative assays. To the best of our knowledge, this report is the first to investigate the antigenotoxic properties of these three *Pinus* species.

## 2. Materials and methods

### *2.1. Essential oils*

Essential oils (Farmalabor srl, Assago, Italy) were dissolved in dimethyl-sulfoxide (DMSO) at 50 mg/mL to obtain complete solubilization and further diluted in the medium for *in vitro* experiments.

### *2.2. The protective activity of essential oils against hydroxyl and peroxyl radicals induced DNA damage*

The protective effect of commercial essential oils (25, 50, 100, 200, and 400 μg/mL) against hydroxyl and peroxyl radicals induced DNA damage was assessed *in vitro* using the salmon sperm DNA [13, 14]. In both assays, quercetin (100 μg/mL) was used as a standard drug [15]. 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).

### *2.3. Statistical analysis*

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

## 3. Results and Discussion

To explore the potential DNA protective activities of three commercial EOs against the hydroxyl radicals, formed in a Fenton-type decomposition of $H_2O_2$ catalysed by

$FeSO_4$, as well as against the peroxyl radicals, formed after the *in situ* oxidation of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) with molecular oxygen, two oxidative DNA damage protective activity assays were performed.

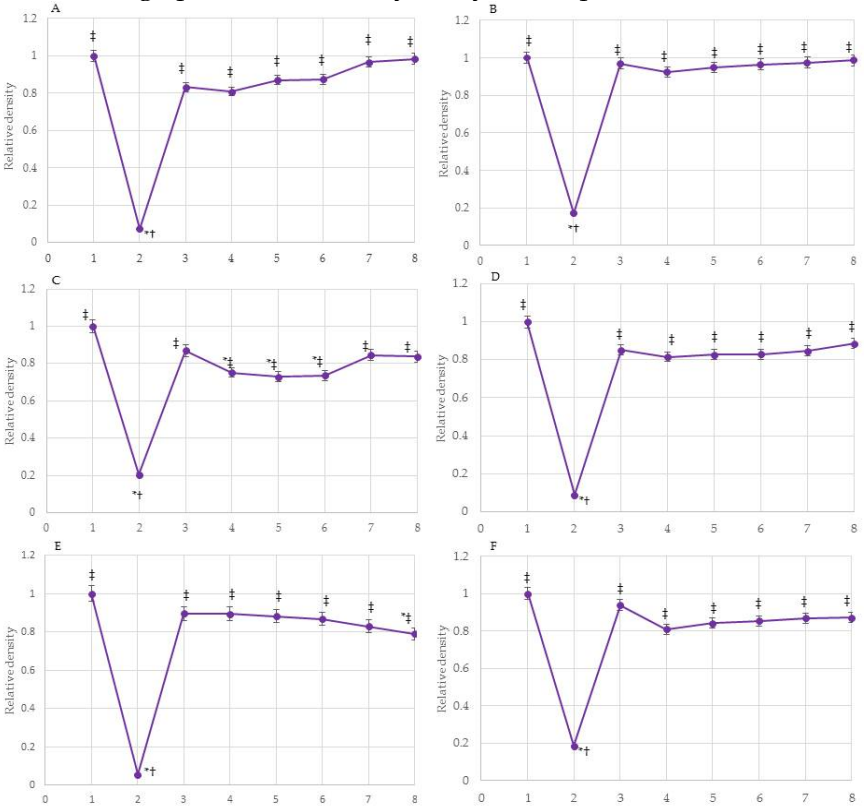


**Figure 1.** Protective effect of essential oils of *P. mugo* (A and B), *P. sibirica* (C and D), and *P. silvestre* (E and F) against hydroxyl and peroxyl radicals-induced DNA damage. DNA from salmon sperm (1, negative control), DNA damage control (2, positive control), quercetin (3, 100 μg/mL, standard), essential oil in concentrations of 25, 50, 100, 200, and 400 μg/mL (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.

The DNA protective activity against the damage induced by hydroxyl (Figure 1A) and peroxyl (Figure 1B) radicals of different concentrations of *P. mugo* EOs has been dose-dependent, increasing with the rise of concentrations applied. In concentrations from 25 to 400 μg/mL, *P. sibirica* essential oil showed effective and concentration-dependent reduction in the DNA damage induced by both radicals, which was better as the administered doses increased (Figure 1C and D). Distinct EO at high concentrations (200 and 400 μg/mL) showed better DNA protective activity against DNA damage induced by peroxyl than for hydroxyl radicals. *P. silvestre* EO at high concentrations (200 and 400 μg/mL) had a weak scavenging activity on hydroxyl radicals (Figure 1E) and was significantly more effective in scavenging peroxyl radicals (Figure 1F). According to the experimental findings, the highest DNA protective activity against free radicals was found in *P. mugo* EO, and the lowest DNA protective potential against peroxyl radicals was determined in *P. sibirica* and *P. silvestre* EOs.

## 4. Conclusions

The results from the present study indicated that the tested essential oils are valuable natural agents with a high degree of hydroxyl radical scavenging activity and ability to scavenge peroxyl radicals.

## Acknowledgment

This research is funded by the Ministry of Science, Technological Development, and Innovation, Republic of Serbia, Grants: Nos. 451-03-47/2023-01/200122 and 451-03-47/2023-01/200378.

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