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# Biological activity of *Pinus nigra* terpenes—Evaluation of FtsZ inhibition by selected compounds as contribution to their antimicrobial activity

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ABSTRACT

In the current work, *in vitro* antioxidant, antibacterial, and antifungal activites of the needle terpenes of three taxa of *Pinus nigra* from Serbia (ssp. *nigra*, ssp. *pallasiana*, and var. *banatica*) were analyzed. The black pine essential oils showed generally weak antioxidative properties tested by two methods (DPPH and ABTS scavenging assays), where the highest activity was identified in *P. nigra* var. *banatica* ($IC_{50}$=25.08 mg/mL and VitC=0.67 mg (vitamin C)/g when tested with the DPPH and ABTS reagents, respectively). In the antimicrobial assays, one fungal (*Aspergilus niger*) and two bacterial strains (*Staphylococcus aureus* and *Bacillus cereus*) showed sensitivity against essential oils of all three *P. nigra* taxa. The tested oils have been shown to possess inhibitory action in the range from 20.00 to 0.62 mg/mL, where var. *banatica* exhibited the highest and ssp. *nigra* the lowest antimicrobial action. In order to determine potential compounds that are responsible for alternative mode of action, molecular docking simulations inside FtsZ (a prokaryotic homolog of tubulin) were performed. Tested compounds were the most abundant terpenoid (germacrene D-4-ol) and its structurally similar terpene (germacrene D), both present in all three essential oils. It was determined that the oxygenated form of the molecule creates stable bonds with investigated enzyme FtsZ, and that this compound, through this mechanism of action participates in the antimicrobial activity.



## 1. Introduction

*Pinus* (Pinaceae), with more than 200 generally recognized species, is the largest extant genus of Pinopsida [1]. Economically, pines are one of the most important tree species valued for wood, paper, resins, charcoal, food (particularly seeds), and ornamentals [2]. Moreover, most pines are considered as significant source of structurally diverse, bioactive compounds, and have provided contributions to the discovery of pharmaceutical agents and other biomedical applications [3]. For instance, turpentine have a long tradition of remedial utilization as topical rubefacient for the treatment of rheumatic diseases while pine bark extract is used in anti-aging cosmetics [4]. However, essential oils from *Pinus* species have been reported to have a variety of therapeutic properties. According to Dervendzi [5] pine needle oils are mostly used in folk medicine for the healing of respiratory infections accompanied by cough, bronchitis, bronchial asthma, emphysema, tracheitis, sinusitis, laryngitis, pharyngitis, and influenza. Moreover, pine oils are widely used as flavoring additives for food and beverages. In various household cleaning products they are well known as scenting and disinfectant agents. Also, these oils are used in the pharmaceutical industry as aroma or natural source of intermediates in the synthesis of perfume components [6].

The European black pine (*Pinus nigra* J.F.Arnold) is a tertiary relict belonging to the group of Mediterranean pines [7]. It is one of the most widespread and polymorphic conifers in Europe with highly fragmented distribution range that extends from North Africa through the northern Mediterranean and eastwards to the Black Sea. In the Flora of Serbia [8], two subspecies of black pine (ssp. *nigra* and ssp. *pallasiana*) are distinguished and within them several varieties (var. *nigra*, var. *zlatiborica*, var. *gocensis*, and var. *banatica*). However, recent studies on the chemotaxonomy of *P. nigra* in Serbia, based on both the *n*-alkane [9] and terpene [10] variability, recognized only three distinct chemotypes (*nigra*, *pallasiana* i *banatica*) within Serbian black pine populations. According to Šarac et al. [10], the major components in the needle

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**Table 1**
Chemical composition of the needle essential oils of three infraspecific taxa of *Pinus nigra* from Serbia [10].

| Entry | Compound | Content [%][a] | | | | Average content [%][b] |
|---|---|---|---|---|---|---|
| | | *P. nigra* taxa<br>Location | ssp. *nigra*<br>Zmajevački potok | ssp. *pallasiana*<br>Jarešnik | var. *banatica*<br>Lazareva reka | |
| *1* | Tricyclene | | 0.16 (0.07) | 0.40 (0.61) | 0.18 (0.06) | 0.26 (0.41) |
| *2* | α-Thujene | | 0.41 (0.30) | 0.41 (0.40) | 0.79 (0.34) | 0.48 (0.37) |
| ***3*** | **α-Pinene** | | **45.93 (10.46)** | **42.33 (8.59)** | **50.83 (7.39)** | **45.40 (9.60)** |
| *4* | Camphene | | 0.95 (0.29) | 1.95 (2.64) | 0.92 (0.36) | 1.35 (1.75) |
| *5* | Sabinene | | 0.31 (0.18) | 0.26 (0.18) | 0.33 (0.05) | 0.29 (0.17) |
| ***6*** | **β-Pinene** | | **6.90 (7.04)** | **5.15 (5.90)** | **3.10 (2.31)** | **5.47 (6.03)** |
| *7* | Myrcene | | 0.88 (0.21) | 0.45 (0.32) | 1.21 (0.26) | 0.77 (0.39) |
| *8* | α-Phellandrene | | 0.00 (0.00) | 0.01 (0.03) | 0.00 (0.00) | 0.00 (0.02) |
| *9* | $\Delta^3$-Carene | | 0.03 (0.12) | 0.70 (2.65) | 0.09 (0.31) | 0.31 (1.71) |
| *10* | α-Terpinene | | 0.00 (0.00) | 0.00 (0.02) | 0.01 (0.02) | 0.00 (0.02) |
| *11* | Limonene | | 2.16 (1.98) | 3.08 (5.61) | 5.04 (2.82) | 3.08 (4.07) |
| *12* | (*E*)-β-Ocimene | | 0.86 (0.47) | 0.27 (0.42) | 0.51 (0.38) | 0.55 (0.51) |
| *13* | γ-Terpinene | | 0.00 (0.00) | 0.00 (0.02) | 0.01 (0.02) | 0.00 (0.02) |
| *14* | Terpinolene | | 0.57 (0.41) | 0.20 (0.22) | 0.75 (0.30) | 0.46 (0.39) |
| *15* | *trans*-Verbenol | | 0.00 (0.00) | 0.01 (0.03) | 0.00 (0.00) | 0.00 (0.02) |
| *16* | α-Terpineol | | 0.00 (0.00) | 0.00 (0.00) | 0.05 (0.07) | 0.01 (0.03) |
| *17* | Linalool acetate | | 0.05 (0.13) | 0.04 (0.11) | 0.12 (0.08) | 0.06 (0.12) |
| *18* | Bornyl acetate | | 0.63 (0.52) | 0.62 (1.17) | 0.57 (0.61) | 0.61 (0.85) |
| *19* | *trans*-Verbenyl acetate | | 0.03 (0.07) | 0.01 (0.03) | 0.00 (0.00) | 0.02 (0.05) |
| *20* | *trans*-Pinocarvyl acetate | | 0.01 (0.03) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.02) |
| *21* | Myrtenyl acetate | | 0.03 (0.10) | 0.00 (0.02) | 0.09 (0.09) | 0.03 (0.08) |
| *22* | δ-Elemene | | 0.00 (0.00) | 0.02 (0.07) | 0.00 (0.00) | 0.01 (0.04) |
| *23* | α-Terpinyl acetate | | 0.48 (0.95) | 0.14 (0.27) | 0.83 (0.36) | 0.41 (0.69) |
| *24* | α-Cubebene | | 0.00 (0.00) | 0.01 (0.04) | 0.00 (0.00) | 0.01 (0.02) |
| *25* | α-Ylangene | | 0.00 (0.00) | 0.00 (0.00) | 0.01 (0.03) | 0.00 (0.01) |
| *26* | α-Copaene | | 0.02 (0.05) | 0.03 (0.04) | 0.07 (0.07) | 0.03 (0.05) |
| *27* | Geranyl acetate | | 0.00 (0.00) | 0.02 (0.09) | 0.00 (0.00) | 0.01 (0.05) |
| *28* | β-Bourbonene | | 0.02 (0.06) | 0.04 (0.05) | 0.08 (0.09) | 0.04 (0.07) |
| *29* | β-Cubebene | | 0.00 (0.00) | 0.04 (0.06) | 0.05 (0.06) | 0.02 (0.05) |
| *30* | β-Elemene | | 0.03 (0.11) | 0.01 (0.03) | 0.00 (0.00) | 0.02 (0.07) |
| ***31*** | **(*E*)-Caryophyllene** | | **8.13 (3.47)** | **7.43 (2.70)** | **7.31 (0.66)** | **7.69 (2.81)** |
| *32* | β-Copaene | | 0.02 (0.07) | 0.10 (0.18) | 0.06 (0.07) | 0.06 (0.13) |
| *33* | 6,9-Guaiadiene | | 0.00 (0.00) | 0.02 (0.07) | 0.00 (0.00) | 0.01 (0.05) |
| *34* | α-Humulene | | 1.24 (0.52) | 1.07 (0.41) | 1.09 (0.11) | 1.14 (0.43) |
| *35* | (*E*)-β-Farnesene | | 0.01 (0.04) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.03) |
| *36* | γ-Muurolene | | 0.34 (0.38) | 0.37 (0.34) | 0.60 (0.45) | 0.47 (0.43) |
| ***37*** | **Germacrene D** | | **27.50 (5.89)** | **30.59 (11.15)** | **23.69 (7.29)** | **28.03 (8.89)** |
| *38* | β-Selinene | | 0.01 (0.03) | 0.02 (0.08) | 0.00 (0.00) | 0.01 (0.05) |
| *39* | Phenylethyl 3-methylbutanoate | | 0.12 (0.60) | 0.00 (0.00) | 0.00 (0.00) | 0.05 (0.38) |
| *40* | γ-Amorphene | | 0.15 (0.26) | 0.00 (0.00) | 0.15 (0.14) | 0.09 (0.19) |
| *41* | Bicyclogermacrene | | 0.19 (0.88) | 0.62 (1.41) | 0.00 (0.00) | 0.33 (1.08) |
| *42* | α-Muurolene | | 0.08 (0.11) | 0.22 (0.35) | 0.14 (0.12) | 0.15 (0.25) |
| *43* | Germacrene A | | 0.09 (0.33) | 0.05 (0.18) | 0.00 (0.00) | 0.06 (0.24) |
| *44* | γ-Cadinene | | 0.25 (0.32) | 0.37 (0.44) | 0.35 (0.30) | 0.32 (0.37) |
| *45* | δ-Cadinene | | 0.61 (0.52) | 0.69 (0.55) | 0.84 (0.59) | 0.69 (0.54) |
| *46* | *trans*-Cadina-1,4-diene | | 0.00 (0.00) | 0.00 (0.00) | 0.01 (0.02) | 0.00 (0.01) |
| *47* | α-Cadinene | | 0.00 (0.01) | 0.00 (0.00) | 0.01 (0.02) | 0.00 (0.01) |
| ***48*** | **Germacrene D-4-ol** | | **0.57 (1.44)** | **1.93 (4.77)** | **0.01 (0.03)** | **1.02 (3.24)** |
| *49* | Caryophyllene oxide | | 0.08 (0.33) | 0.04 (0.07) | 0.02 (0.04) | 0.05 (0.22) |
| *50* | 1,7-Diepi-α-Cedrenal | | 0.00 (0.00) | 0.06 (0.13) | 0.00 (0.00) | 0.02 (0.08) |
| *51* | T-Cadinol | | 0.03 (0.11) | 0.01 (0.06) | 0.00 (0.00) | 0.02 (0.08) |
| *52* | α-Cadinol | | 0.03 (0.13) | 0.02 (0.07) | 0.00 (0.00) | 0.02 (0.10) |
| *53* | Germacra-4(15),5,10(14)-trien-1-alpha-ol | | 0.00 (0.01) | 0.13 (0.22) | 0.00 (0.00) | 0.05 (0.15) |
| *54* | Oplopanone | | 0.02 (0.09) | 0.00 (0.00) | 0.00 (0.00) | 0.01 (0.06) |
| *55* | Unknown components | | 0.05 (0.02) | 0.05 (0.01) | 0.08 (0.08) | 0.06 (0.04) |
| | Total [%] | | 100 | 100 | 100 | 100 |

[a] Contents are given as percentages (mean ± SD) of the total terpene content.
[b] Average contents of the indicated populations.

essential oils of the infraspecific taxa of *P. nigra* from Serbia were α-pinene, germacrene D, (*E*)-caryophyllene, and β-pinene as non-polar and germacrene D-4-ol as polar compound (Table 1).

One of the most studied properties of essential oils are their antimicrobial and antioxidant activity, important for both food preservation and control of human and animal diseases of microbial origin. Although the chemical composition and variability of the essential oils of black pine have been intensively studied in many parts of its habitat [10–15], *etc.*, there is little information about biological activities of *P. nigra* oils. According to the literature data, antimicrobial and antioxidant properties of *P. nigra* essential oils have been examined only for endemic Dalmatian black pine (*P. nigra* ssp. *dalmatica*) [3,16] and Crimean black pine (*P. nigra* ssp. *pallasiana*) from Turkey [17,18].

It is well known that essential oils possess antimicrobial activity owing to their lipophylicity and, thus, easy incorporation into bacterial membranes. For this reason, the most common mode of their action is membrane permeability alteration, which leads to