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Metabolomic Variability in the Volatile Composition of Essential Oils from *Pinus pinea* and *P. pinaster* †

Jorge M. S. Faria [1,*] and Ana Margarida Rodrigues [2]

[1] MED, Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, Évora University, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal
[2] Plant Metabolomics Laboratory, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal; (email 1)
* Correspondence: fariajms@gmail.com
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Abstract: In the Mediterranean basin, *Pinus pinaster* (maritime pine) and *P. pinea* (stone pine) are highly economically important pine species, namely as a source of raw material for various industries, such as wood, paper, resin, pine nuts (stone pine) and extraction of essential oils (EOs). Research on these species reports a large genetic and phenotypic intra-species variability that ultimately hinders the comparison between different studies. The present work reviews the available bibliography on *P. pinaster* and *P. pinea* EO composition and identifies the compounds responsible for the highest variation. The chemical profiling of EOs extracted from the aerial parts of these species was obtained from a total of 30 publications. Cluster analysis based on the similarity of EOs relative composition indicated a higher geographic influence in *P. pinaster* than in *P. pinea*. The EO components that showed the highest variations in relative amounts were limonene, α-pinene, β-pinene, *trans*-β-caryophyllene, germacrene D and β-myrcene for *P. pinea*, and α-pinene, β-pinene, *trans*-β-caryophyllene, germacrene D and β-myrcene for *P. pinaster*. A considerable degree of chemical variability was detected for these species. Research performed in the field or in greenhouse conditions should first ascertain chemical variability on these species.

Keywords: forest tree species; germacrene D; limonene; β-myrcene; Pinaceae; pine trees; α-pinene; β-pinene; *trans*-β-caryophyllene; volatile chemical diversity

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1. Introduction

Pines, conifers of the genus *Pinus*, are the largest group in the Pinaceae family. These gymnosperms are evergreen resinous trees that mostly populate temperate mountainous areas of the Northern Hemisphere but can also occur in semi-arid desert or even rainforests in a few parts of the tropics in the Southern Hemisphere [1]. Pines are largely responsible for shaping the forest ecosystems of the Mediterranean and are economically important species, valued for the timber, wood pulp and resin industries, used as ornamentals, as a source of edible pine nuts and essential oils (EOs). Pine EOs are obtained exclusively by hydro-, steam- or dry distillation, and consist mostly of terpenes (mono-, sesqui- and diterpenes) and aromatic compounds, usually with one to three major components at relatively high concentrations (20–70%). The terpenic compounds occurring in pine EOs have high-value commercial uses, such as industrial and household cleaning products, disinfectants, solvents, fragrances, medicine, and aromatherapy [2,3].

A high genetic variability has been described among populations of *P. pinaster* from different provenances. This variability has been further linked to intraspecific variation in the response to environmental stress; namely, drought, and resistance to biotic stress factors [4,5]. This natural variation found in *P. pinaster* allows to explore the potential of this species for reforestation purposes in the context of the current climate change scenario (e.g., increasing temperatures, prolonged drought events) [6]. In contrast, *P. pinea* is reported to display very low genetic variation but a remarkable degree of phenotypic plasticity; namely, in the response to heat and drought events [7,8]. The variability and/or phenotypic plasticity appears to be the reason for these *Pinus* species high colonization ability, taking a central role in Mediterranean ecosystems [9].

The occurrence of EO chemotypes (i.e., qualitative and quantitative differences in the chemical composition between populations within the same species) is fairly common, namely in forest trees. EO chemotypes are another source of variation among species and have been strongly linked with geographical variation, being able to reflect the diverse environmental conditions to which pines are exposed [3,10–12]. Chemical diversity in these species can lead to increased variability in research results and ultimately influence comparisons between different studies.

The present work explores the chemical variation found in *P. pinaster* and *P. pinea* EOs reported from different geographical locations, according to the available literature. The main EO components responsible for variation were identified.

2. Bibliographic Data

Research on bibliographic data was performed on Google Scholar® search engine using the keywords "essential oil" and "pinea" or "pinaster". Selected publications reported detailed EO composition of the aerial parts (needles or branches) of *P. pinea* or *P. pinaster*. Selection was also based on the definition of EO by the International Organization for Standardization (ISO), specifically a "product obtained from natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase, if any, by physical processes" [13]. From each report, the country of origin of EO pine sources and the EO components with amounts ≥ 1% were compiled and analyzed.

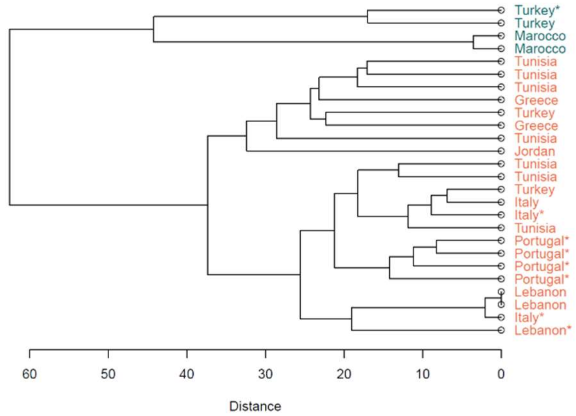
The compiled EO main compositions, compounds with amounts ≥1%, were used to determine relationship levels of different samples by cluster analysis. Analysis was performed using R studio (RStudio Team, 2018 [14]; R Core Team, 2019 [15]) software, using the package "dendextend" [16]. Components with the highest variability were identified by determining percentage of variation. Component variability was assessed by determining the range of values and calculating its percentage of the maximum value {100*[(Max-Min)/Max]}. Components with variability ≥90% were considered to be the highest variable compounds while those with values ≤10% the most stable compounds, across EO compositions. Variability in EO components was considered only if determined from more than 3 samples from different references.

3. Essential Oil Chemical Compositions

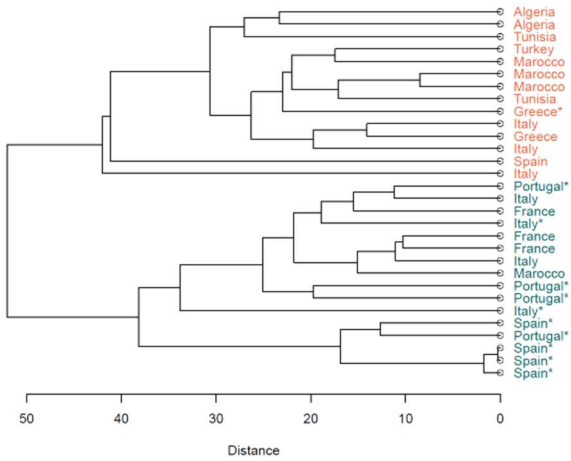
A total of 56 samples (26 for *P. pinea* and 30 for *P. pinaster*) were reported from 30 publications [3,12,17,18–44]. The pine sources of EOs were mainly located in Mediterranean countries, namely Algeria, France, Greece, Italy, Jordan, Lebanon, Morocco, Portugal, Spain, Tunisia and Turkey. *Pinus pinea* EOs summed 78 EO components with amounts ≥ 1% while *P. pinaster* EOs amounted 68 compounds.

3.1. Pinus Pinea

Cluster analysis was performed to determine the similarity between EO compositions from samples of *P. pinea* from different provenances (Figure 1a). Two main clusters were obtained, EOs from Moroccan pine trees grouped exclusively in one cluster along with some EOs from Turkey, while the remaining EOs grouped in a second cluster. Overall, there appears to be no consistent geographic pattern from the analysis of the distance dendrogram.



(**a**)



(**b**)

Figure 1. Dendrogram obtained by cluster analysis of the percentage composition (≥1%) of essential oils from the areal parts of *Pinus pinea* (**a**) and *Pinus pinaster* (**b**), based on distance and using unweighted pair-group method with arithmetic average (UPGMA) method. Samples with * correspond to EOs extracted from branches while the remaining from needles.

From a total of 78 components of *P. pinea* EOs, six showed very high variability, namely limonene (99%), β-pinene (98%), α-pinene (97%), *trans*-β-caryophyllene (94%), germacrene D (90%) and β-myrcene (90%) (Table 1).

Table 1. *Pinus pinea* essential oil compounds with variability ≥ 90%, across 26 samples.

Compound	Variability (%) [1]	Average (%)	Standard Deviation (%)
Limonene	99	45.1	21.4
β-Pinene	98	8.8	11.9
α-Pinene	97	10.7	10.3
trans-β-Caryophyllene	94	4.5	3.8
Germacrene D	90	3.8	2.7
β-Myrcene	90	2.6	2.3

[1] Range percentage of the maximum value {100*[(Max-Min)/Max]}.

The sum of these compounds varied between 25% and 92% of the total EOs compositions, and in 70% of the EOs they amounted to more than 50% of the total EOs compositions. Limonene varied between 1% and 75%, β-pinene between 1% and 42%, α-pinene between 1% and 37%, *trans*-β-caryophyllene between 1% and 17%, germacrene D between 1% and 10% and β-myrcene between 1% and 11%. Other EO compounds present in high amounts (10–15%) were thymol (13%), guaiol (13%), β-phellandrene (14%), 9-octadecenoic acid (13%) and abienol (12%).

The monoterpenoid 1,8-cineole was the EO compound with the least variability (7%), varying between 4.0% and 4.3% over 3 EOs from 3 different publications.

3.2. Pinus Pinaster

Compiled compositions of *P. pinaster* EOs were grouped by similarity through cluster analysis (Figure 1b). Two main clusters are observed, one grouped all Portuguese and French *P. pinaster* EOs. The EOs from Moroccan, Italian and Spanish *P. pinaster* were present in both groups but the Italian and Spanish ones were mainly in the first while the Moroccan mainly in the second. The second cluster included all the Turkish, Greek, Algerian and Tunisian EOs. Influence of geographical position can be proposed in *P. pinaster* EO chemical composition.

From the total 68 main components in *P. pinaster* EOs, five components showed a very high variability, namely β-pinene (99%), α-pinene (98%), *trans*-β-caryophyllene (97%), β-myrcene (92%) and germacrene D (91%) (Table 2).

Table 2. *Pinus pinaster* essential oil compounds with variability ≥ 90%, across 30 samples.

Compound	Variability (%) [1]	Average (%)	Standard Deviation (%)
β-Pinene	99	27.6	22.1
α-Pinene	98	25.2	13.6
trans-β-Caryophyllene	97	13.4	9.2
β-Myrcene	92	3.2	2.5
Germacrene D	91	6.8	6.2

[1] Range percentage of the maximum value {100*[(Max-Min)/Max]}.

The sum of these compounds varied between 13% and 97% of the total EO composition, and in almost 70% of the EOs they amounted to more than 50% of the EO composition. β-Pinene varied between 1% and 67%, α-pinene between 1% and 62%, *trans*-β-caryophyllene between 1% and 31%, germacrene D between 2% and 20% and β-myrcene between 1% and 12%.

Other EO compounds present in high amounts (10–30%) were δ-3-carene (18%), α-amorphene (11%), abieta-8,13-diene (11%), abieta-7,13-diene (32%), sclarene (18%), abietadiene (11–15%) allo-aromadendrene (13%), β-selinene (13%) and isoabienol (20%).

4. Discussion

Overall, a high chemical variability was found in the EOs from both species. This may be due not only to genetic variation but also to a strong component of variation resulting from different environmental conditions. EO composition is known to vary greatly depending on plant physiological parameters, environmental conditions, such as climate, pollution, type of soil and pests and diseases, and ultimately to geographic localization [45]. Some geographic influence in variability was detected in *P. pinaster*. In this species, EOs from Portuguese and French trees were grouped in one main cluster while the more distant Turkish, Greek, Algerian and Tunisian in a different cluster. Latitude can differently influence some phenotypic traits in relation to phylogenetic or environmental variations [1]. Cluster analysis of *P. pinaster* EOs, from different provenances, also revealed a higher dissimilarity in composition (i.e., higher variability), as some clusters are fused at higher distances, when compared to those from *P. pinea*. Nonetheless, the EO components responsible for this variability are, with the exception of limonene in *P. pinea*, the same between species, which may indicate the influence of limited biosynthetic pathways.

5. Conclusions

The preliminary results described on the chemical variability of EOs from *P. pinaster* and *P. pinea* from different provenances indicate that variability in *P. pinaster* may be more dependent on geographic localization than in *P. pinea*, but the compounds responsible for this variation are fundamentally the same in both species, namely, β-pinene, α-pinene, *trans*-β-caryophyllene, β-myrcene and germacrene D. However, further studies with a higher number of biological replicates are needed to improve the statistical analysis.

The analysis of EOs can provide a preliminary assessment of pine chemical variability without the need to sacrifice the plant. This may be useful for research where chemical homogeneity is important. There is an urgent need for standardization when performing field studies. Variations in environmental conditions and/or sampling procedures can deeply impact the data and thus, metadata should be greatly detailed to enable data usage with a higher reliability.

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