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A comparative analysis of the anatomical variability in *Pinus brutia* Ten. essential oils†

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Abstract: *Pinus brutia* Ten. is distributed throughout the Eastern Mediterranean, mainly in Turkey and bordering countries. Pine essential oils (EOs), mainly comprised of terpenoid volatiles, have high commercial value and a wide range of biological activities as repellents, insecticides, antivirals, antimicrobials and antioxidants. The present work reviewed the chemical variability of EOs reported for *P. brutia* trees and related bioproducts. The major EO components (≥ 20 %) reported were α-pinene, β-pinene and δ-3-carene, that generally comprised 50 – 90 % of the EO composition. δ-3-Carene showed the highest variability suggesting the occurrence of chemotypes. Assessing the variability of EOs extracted from different tree parts or tree bioproducts can provide useful information for guided *P. brutia* EO extraction, according to its intended purpose.

Keywords: chemical diversity; chemotypes; essential oil; *Pinus brutia*; volatiles; α-pinene; β-pinene; δ-3-carene

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1. Introduction

In the Mediterranean, conifers play important ecological and cultural roles. Pines (Pinaceae family), are the most numerous and widespread conifers in forest ecosystems, and occupy over 25 % of the total forest area [1]. Five main species dominate the landscape, *Pinus halepensis* Mill., *P. brutia* Ten., *P. pinea* L., *P. pinaster* Aiton and *P. sylvestris* L. *P. brutia* is commonly known as Brutian pine, Calabrian pine or Turkish pine and is largely distributed throughout the Eastern Mediterranean, mainly in Turkey and nearby countries, namely Azerbaijan, Bulgaria, Cyprus, Georgia, Greece, Iran, Iraq, Israel, Jordan, Lebanon and Syria. *P. brutia* often hybridizes with *P. halepensis*, despite the latter be distributed mainly in the Western Mediterranean [2]. Brutian pine is a rapidly growing tree that can reach 35 m in height and presents a usually open crown of irregular branches. In its native range, *P. brutia* has a high economic value for the raw products it provides, namely wood, bark, cones and also the extracted resin [3]. In conifers, the release of oleoresin is commonly produced in the response to abiotic factors but more pronouncedly to biotic attacks, such as the activity of herbivores, stem-boring insects, and pathogenic microorganisms. The production of oleoresin is stimulated by the wounds induced by these pathogens, and results in the release of strong chemical volatile deterrents and in the sealing of the open wounds [4]. Industrially, the oleoresin can be obtained by tapping the bark (also known as bark chipping) and collecting exudates from the pine trees. The crude oleoresin is then converted to turpentine (volatile fraction) and rosin (non-volatile fraction) by steam distillation, and can be further processed into valuable chemical industrial products, namely, adhesives, cleaners, coatings, disinfectants, flavorings, food gums, fragrances, pharmaceuticals and printing inks [1]. Pine essential oils (EOs), complex mixtures of volatile compounds, are another important bioproduct with high commercial value that can be obtained, by hydro-, steam- or dry distillation, from several parts of the pine tree. In addition to their cultural and ethnobotanical importance, pine EOs have strong biological activities as antimicrobials, antioxidants, antiparasitics, antivirals and insecticides [5].

2. Essential oils

EOs are defined by the International Organization for Standardization (ISO 9235) as a product obtained from natural raw material of plant origin, by hydro-, steam- or dry distillation, and by mechanical processes from the epicarp of citrus fruits. The result is a concentrated hydrophobic liquid, at room temperature, containing volatile compounds, slightly soluble in water and highly soluble in organic solvents [6]. The chemical composition of most EOs is dominated by mono- and sesquiterpenes, and phenolic compounds (e.g., phenylpropanoids), although other groups of compounds can also occur in relevant amounts. Terpenes and terpenoids occurring in pine EOs have a high commercial value and a wide range of practical applications, such as industrial and household cleaning products, disinfectants, solvents, fragrances, medicine, and aromatherapy [7]. Although, pine EOs are comprised of usually one to three major components at relatively high concentrations (20–70%), a great chemical variability can occur between and within species (e.g., the occurrence of chemotypes) [8].

The present work aimed at screening the EO variability from various parts of *P. brutia* trees and derived bioproducts reported in the literature. Mapping the chemical variability of EOs from different parts of the tree and related bioproducts can provide useful information for guided *P. brutia* EO extraction, according to its intended purpose.

3. Bibliographic data

Research was performed with Web of Science search engine, in all available databases, on published works reporting EO chemical composition, using the topics "*Pinus*", "*brutia*" and "essential oil"

A total of 17 works was retrieved reporting full EO compositions from various *P. brutia* tree parts and bioproducts. These reports were published in journals specialized in the scientific fields of Plant sciences (24 %), Applied chemistry (18 %), Food science technology (18 %) and Materials science (paper, wood) (18 %). Publications dated from 1995 to 2019. The listed publications were cited 394 times, by a total of 283 works, with an average of 23 citations per work. These citations were included in journals specialized in the scientific fields of Plant sciences (17 %), Biochemistry and molecular biology (17 %) and Food science technology (16 %). Since 2011, the cumulative number of citations is greatly increasing, revealing a growing research interest in *P. brutia* EOs (Figure 1).

4. *Pinus brutia* EO chemical variability

The production of secondary metabolites in plants can be influenced by various factors, such as those related to plant genetics and physiology (e.g., plant parts and their developmental stage, the growth season and the type of secretory structures present), and those related to environmental conditions (e.g., geographic location, climatic and edaphic conditions, associated pests and diseases and environmental pollution) [9]. Among the genus *Pinus*, variability in secondary metabolites, and terpenoids in particular, is heavily dependent on the species and on geographic locations (i.e., implying different environmental conditions). A high capacity for phenotypic plasticity can be observed through the common occurrence of different chemical phenotypes in some species, also known as chemotypes.

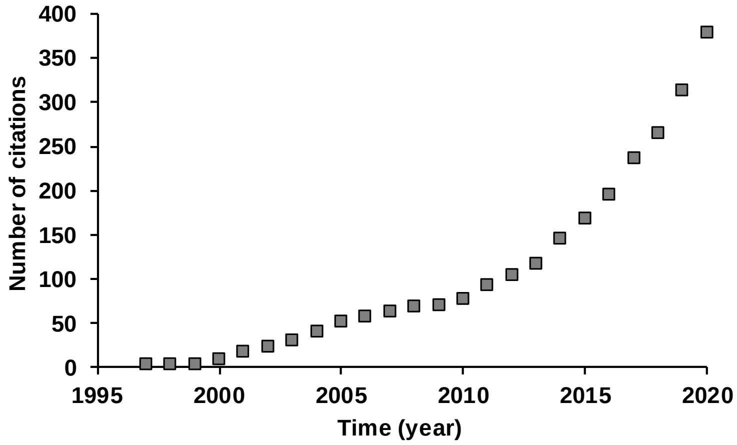

Figure 1. Cumulative number of citations on works reporting on the chemical composition of essential oils of *Pinus brutia* tree parts and derived bioproducts, obtained from Web of Science (https://www.webofknowledge.com), using the keywords "*Pinus* ", "*brutia*" and "essential oil".

The occurrence of chemotypes has been strongly linked with geographical variation, being able to reflect the diverse environmental conditions to which pines are exposed [8,10–12]. Chemical diversity can lead to increased variability in the products obtained from these species and an instability in production, for example from extracted EOs [7]. In *P. brutia*, this degree of variability was reported for EOs obtained from different plant parts but also among EOs obtained from the same plant part.

4.1. Between plant parts and related bioproducts

The compiled bibliography reported EOs from *P. brutia* needles, twigs, bark, flowers, seeds, from extracted resin and sawdust from collected wood. The EOs main composition (≥1 %) was comprised of the compounds α-pinene, α-terpineol, α-terpinyl acetate, β-caryophyllene, β-myrcene, β-pinene, camphene, caryophyllene oxide, δ-2-carene, δ-3-carene, γ-terpinene, germacrene D, limonene, longifolene, neryl acetate, sylvestrene and terpinolene (Table 1).

Table 1. Essential oil composition (≥1 %) reported for *Pinus brutia* needles, twigs, resin, cones, flowers, bark, sawdust, and seeds.

Compounds (%)	Needles[1]	Twigs[2]	Resin[3]	Cones[4]	Flowers	Bark	Sawdust	Seeds
α-pinene	14.4 - 20.3	14.5 - 20.6	19.7 - 49.1	30.9 - 40.7	24.2	14.9	77.3	25.0
camphene		1.1	2.2 - 5.0				1.3	1.6
β-pinene	29.5 - 47.7	17.5 - 31.7	9.7 - 19.1	28.3 - 39.6	35.2	5.7	12.2	38.2
β-myrcene	1.6 - 2.9	2.1 - 11.0	2.2 - 15.3	1.1 - 2.3	11.9		1.8	2.4
δ-3-carene	1.9 - 3.8	1.4 - 25.1	3.7	7.8 - 13.4	11.2	9.6		1.4
limonene	1.0 - 4.0	1.5 - 13.4	2.2 - 6.2	1.0 - 3.7	5.6	2.9	1.4	1.1
sylvestrene	1.6		7.4					
γ-terpinene			10.2	2	1.7			
terpinolene	1.0 - 1.3	1.8 - 3.5	6.2			1.0		
α-terpineol	1.1 - 4.4	1.0 - 2.7	3.0	1.0 - 2.9		6.7		
α-terpinyl acetate	2.5	5.3						
β-caryophyllene	4.6 - 12.0	6.8 - 14.5	2.8 - 7.8	2.7 - 5.0	1.9	11.2		15.2
germacrene D	7.5 - 17.9	1.4 - 6.1						4.3

caryophyllene oxide		1.5	1.6	6.9
longifolene	1.1 - 1.2	9.9		
neryl acetate				5.9
δ-2-carene		6.9		

[1] retrieved from 8 EOs [1,13–19]. [2] retrieved from 4 EOs [16,19–21]. [3] retrieved from 3 EOs [3,4,22]. [2] retrieved from 2 EOs [2,23].

The monoterpenes α-pinene and β-pinene were present in all samples (Table 1). For α-pinene, the highest amounts were detected in resin, cones and sawdust samples (20 - 77 %), while for β-pinene, needles, cones and seeds showed the highest amounts (38 - 48 %). Other compounds present in relatively high amounts (≥15 %) include the monoterpene δ-3-carene (25 % in pine twigs), the sesquiterpene germacrene D (18 % in pine needles), the monoterpene β-myrcene (15% in pine resin) and the sesquiterpene β-caryophyllene (15 % in pine seeds) (Table 1).

4.2. Among samples of the same plant part

Variability in the amounts of volatile compounds present in P. *brutia* EO was determined as the range of values percentage of the maximum value, in the plant parts where more than one sample was reported (namely needles, twigs, resin or cones). Apart from longifolene, all compounds showed at least 20 % variability between samples (Figure 2). The highest variabilities (≥80 %) were detected for δ-3-carene (94 %) and limonene (89 %) in twigs EOs, and β-myrcene in EOs from resin (86 %) and twigs (81 %).

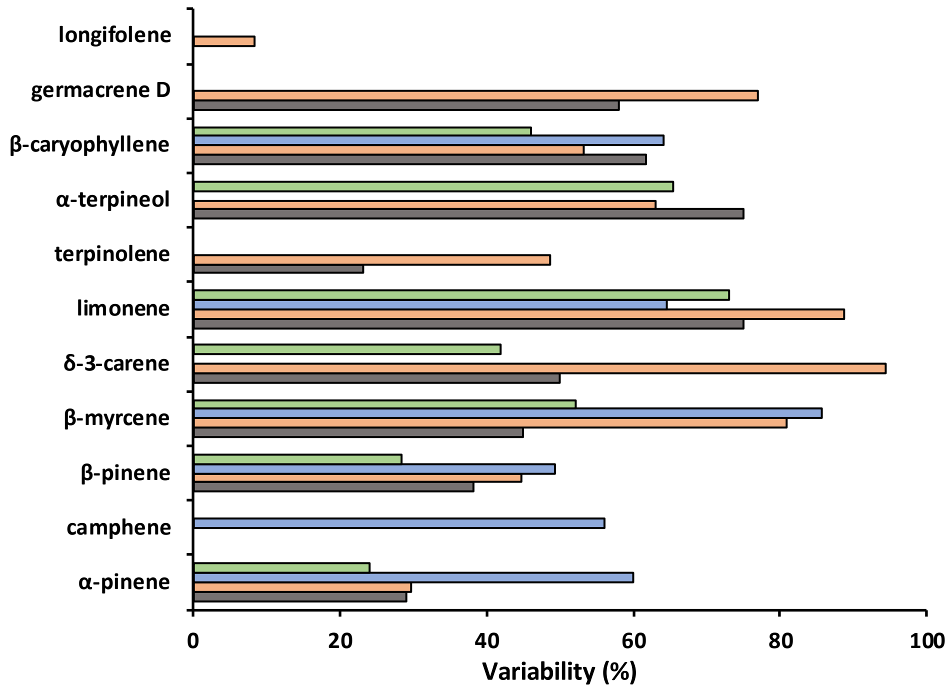

Figure 2. Variability (range percentage of the maximum value {100*[(Max-Min)/Max]}) of essential oil compounds in *Pinus brutia* needles (grey bars), twigs (orange bars), resin (blue bars) and cones (green bars).

It is noteworthy that the composition of EOs extracted from *P. brutia* resin samples share a resemblance with other resin producing pines like *P. pinea*, being rich in α-pinene (21 to 25%), β-pinene (10%), and caryophyllene (5 to 9%) [4]. Additionally, both types of EOs were seen to possess similar antimicrobial, insecticidal, phytotoxic and antioxidant activities.

5. Conclusions

Analysis of the chemical composition of EOs extracted from various tree parts and bioproducts obtained from *P. brutia* allows to select the most suitable raw material according to the desired volatile components. A higher number of studies are necessary for a detailed description of the anatomic volatile composition of *Pinus brutia*.

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