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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM *Pinus brutia* (CALABRIAN PINE) GROWING IN LEBANON

Monica Rosa Loizzo,[1*] Antoine Mikhael Saab,[2] Rosa Tundis,[1] Federica Menichini,[1] Marco Bonesi,[1] Giancarlo Antonio Statti,[1] and Francesco Menichini[1]

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The genus *Pinus* belongs to the Pinaceae family and comprises about 250 species [1]. *Pinus brutia* Ten. (Calabrian pine) and *Pinus halepensis* (Aleppo pine) were historically considered two varieties of *Pinus halepensis* [2]: however, morphological and biochemical analyses have confirmed that they are two distinct species [3].

Pinus brutia is commonly found in ecosystems of the eastern Mediterranean region [4]. Pine is used in ethnomedical practice throughout the world [5, 6]. For example, Indians use a boiled extract of the inner bark from *P. strobes* (White pine) as an astringent for diarrhea or in cough remedies, but they mainly soak the bark and apply it to wounds as a soothing plaster. A boiled extract of the gum of white pine is also used as a pain reliever for rheumatism, and a syrup made from the resin, for colds. In 19th century North America *P. sylvestris* (Scots pine) was employed as a diuretic and to induce perspiration and thus help break a fever. It was also specified for constipation and chronic bronchitis. Externally, the tar was incorporated into an ointment, or tar water, and employed as a remedy for such chronic skin diseases such as psoriasis and eczema, and for open sores. Scotch pine pitch, the result of distilling the tar, also yields medicinal preparations for eczema and similar skin problems and is recommended internally for skin diseases and hemorrhoids as well. *P. brutia* is used in folk medicine in Turkey, and recently the antimicrobial activity of tar obtained from the roots and stems of *P. brutia* against *Staphylococcus aureus*, *Streptococcus pyrogenes*, *Escherichia coli*, and *Candida albicans* was reported [7]. Previous studies on *Pinus* species reported the terpene [8–14], flavonoid [15], and alkaloid [16] content. Only two studies reported the chemical composition of needles and wood from this conifer [17, 18], but no previous report analyzed and discussed the chemical composition of the essential oils from *P. brutia* cones and flowers and their antimicrobial activity, and this is the object of this work.

The composition of the *P. brutia* oils and the retention times of the components listed in order of elution in a nonpolar column are presented in Table 1. The cone and flower essential oils of *P. brutia* were obtained by hydrodistillation in 1.2% and 1.5% yield, respectively. The hydrodistillate from cones (13 compounds amounting to 99.23% of the total oil) consisted of ca. 95.93% of monoterpenoids (0.39% oxygenated monoterpenes and 95.34% monoterpene hydrocarbons) and ca. 3.30% of sesquiterpene hydrocarbons. The main compounds are the monoterpene hydrocarbons α-pinene (40.70%), β-pinene (28.27%), and δ-3-carene (13.36%). The only oxygenated monoterpene is terpinen-4-ol (0.39%), while the sesquiterpene hydrocarbons are β-caryophyllene (2.74%) and α-humulene (0.56%). The oil obtained from flowers comprises ca. 93.23% of the monoterpenoids (0.21% oxygenated monoterpenes and 93.02% monoterpene hydrocarbons) and ca. 2.27% of sesquiterpene hydrocarbons. The monoterpene hydrocarbon fraction is the most representative, as well as α-pinene (24.21%), β-pinene (35.18%), β-myrcene (11.90%), and δ-3-carene (11.20%). Terpinen-4-ol (0.21%) is the only oxygenated monoterpene, while β-caryophyllene (1.85%) and α-humulene (0.42%) represent the sesquiterpene hydrocarbon fraction. From a comparison of the essential oil composition of the flower oil and the cone oil, we detected the presence of two monoterpene hydrocarbons, sabinene (0.52%) and γ-terpinene (0.32%), in the flower oil. The flower oil is richer in β-myrcene content than the cone oils.

1) Department of Pharmaceutical Science, Faculty of Pharmacy and Nutrition and Health Sciences, University of Calabria, I-87030 Rende (CS) Italy, fax: +39(0)984493298, e-mail: mr.loizzo@unical.it; 2) Chemistry Department, Faculty of Sciences II, Lebanese University, P.O. Box: 90656 Fanar, Beirut, Lebanon, e-mail: antsaab@gmail.com. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 633-634, November-December, 2008. Original article submitted March 12, 2007.

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TABLE 1. Composition of Essential Oils Obtained from *Pinus brutia* Cones and Flowers

Compound[a]	t_R[b]	Cones[c]	Flowers[c]	Compound[a]	t_R[b]	Cones[c]	Flowers[c]
α-Pinene	7.13	40.70±0.63	24.21±0.03	α-Terpinolene	9.94	2.00±0.22	1.70±0.04
Camphene	7.38	0.75±0.04	0.46±0.04	Terpinen-4-ol	11.28	0.39±0.01	0.21±0.02
Sabinene	7.94	-	0.52±0.03	α-Terpineol	11.42	2.94±0.11	0.65±0.04
β-Pinene	7.96	28.27±0.44	35.18±0.01	β-Caryophyllene	14.17	2.74±0.18	1.85±0.02
β-Myrcene	8.21	2.29±0.07	11.90±0.12	α-Humulene	14.43	0.56±0.11	0.42±0.01
Δ-3-Carene	8.62	13.36±0.12	11.20±0.06				
p-Cymene	8.86	0.54±0.02	0.36±0.05	Monoterpene hydrocarbons		95.54	93.02
β-Phellandrene	8.90	1.00±0.01	0.67±0.03	Oxygenated monoterpenes		0.39	0.21
Limonene	8.94	3.69±0.02	5.55±0.01	Sesquiterpene hydrocarbons		3.30	2.27
γ-Terpinene	9.45	-	0.32±0.03	Total area of identified compounds		99.23	95.50

[a]Compounds listed in order of elution from an SE 30 nonpolar column. [b]t_R: retention time (as min). [c]Relative area percentage (peak area relative to total peak area %).

TABLE 2. Antimicrobial Activity and MIC for the *Pinus brutia* Cones and Flowers Essential Oil (EO) Using the Agar Method

Microrganism	Diameter of inhibition, mm		MIC (μL/mL)	
	Cones EO	Flowers EO	Cones EO	Flowers EO
Gram positive				
Staphylococcus aureus (ATCC 25923)	10	9	8	6
Bacillus subtilis (ATCC 6633)	12	8.5	7	9
Gram negative				
Escherichia coli (ATCC 25922)	52	56	8	9
Pseudomonas aeruginosa (ATCC 27853)	-	-	>10	>10
Yeast				
Candida albicans (ATCC 10231)	8	7.4	7	9

The results obtained in the antimicrobial assay are shown in Table 2 [19, 20]. From the disc diffusion assay, *P. brutia* cone essential oil exhibited a higher growth reduction of gram negative pathogen, such as *Escherichia coli* (inhibition zone 52 mm), and gram positive *Staphylococcus aureus* (inhibition zone 10 mm) and *Bacillus subtilis* (inhibition zone 12 mm). The flower essential oil is less active on gram positive bacteria inhibition zones of 9 and 8.5 mm for *Staphylococcus aureus* and *Bacillus subtilis,* respectively. Both oils are able to inhibit yeast *Candida albicans* inhibition zones of 8 and 7.4 mm for cones and flowers, respectively. These results were confirmed by the minimum inhibitory concentration (MIC) assay in which the flower essential oil exhibited a high MIC value of 6 μL/mL on *Staphylococcus aureus* and the cone oil exerted the highest activity against the yeast *Candida albicans* (MIC 7 μL/mL). Our results demonstrate that *P. brutia* cones and flower oils have a broad spectrum of activity on various microbial infections, thus supporting the traditional folk medicine uses of this conifer.

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