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RESEARCH ARTICLE

Characterization and Antimicrobial Evaluation of the Essential Oil of *Pinus pinea* L. from Turkey¥

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Abstract

Pinus pinea L. is commonly known as Stone or Umbrella Pine, which is a member of the family Pinaceae and grows natively in the northern Mediterranean and Aegean coastal regions; southern Europe, north Africa, Spain to Turkey. *P. pinea* is also cultivated for its edible pine nuts, as ornamental trees and commonly planted in gardens and parks. Its essential oil is used for a variety of skin complaints, wounds, sores, burns, in herbal steam baths and various inhalers. Air dried needles collected from Ortanca-Muğla were subjected to water-distillation using a Clevenger-type system. The resulting essential oil was analysed by GC-FID and GC-MS, simultaneously. Overall, thirty components were characterized. Limonene (54.6 %), α-pinene (4.0 %), myrcene (2.4 %) and α-phellandrene (2.4 %) were characterized as major constituents. The essential oil was also screened against 8 different human pathogenic microorganisms, where the minimal inhibitory concentrations (MIC) were determined using a microdilution method. The oil showed the same inhibitory activity against *Escherichia coli, Staphyloccocus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus vulgaris* and *Salmonella thyphimurium* (MIC>0.75 mg/ml). Its antifungal susceptibility against *Candida parapsilosis* was relatively more than that of the pathogen *Candida albicans* with a MIC value of 0.375 mg/ml, when compared with the antifungal standards.

Keywords *Pinus pinea*, GC-FID/GC-MS, limonene, antimicrobial

Introduction

Pinus pinea L. a member of the Pinaceae family, is commonly known as Stone Pine or Umbrella Pine, which grows natively in the northern Mediterranean and Aegean coastal regions; southern Europe, north Africa, Spain to Turkey. *P. pinea* is also cultivated for its edible pine nuts, as ornamental trees and commonly planted in gardens and parks. Its essential oil is used for a variety of skin complaints, wounds, sores, burns, in herbal steam baths and various inhalers.

Phytosterols are triterpenes that are important structural components of plant membranes and they are also signaling molecules. Sterol and aliphatic alcohol contents and compositions of seed samples for *Pinus pinea* are described in literature. β-sitosterol was found as the most abundant (74%) phytosterol whereas octacosanol and hexacosanol (41%) for aliphatic alcohol, which were described for *Pinus pinea* seed oils (Nasri et al, 2007). Resin from *P. pinea* is a complex mixture of many organic compounds tapped by wounding its bark. It is also used for timber and resin production and its wood is well known to be stable even at high humidity.

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It can be used for construction purposes, furniture making and to a lesser extent for the pulp and paper industry. Resin also contains turpentine which can be used as an antiseptic, as a remedy for kidney, bladder, and respiratory problems and also for skin treatments (Nergiz and Dönmez, 2004; Arshad et al, 2010).
Except for these industrial uses, *P. pinea* is much appreciated for its seed production which is widely used in food preparation and particularly in cake-pastry. They are commonly added to meat, salads and into bread, since Pine nuts are a good source of nutrients. They contain vitamins, particularly B1, B2, C. E, K and also minerals, especially potassium, calcium, iron, magnesium and phosphorus. It is reported that the seeds of *P. pinea* show a composition of 5.6 % moisture, 31.1% protein, 47.4% fat, 10.7% carbohydrate and 4.3% ash *Pinus pinea* has many fatty acids, such as linoleic acid is the major fatty acid followed by oleic, palmitic and stearic acids (Nergiz and Dönmez, 2004; Nasri et al, 2005). Additionally, tocopherol and triacylglycerol contents in *P. pinea* L. seeds have been reported (Nasri et al, 2009).
In our study, we aimed to evaluate chemical composition of *Pinus pinea* essential oil. Hence, needles were distilled by a Clevenger-type apparatus and analysed by GC/GC-MS systems, simultaneously. Limonene (55.0 %) was found the main compound. *In vitro* antimicrobial activity of the essential oil against 8 different human pathogenic microorganisms was determined.

Materials and Methods

Plant Material

Needles (leaves) were collected from Ortanca-Muğla in May of 2009.

Isolation of the Essential Oil

The air dried needles were water distilled for 3 h using a Clevenger-type apparatus. Chemical composition of the essential oil is shown in Table 1.

GC–MS Analysis

The GC-MS analysis was carried out using an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450. *n*-Alkanes were used as reference points in the calculation of the relative retention indices (RRI).

GC-FID Analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts (%) of the separated compounds were calculated from FID chromatograms. The result of the analysis is shown in Table 1.

Identification of Components

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention indices (RRI) to series of *n*-alkanes. Computer matching against commercial (McLafferty and Stauffer, 1989; Koenig et al, 2004) and in-house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils was performed. Additionally, MS literature data (Joulain and Koenig, 1998; ESO 1999) was also used for the identification of components.

Antimicrobial Activity

The essential oil was examined against a panel of 6 different human pathogenic bacterial and 2 *Candida* standard strains using the micro-dilution method *versus* standard antimicrobial agents. In this study *Escherichia coli* NRRL B-3008, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* NRRL B-123*, Enterobacter aerogenes* NRLL 356, *Salmonella typhimurium* ATCC 13311, *Candida albicans* NRRL Y-12983 and *Candida parapsilosis* NRRLY 12696 acquired from various culture collections were used for antimicrobial activity evaluations. The microorganisms were stored at -85˚C in glycerol until inoculation and purity check. Stock solution of the essential oil and the antimicrobial standard agent were prepared in 25 of % dimethyl sulfoxide (DMSO). The diluted essential oil (200 µL) was added to wells of row A, while the remaining wells in rows B to H received 100 µl of MHB. Microbial suspensions were grown overnight in double strength Mueller-Hinton broth (MHB, Merck) standardized to 10^8cfu /mL for bacteria and 10^6cfu/mL for *Candida* species (corresponding to McFarland no: 0.5) using a turbidometer (Bioland, Turkey). Each microbial suspension was added to the appropriate well. After incubation at 37˚C for 24h the first well without turbidity was determined as the minimal inhibition concentration (MIC, mg/mL). Antimicrobial standard chloramphenicol and ketoconazole (Sigma–Aldrich) were used for this assay. Antimicrobial activity results were shown in Table 2.

Results and Discussion

Water distilled essential oil from the air-dried needles of *Pinus pinea* L. from Ortanca-Muğla was analysed both by GC and GC-MS systems, simultaneously. Overall, thirty one components were characterized, where limonene (54.6 %), β-phellandrene (7.4 %), α-pinene (4.0 %), β-caryophyllene (4.0 %) myrcene (2.4 %) and α-phellandrene (2.4 %) were identified as major constituents. The essential oil was also screened against 8 different human pathogenic microorganisms, where the minimal inhibitory concentrations were determined using a microdilution method. The oil showed the same inhibitory activity against *Escherichia coli, Staphyloccocus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus vulgaris* and *Salmonella thyphimurium* (MIC >0.75 mg/ml). Its antifungal susceptibility against *Candida parapsilosis* was relatively more than that of the pathogen *Candida albicans* with a MIC value of 0.375 mg/mL, when compared with the antifungal standards.

Table 1. Chemical composition of *Pinus pinea* L. essential oil

RRI	Compounds	%
1032	α-pinene	4.0
1118	β-pinene	1.7
1132	Sabinene	0.1
1174	Myrcene	2.4
1176	*α*-Phellandrene	2.4
1203	Limonene	55.0
1210	β-Phellandrene	7.4
1266	(*E*)-β-ocimene	1.7
1280	*p*-Cymene	0.2
1290	Terpinolene	0.4
1479	*δ*-Elemene	0.5
1553	Linalool	0.4
1583	Longifolene	1.0
1590	Bornyl acetate	0.3
1612	β-Caryophyllene	4.0

Table 1. cont.

RRI	Compounds	%
1617	6,9-Guaiadiene	0.6
1637	*p*-Menth-1-en-9-ol	0.5
1596	α-Guaiene	0.9
1687	α-Humulene	0.9
1704	γ-Muurolene	0.2
1706	α-Terpineol	1.6
1707	δ- Selinene	
1726	Germacrene D	2.2
1868	(*E*)-Geranyl acetone	0.7
2103	Guaiol	2.1
2183	Selina-6-en-4-ol	0.7
2185	γ-Eudesmol	0.3
2503	Dodecanoic asit (lauric acid)	2.3
2622	Phytol	1.0
2705	Tetradecanoic acid (myristic acid)	0.7
2931	Hexadecanoic acid (palmitic acid)	1.9
	Total	**98.1**

RRI: Relative retention indices calculated against *n*-alkanes,
%: percentages were calculated from FID data, tr Trace (< 0.1 %)

According to previous research reports, the chemical composition of Tunisian *Pinus pinea* essential oils showed limonene (54.1 %), α-pinene (7.7 %), and β-pinene (3.4 %) as major constituents. Additionally antifungal activity evaluations on the oil of *P. pinea* showed that it significantly inhibited the growth of ten plant pathogenic fungi (Amri et al, 2012). In another previous study, main constituent of the essential oils from needles, branches and female cones of *P. pinea* was found as limonene (59.8 %, 62.5 % and 61.6 %, respectively) in agreement with our findings (Macchioni et al, 2003).

Table 2. Minimum inhibitory concentrations (MICs/mg/ml) obtained from essential of *Pinus pinea* against 8 different microorganisms

Microorganism Strains	***P. pinea* essential oil**	Chloramphenicol	Ketoconazole
E. coli NRRL B-3008	>0.75	0.0312	-
S. aureus ATCC 6538	>0.75	0.0156	-
P. aeruginosa ATCC 27853	>0.75	0.5	-
E. aerogenes NRLL 3567	>0.75	0.0312	-
P. vulgaris NRRL B-123	>0.75	0.0078	-
S. typhimurium ATCC 13311	>0.75	0.0039	-
C. albicans NRRL Y-12983	0.375	-	0.125
C. parapsilosis NRRLY 12696	0.75	-	0.125

In another report on chemical composition of the essential oils isolated from the needles of *Pinus halepensis*, *P. canariensis*, *P. pinaster*, *P. pinea* and *P. brutia* from Morocco, the most abundant compound in *P. pinea* oil was found as α-pinene (37.0 %). Furthermore, the oils as well as the major constituents α-pinene, myrcene and β-caryophyllene were tested for their inhibitory effect against 21 bacterial strains. Examination of the antibacterial activity revealed that only *P. pinaster* and *P. pinea* oils exhibited a definite activity against all the organisms tested (Hmamouchi et al, 2001).

The essential oil of *P. pinea* was investigated against *Citrus* pathogens; *Botrytis cinerea, Penicillium digitatum* and *Geotrichum citri-aurantii* and *Phytophthora citrophthora* according to another previous report (Bouchra et al, 2003). The acetone, ethyl acetate, and ethanol extracts and essential oils of the twigs and needles of *P. pinea* were examined for their inhibitory effects against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and antioxidant activity (Üstün et al, 2012).

Another study on the essential oils from the cones and needles of five different *Pinus* species (*P. brutia* Ten., *P. halepensis* Mill., *P. nigra* Arn., *P. pinea* L. and *P. sylvestris* (L.) from Turkey evaluated the *in vivo* wound healing and anti-inflammatory activities. The essential oils obtained from the cones of *Pinus pinea* and *Pinus halepensis* demonstrated the highest effects on the wound healing activity models (Süntar et al, 2012).

As a conclusion, the essential oil from *Pine* sp. and in particular *P. pinea* from Turkey may constitute an important resource as antimicrobial agents.

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REFERENCES

Amri, I., Gargouri, S., Hamrouni, L., Hanana, M., Fezzani, T., Jamoussi, B. (2012). Chemical composition, phytotoxic and antifungal activities of *Pinus pinea* essential oil. Journal of Pest Science, 85(2), 199-207.

Arshad, M.A., Khan, M.A., Muhammad, Z., Sarwat, J., Shahzia, S. (2010). Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province Pakistan. J Ethnopharmacol 128,322–335.

Bouchra, C., Mohamed, A., Mina, I.H, Hmamouchi, M. (2003). Antifungal activity of essential oils from several medicinal plants against four postharvest citrus pathogens. Phytopathologia Mediterranea, 42(3), 251-256.

ESO. (1999). The Complete Database of Essential Oils, Boelens Aroma Chemical Information Service, The Netherlands.

Hmamouchi, M., Hamamouchi, J., Zouhdi, M., Bessiere, J. (2001). Chemical and antimicrobial properties of essential oils of five Moroccan Pinaceae. Journal of Essential Oil Research, 13(4), 298-302.

Joulain, D., W.A., Koening. (1998). The Atlas of Spectra Data of Sesquiterpene Hydrocarbons, EB-Verlag,Hamburg.

Koenig, W.A, Joulain, D., Hochmuth, D.H. (2004). Terpenoids and Related Constituents of Essential Oils. MassFinder 3. Hochmuth DH (ed). Convenient and Rapid Analysis of GCMS, Hamburg, Germany

Macchioni, F., Cioni, P.L., Flamini, G., Morelli, I., Maccioni, S., Ansaldi, M. (2003). Chemical composition of essential oils from needles, branches and cones of *Pinus pinea, P. halepensis, P. pinaster and P. nigra* from central ltaly. Flavour and Fragrance Journal, 18,139-143.

McLafferty, F.W, Stauffer, D.B. (1989).The Wiley/NBS Registry of Mass Spectral Data, J Wiley and Sons: New York.

Nasri, N., Fady, B., Triki S. (2007). Quantification of Sterols and Aliphatic Alcohols in Mediterranean Stone Pine (*Pinus pinea* L.) Populations. Journal of Agriculture and Food Chemistry, 55, 2251-2255.

Nasri, N., Khaldi, A.,Fady, B., Triki S. (2005). Fatty acids from seeds of *Pinus pinea* L.: Composition and population profiling. Phytochemistry 66, 1729–1735.

Nasri, N., Tlili, N., Ammar, K.M., Khaldi, A.,Fady, B., Triki S. (2009). High tocopherol and triacylglycerol contents in *Pinus pinea* L. seeds. International Journal of Food Sciences and Nutrition. 60 (S1),161-169.

Nergiz, C., Dönmez, I. (2004). Chemical composition and nutritive value of *Pinus pinea* L. seeds. Food Chemistry. 86, 365–368.

Süntar, I., Tümen, I., Üstün, O., Keleş, H., Küpeli, A. E. (2012). Appraisal on the wound healing and anti-inflammatory activities of the essential oils obtained from the cones and needles of *Pinus* species by in vivo and *in vitro* experimental models. Journal of Ethnopharmacology, 139(2), 533-54

Üstün, O., Şenol, F.S, Kürkçüoğlu, M, Orhan, I.E, Kartal, M, Baser, K.H.C. (2012). Investigation on chemical composition, anticholinesterase and antioxidant activities of extracts and essential oils of Turkish *Pinus* species and pycnogenol. Industrial Crops and Products, 38, 115-23.