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Full Length Research Paper

Chemical composition and antifungal activity of Aleppo pine essential oil

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The composition of spine essential oil of Aleppo pine tree from Ghazaouet (Tlemcen) extracted by hydro-distillation (yield: 0.3%) was investigated by GC_MS. Twenty-two compounds, representing 93.38% of the essential oil were identified. The main constituents are caryophyllene oxide (52%), thumbergol (9%), and humulene oxide (7.2%). The antifungal activity of this essential oil against *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer* was evaluated by the disc diffusion method.

Key words: Aleppo pine (*Pinus halepensis*), Algéria, GC_MS, antifungal activity.

INTRODUCTION

Recently, there has been an alarming increase in fungal infections, especially for immuno-compromised individuals. Among them, opportunistic systemic mycoses have been associated with high mortality rates. There is an increasing awareness amongst clinicians and microbiologists pertaining to the importance of infection caused by opportunistic fungi (Sunita and Mahendra, 2008; Sadeghi-Nejadt et al., 2010). Many drugs to treat fungal diseases have been developed over the years. Yet, there is a limited number of efficient ones, due to the general undesirable side effects and low sensitivity against the fungi (Sadeghi-Nejadt et al., 2010).

On the other hand, opportunistic molds are able to colonize diverse substrates including food. These microorganisms can cause a high degree of deterioration in foods and can be responsible for considerable economic losses. Furthermore, they can act as potential producer of toxic mycotoxins, which are potential damaging agents to consumer's health (Evandro et al., 2005). To retard molds growth and mycotoxin production, chemical preservatives are used. Currently, there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity (Omidbeygi et al., 2007). This situation led scientists to search for new antimicrobial agents.

Traditional medicinal plants can be used for their antifungal activities. The antimicrobial properties of these plants essential oils have been recognized and experimentally evaluated for many years. Moreover they found applications agents in various fields,including pharmacology, pharmaceutical botany, phytoptherapy, medical and clinical microbiology, food preservation,etc (Maridars, 2009)

Pinus halepensis (Pinaceae) is one of the many trees that are known for their medicinal properties (Delille, 2007) as well as for their economical importance (Kurose et al., 2007). To the best of our knowledge, few studies were achieved to determinate the bioactive compounds of Mediterranean *Pinus* species (Hmamouchi et al., 2001; Lahlou, 2004).

The aim of this study was to evaluate the sensitivity of some moulds strains to essential oil of *P. halepensis* obtained from the area of Ghazaouet (North-west of Algeria) for a possible future use as alternative antimould compounds.

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MATERIALS AND METHODS

Chemicals

The spines of *P. halepensis* were collected in April 2008 at the forest of Ghazaouet (Tlemcen). This species was identified by both the ecology laboratory and the Forestry department (Tlemcen). The spines (100 g) were shade-dried (away from humidity, at room temperature for 20 days) and subjected to Hydro-distillation using a Clevenger-type system for 2 h. The obtained essential oil was stored away from light at 4°C. GC/MS analyses were performed on a CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm, coating thickness 0.25 m and a Varian Saturn 2000). Analytical conditions were as follows: carrier gas helium at 1 ml/mm; injection of 0.02 µL; split ratio 1:80; oven temperature programmed from 50 to 220°C at 4°C /min. (The analysis of *P. halepensis* essential oil was achieved in the laboratory of the Ecole normale supérieure de chimie (Montpellier).

Microorganisms

The evaluation of the antifungal activity of *P. halepensis* essential oil (EO) was performed against four strains isolated from cereals, namely *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, and Rhizopus stolonifer*. Strains molds come from a 3 days culture (*R. stolonifer*) and a 5 day culture (*F. oxysporum, A. flavus, A. niger*) at 25°C on Petri dishes containing potato dextrose agar (PDA). The dilution method was used to evaluate this activity. Because of the non-miscibility of EO with water, the emulsification was carried out using a 0.2% agar solution (Lahlou, 2004; Ouraini et al., 2005). 100, 200 and 300 µL of EO were added to 1900, 1800 and 1700 µL of 0.2% agar solution, respectively. A total volume (2 mL) of each dilution was added aseptically to 18 mL of cultural medium potato dextrose agar (PDA). The tubes were sterilized in an autoclave for 20 min at 120°C then stirred using a vortex tube in order to disperse the EO. Finally, seeding was achieved by filing 100 µl of sporal suspension containing 10^6 spores/mL for *A. Niger* and *A. flavus* [the inoculums is obtaining by pooting spore in distilled water with 0.1% tween 80 and was adjusted to 10^7 spores/mL] (Tantaoui-Dlaraki and Baroud, 1992) and disk of mycelium of about 0.6 cm diameter in the center of the Petri disk (Kordali et al., 2008) for *F. oxysporum* and *R. stolonifer*.

Final concentrations of 15, 10, and 5µL/mL were obtained. The results were determined after a 5 days incubation time at 25°C. Both the growth diameter and the antifungal index were determined.

$$\text{Antifungal index} = \frac{(Da-Db)\ 100}{Db}$$

Db: growth diameter (control) and Da: growth diameter (test) (De Billerbeck, 2007).

RESULTS AND DISCUSSION

GC/SM

The Hydro-distillation of *P. halepensis* spines yielded 0.3% of the essential oil. The chromatographic profile showed the oil to be mainly constituted with sesquiterpene hydrocarbons. More than 22 compounds were identified accounting for 92.38% of the total oil. The major constituent in our EO is caryophyllene oxide (48.15%), followed by thumbergol, humulene oxide, phenethyl valerate and caryophyllene. Smaller amounts of terpine-4-ol, p-cymen-8-ol were also detected (Table1). These data are in agreement with those obtained by Dob et al. (2007) who showed predominance of β-caryomphyllene (70.31%) and α-humulene (7.92%) in the Algerian *P. halepensis* essential oil. Roussis et al. (1995) have reported that monoterpene hydrocarbons (myrcen) constitute 50% or more of the Greek volatile oil. Macchioni et al. (2003) reported the main compounds of the spine oil of Aleppo pine grown in Italy as being myrcen, α-pinene and β-caryophyllene with 73.2% of monoterpenes and 21.2% of sesquiterpenes. On the other hand, a study achieved in Tissimsilet and Djelfa (Algeria) showed α-pinene (17.56%), phenyl-ethyl-2-methyl butyrate (10.29%), and myrcene (8.6%) as major compounds (Badjah-Hadj-Ahmed et al., 1993). It is noteworthy that caryophyllene_oxide is either absent or in traces the spines of Aleppo pine of other countries. A total absence is noted for the thumbergol, humulene oxide and phenethyl-valerate. Our results showed qualitative and quantitative differences in the composition of the EO pine with those of both Italian and Greek origins, which are characterized by the presence of hydrocarbons and oxygenated monoterpenes. On the other hand, our results were in agreement with other studies on EO pine of Algerian origins, revealing a sesquiterpene hydrocarbon composition. Our essential oil is characterized by the presence of important concentrations of caryophyllene oxide, thumbergol, humulene oxide and phenethyl valerate, which are characteristics of the Ghazaouet region. These data may be sufficient to distinguish the chemotype of such a region, which is likely the result of an adaptive process to particular ecological conditions.

Microbiological study

The EO *P. halepensis* showed a moderate activity on molds (*A. niger, A. flavus, F. oxysporum*) which were inhibited at 35, 31.51 and 35.71% at concentration of 15 µL/mL and 44.23% at the same concentration (Table 2).

These results of Krauze–Baranowska et al. (2002) showed that the *Pinus* essential oil (*P. ponderosa, P. resinossa, P. strobus)* has strong activity against *fusarium solani, F. poae, F. culmorum* with percentages of inhibition of 100, 78 and 100%, respectively (Figure 1).

In another study (Sacchetti et al., 2005), *Pinus radiata* showed activity against *Candida albicans ATCC 48 274, Rhodotorula glutinis* ATCC 16 740, *Schizosaccharomyces pombe* ATCC 60232, *Saccharomyces cerevisiae* ATCC 2365, *Yarrowia hypolitica* ATCC 16617 with CMI of 0.14 to 0.09 to 0.02 to 0.06 to 0.29 mg/ml, respectively (Sacchetti et al., 2005).

Table 1. Chemical composition of spine oils of *Pinus halepensis* by CG_MS.

Compound	Rétention time	Constituent percentage
Terpinen-4-ol	18.39	0.3704
p-cymen-8-ol	16.66	0.5556
α-Copaene	26.93	0.2778
Caryophyllene	28.77	2.9632
Humulene	30.23	0.7408
Phenyl-isovalerate	31.51	0.5556
Phenethyl-valerate	31.72	5.7875
Cubebol	31.96	0.5556
α-Numulene	32.12	0.2778
1-epi-Cubebol	32.76	0.7408
Bourboneol	34.23	2.1298
Caryophyllene-Oxide	35.5	48.152
Humulene-oxide	36.6	6.6672
Tetradecanal	39.06	1.7594
Tetradecanal	39.38	1.2364
Tetradecanal	39.9	0.1668
Pentadecanal	41.02	1.2038
Pentadecanal	41.63	0.3704
Ledol	44.46	1.2038
Phenthyl ester	46.03	0.0986
Thumbergol	50.27	8.334
Unknown	52.28	1.389

Table 2. Antifungal activity of the *Pinus halepensis* essential oil.

	Control	EO 5 µL/ML	10 µL/ML	15 µL/ML
A. niger	d:3	d:2.5 Ia:16.67%	d:2.O5 Ia:31.67%	d:1.95 Ia:35%
A. flavus	d:3.75	d:3.35 Ia:10.67%	d:2.75 Ia:26.67%	d:2.5 Ia:33.33
Rhizopus spp	d:9	d:6.75 Ia:25%	d:4.95 Ia:45%	d:4.35 Ia:51.66%
Fusarium oxysporum	d:3.5	d:2.5 Ia:28.57%	d:2.4 Ia:31.43%	d:2.25 Ia:35.71%

Ia: Antifungal index and d: diameter of inhibition.

Several authors who studied the antimicrobial activity of Pinacee (Recio and Rios, 1989; Karaman et al., 2001; Nedorostova et al., 2009), reporting that monoterpenes (α pinene, β pinene….) possess a high antifungal activity. The absence of monoterpenes in our EO can explain the absence of a very high activity of EO Aleppo pine. On the other hand, the antifungal activity of our EO may due to the presence of caryophyllene_oxide. In fact, this compound is known for its use as preservative in foods, drugs and cosmetics. It is tested as an antifungal against

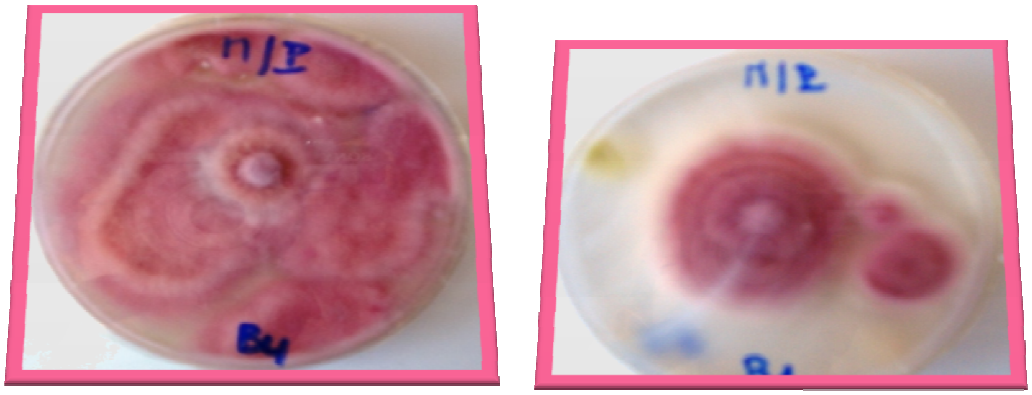

Figure 1. *Fusarium spp* with 0 µl (control) and 300 µl (test) of *Pinus halepensis* EO.

dermatophytes in onychomycoses with significant results (Yang et al., 1999).

Conclusion

The results of this work show that the *P. halepensis* essential oil possesses antifungal activity against *A. flavus, A. niger, F. oxysporum, R. stolonifer* and thus can be used as a natural treatment for fungal infections, as well as natural preservative in food. It is clear that such an activity is moderate, but the studied essential oil could be used to determinate the active species, thus leading to the discovery of a lead compound and the use of combinatorial chemistry. However, as far as the medicinal usage, it will be necessary to study the medicinal aspect of such oil.

REFERENCES

Badjah-Hadj-Ahmed Y, Tazerouti F, Meklati Y (1993). Analysis of Pine Needles Essential Oils by GC-MSand GC-FTIR.

De Billerbeck (2007). Essential oils and bacteria resistant to ATB. Phytotherapy, 5: 249-253.

Delille L (2007). Plants in Algeria. Berti edition.

Dob T, Berramdane T, Chelgoum C (2007). Chemical composition of essential oil of *Pinus halepensis* Miller growing in Algeria. C. R. Chimie., 1939-1945.

Evandro LS, Edeltrudes DOL, Kristerson RLF, Cristina PS (2005). Inhibitory Action of Some Essential Oils and Phytochemicals on the Growth of Various Moulds Isolated From Foods. Brazilian Arch. Biol. Technol., 48(2): 245-250.

Hmamouchi M, Hamamouchi J, Zouhdi M, Bessiere J (2001). Chemical and Antimicrobial Properties of Essential Oils of Five Moroccan Pinaceae. J. Essent. Oil Res., 13(4): 298-302.

Karaman S, Digrak M, Ravid U, Ilcim A (2001). Antibacterial and antifungal activity of the essential oils of Thymus revoltus celak from turkey. J. Ethnopharmacol., p. 76.

Kordali S, Cakir A, Ozer H, Cakmazan R, Kesdek M, Mete E (2008). Antifungal phytotoxic and insecticidal properties of essential oil isolated. Bioresour. Technol., 99: 8788-8795.

Krauze –Baranowska M, Maradarowiez M, Wiwart M (2002). Antifungal activity of the essential oils from some species of the genus Pinus. Z. Naturforsh., 57: 478-482.

Kurose K, Okamura D, Yatagai M (2007). Composition of the essential oils from the leaves of nine Pinus species and the cones of three of Pinus species. Flavour Fragr. J., 22(11): 10-20.

Lahlou M (2004). Méthods to study the phytochemistry and bioactivity of essential oils. Phytother. Res., 18: 425-448.

Macchioni F, Cioni L, Flamini G, Morelli I, Maccioni SA (2003). Chemical composition of essential oils from needles, branches and ones of *Pinus pinea*, *P. halepensis*, *P. pinaster* and *P. nigra* from central Italy. Flavour Fragr. J., 18: 139-143.

Maridars (2009). Screening of antifungal activities of barkes of cinnamomum species. Thail. J. Pharm. Sci., 33: 137-143.

Nedorostova L, Kloucek P, Kokoska L, Stolcova M, Pulkraibek J (2009). Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. Food Control, 20: 157-160.

Omidbeygi M, Mohsen BA, Hamidi A, Naghdibadi H (2007). Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. Food Control, 18(12): 1518-1523.

Ouraini D, Agouni A, Alaoui MI, Alaoui K (2005). Therapeutic approach of dermatophytes by HE Moroccan herbs. Herbal Medicine. Phytother., 1: 3-12.

Recio MC, Rios JL (1989). A review of some antimicrobial compounds isolated from medicinal plants reported in the literature 1978_1988. Phytother. Res., 3(4): 1989.

Roussis V, Petrakis P, Ortiz A, Mazomenos B (1995). Volatile constituents of five *Pinus* species grown in Greece. Phytochemistry, 39(2): 357-361.

Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini N, Radice M, Bruni R (2005). Comparative evaluation of 11 essentials oils of different origin as functional antioxidants, antiradicals. Food Chem., 91: 621-632.

Sadeghi-Nejadt B, Shiravi F, Ghanbari S, Alinejadi M, Zarrin M (2010). Antifungal activity of *Satureja khuzestanica* (Jamzad) leaves extracts *Jundishapur*. J. Microbiol., 3(1): 36-40.

Sunita B, Mahendra R (2008). Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic *Aspergillus fumigatus* and *A. niger*. World J. Med. Sci., 3(2): 81-88.

Tantaoui-Dlaraki A, Baroud L (1992). Inhibition of growth and aflatoxin production in Aspergillus parasiticus NRRL.2999 by essential oils of selected plant materiels. Thai J. Toxicol., 8: 51-59.

Yang D, Michel L, Chaumont JP, Millet-Clere J (1999). Use of caryophyllene oxide as an antifungal agent in an *in vitro* experimental model of unychomycosis. Mycopathologia, 148: 79-82.