African Journal of Biotechnology Vol. 8 (19), pp. 5021-5024, 5 October, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Antimicrobial activity of the essential oils of rosin from cones of *Abies cilicica* subsp. *cilicica*

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Accepted 7 July, 2009

Essential oil from the rosin of cones of *Abies cilicica* (Ant. Et Kotschy.) subsp. *cilicia* Carr. grown in Turkey was obtained by the hydro-distillation method and the antimicrobial activities of the oil were evaluated. The antimicrobial results showed that the microbial activities of the oil were considerably dependent upon concentration and its bioactive compounds. The oil inhibited the growth of all the bacteria and yeasts except for *Escherichia coli* at an oil concentration of 4 µl/disc. *Saccromyces cerevisiae*, *Klebsiella pneumoniae* and *Mycobacterium smegmatis* were the most sensitive microorganisms to the essential oil due to their low MIC values of 0.5 µg/ml. The results indicated that limonene was the most effective constituent on the microbial activities, followed by α-pinene, myrcene, and β-pinene, the most effective antifungal activities were also determined for myrcene.

Key words: Antimicrobial activity, essential oil, *Abies cilicica*, Turkey, cone, rosin.

INTRODUCTION

The art of aromatherapy, or the therapeutic use of essential oils, is among the fast growing segments of the emerging alternative health care industry (Pattnaik et al., 1997; Digrak et al., 1999; Dang et al., 2001). Essential oils can be used in a wide variety of ways for different purposes (Tylor, 1994; Kusmenoglu et al., 1999; Bagci et al., 1999; Grassmann et al., 2000).

Cilician fir [*Abies cilicica* (Ant. and Kotschy) subsp. *cilicica*] is a member of the *Pinaceae* (*Abietaceae*) family. The genus *Abies* contains 10 species and divisible into 2 subspecies: subsp. *cilicica* (Buds not rosinous; young shoots hairy) and subsp. *isaurica* (Buds reinous; young shoots glabrous). *A. cilicia* subsp. *cilicica* is native to mediterranean region of Turkey. The buds of the trees are not rosinous except for its female cones. Cone is subsessile, cylindrical and somewhat tapered above, up to 15 cm or more (Davis, 1967).

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In fall, the cones of *A. cilicica* start to disintegrate and these cones together with their scales pour out *Abies* trees on ground. And then, people collect the solidified rosin on the scales of the cones. The rosin has traditionally been used as antiseptic, anti-inflammatory, antipyretic, antibacterial and antiviral medicines and as chewing gum against some stomach diseases (e.g., ulcer), lip-dryness and asthma and curing the wound in the form of ointment and plaster (Baytop, 1999). Bagci et al. (1999) have stuided the chemical composition of young shoots from 2 subspecies of *A. cilicica* (Ant. et Kotschy) Carr. From Turkey. Studies were carried out on the essential oils from rosin of *A. cilicica* subsp. *cilicica* cones that the studies on essential oils of the root, stems (Kizil et al., 2002) and leaves (Bagci and Digrak, 1994; Bagci and Digrak, 1996) indicated the antibacterial and antifungal activities of nine *Abies* species.

However, antimicrobial activities of the essential oils from the rosin of the cones of *A. cilicica* subsp. *Cilicica* have not been studied so far. Thus, this study was aimed to investigate the antimicrobial properties of essential oil obtained from the rosin of *A. cilicica* subsp. *cilicica* grown

in Turkey.

MATERIALS AND METHODS

Plant and chemical materials

In this study, rosins from the cones (female) of cilician fir [*A. cilicica* (Ant. and Kotschy.) Carr. subsp. *cilicia* (Abietaceae)] were collected on October 27, 2002 from a Turkish state forest established in Baskonus district, South-East mediterranean part of Turkey. The altitude of the location was around 1100 m. Two reference antibiotics, ampicillin sodium (ampicillin 10 µ/disc) and streptomycin sulphate (streptomycin 10 µ/disc) were used as positive control bactericides and nystatin 100 µg/disc was used as a positive control yeastcide. They were purchased from Eczacibasi Chem. Co., Turkey. 4 reference compounds (limonene, α-pinene, myrcene and β-pinene) of the essential oil were purchased from Merck (Darmstard, Germany) to determine and compare MICs activities of essential oils.

Preparation of essential oil

The essential oil of the gum (50 g) of cilician fir was obtained by hydro-distillation method using a clevenger-type apparatus for 3 h. The yield, density (d) and refractive index (nD) of the oil were determined as 3.47%, 0.88 g/cm^3 and 1.4670 - 1.4720, respectively, by conventional methods. The white-colored essential oil was dried over anhydrous sodium sulphate (Na_2SO_4) and stored at -18°C.

Microorganisms

The growth inhibitory activity of the essential oil was tested against 11 bacteria (*Corynebacterium xerosis* UC 9165*, Bacillus brevis* NRS*, Bacillus megaterium* DSM 32, *Bacillus cereus* EU, *M. Smegmatis* CCM 2067, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* Cowan 1, *K. pneumoniae* FMC 5*, Enterococcus faecalis* ATTC 15753*, Micrococcus luteus* LA 2971 and *E. coli* DM) and 3 yeasts (*Kluvyeromyces fragilis* A 230, *Rhodotorula rubra* MC12 and *S. cerevisiae* ATCC 1023). These microorganisms were provided by microbiology laboratory culture collection, Department of Biology, Kahramanmaras Sutcu Imam University, Turkey.

Biological activity

Antimicrobial activities of the essential oils of the gum from *A. cilicica* were determined using the agar-disc diffusion method. The bacteria were first incubated at 37 ± 0.1°C for 24 h in nutrient broth (Difco), and the yeasts were incubated in sabouraud dextrose broth-SDB (Difco) at 25 ± 0.1°C for 24 h. The cultures of the bacteria and yeast with 0.1 ml (10^7 - 10^8 cfu (colony formation unit)/ml for the bacteria and 10^6 cfu/ml for the yeasts) were injected into the petri dishes (9 cm) (NCCLS, 1999). Mueller Hinton agar (MHA) and sabouraud dextrose agar-SDA (sterilized in a flask and cooled to 45 - 50°C) were homogenously distributed into the sterilized petri dishes in the amount of 15 ml. Subsequently, 6 mm diameter sterilized blank paper discs were saturated with 1.0, 2.0 and 4.0 µl of essential oils. The treated discs were placed into the agar plates, which had previously been inoculated. The blank paper discs treated with ampicillin, streptomycin and nystatin-saturated antibiotics were used as positive controls. Afterwards, the plates combined with the discs were left at 4°C for 2 h, the plates injected with yeast were incubated at 25 ± 0.1°C for 24 h and discs injected with bacteria were incubated at 37 ± 0.1°C for 24 h. Inhibition zones appearing around the discs were measured and recorded in mm after 24 h incubation period. The initial number of microorganisms in the suspension was determined for the total yeasts and bacterial count during 24 h at 37°C for bacteria and 48 h at 25°C for yeasts (Collins et al., 1989).

Minimal inhibitory concentration (MIC)

A microdilution broth susceptibility assay was used, as recommended by NCCLS (1999), for the determination of the MICs of essential oils and reference components used (NCCLS, 1999). All tests were performed in Mueller Hinton Broth (MHB) supplemented with Tween 80 detergent (final concentration of 0.5% (v/v), with the exception of the yeasts (SDB + Tween 80). Bacterial strains were cultured overnight at 37°C in MHB and the yeasts were cultured overnight at 30°C in SDB. Geometric dilutions ranging from 0.5 to 6.0 µg of the essential oils were prepared including one growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil). Test tubes were incubated under normal atmospheric conditions at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeasts. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom.

Microbial numbers

Microbial numbers were determined by standard plate counts, using plant count agar and potato dextrose agar (Difco Laboratories). Plates were incubated at 37°C and colonies arising after 24 h were counted (Collins et al., 1989).

RESULTS AND DISCUSSION

In this study, the antimicrobial activities of the essential oil extracted from the rosins of the *A. cilicica* cones, having three different concentrations of 1, 2 and 4 µl/disc, were compared with those of ampicillin, streptomycin and nystatin used as positive controls (Table 1). The antimicrobial (including antibacterial and antifungal) activities obviously increased with increasing the oil concentration from 1 to 4 µl/disc. The all oils inhibited the growths of all the bacteria and yeasts studied except *E. coli* at an oil concentration of 4 µl/disc.

However, the oil with the concentrations of 2 and 4 µl/disc has higher inhibition zone than 7 mm, which is considered as the limit inhibition zone for being reasonable antibiotic (Baytop, 1999), for all the microorganisms studied with the exceptions of *E. coli*, *P. aeruginosa, S. aureus* and *M. luteus*. The antimicrobial activities of the essential oil from *A. cilicica* against the same microorganisms were low as compared to those of ampicillin (10 µg/disc), streptomycin (10 µg/disc) and nystatin (100 µ/disc). The results clearly proved that *M. smegmatis*, *K. pneumoniae* and *S. cerevisiae* were also susceptible to the oil even at the concentration of 1 µl/disc.

The MICs of the oil against several bacteria and yeasts were presented in Table 2. The oils had variable levels of inhibition. *S. cerevisiae*, *K. pneumoniae* and *M. Smeg-*

Table 1. Antimicrobial activities of the essential oil from the rosin of *A. cilicica* subsp. *cilicica* and some positive control antibiotics along with the minimum inhibitory concentration (MIC) of the oil.

Microorganisms	cfu[a]/mL inoculum	MIC (µg/mL)	Diameter Inhibition Zone (DIZ-mm)					
			Essential oil amounts			A10[b]	S10[c]	N100[d]
			1 µl/disc	2 µl/disc	4 µl/disc			
Gram-Positive bacteria								
C. xerosis	8.7×10^8	1.50	-[e]	9	12	12	10	nt[f]
B. brevis	5.2×10^8	1.75	-	7	10	14	16	nt
B. megaterium	6.9×10^8	1.50	-	8	10	11	17	nt
B. cereus	5.5×10^8	1.75	-	9	11	15	18	nt
M. smegmatis	7.8×10^8	0.50	8	10	12	19	15	nt
S. aureus	2.7×10^8	3.50	-	-	8	25	22	nt
M. luteus	5.2×10^8	3.50	-	-	8	33	-	nt
E. faecalis	6.9×10^8	1.75	-	9	11	16	17	nt
Gram-negative bacteria								
P. aeruginosa	9.3×10^7	3.50	-	-	8	10	13	nt
K. pneumoniae	8.5×10^8	0.50	7	10	13	17	16	nt
E. coli	9.5×10^8	>10	-	-	-	11	-	nt
Yeasts								
K. fragilis	6.6×10^7	1.75	-	7	10	nt	nt	15
R. rubra	8.0×10^7	1.50	-	9	12	nt	nt	14
S. cerevisiae	6.5×10^7	0.50	8	11	14	nt	nt	18

[a]Number of Colony Forming Units. [b]Ampicillin (10 µg/disc). [c]Streptomycin (10 µg/disc). [d]Nystatin 100 Unit/disc). [e]No inhibition zone is determined. [f] Not tested. Blanks mean not investigated.

Table 2. Minimum inhibitory concentration (MIC, µg/mL) of model several compounds of the essential oils from the rosin of *A. cilicica* susp. *Cilicica*.

Gram-positive bacteria	MIC (µg/mL)			
	Limonene	α-Pinene	β-Pinene	Myrcene
C. xerosis	3.00	>8.0	>8.0	>8.0
B. brevis	2.50	>8.0	>8.0	>8.0
B. megaterium	2.50	>8.0	>8.0	>8.0
B. cereus	2.50	>8.0	>8.0	>8.0
M. smegmatis	4.00	>8.0	>8.0	>8.0
S. aureus	3.00	>5.0	>8.0	>8.0
M.luteus	1.50	>5.0	>8.0	>8.0
E. faecalis	>5	>8.0	>8.0	>8.0
Gram-negative bacteria				
P. aeruginosa	6.00	>10.0	>8.0	>8.0
K. pneumoniae	2.50	>8.0	>8.0	>8.0
E. coli	2.50	>8.0	>8.0	>8.0
Yeasts				
K. fragilis	>5	>5.0	>8	1.5
R. rubra	>5	>5.0	>8	2.0
S. cerevisiae	4.00	>8.0	>8	2.0

matis were the most sensitive microorganisms to the essential oil due to their low MIC values of 0.5 µg/ml The MIC value (1.75 µg/ml) of oil were the same against the *B. brevis, B. cereus*, *E. faecalis and K. fragilis.* The highest MIC value (3.5 µg/ml) was determined against *P. aeruginosa* and *K. pneumoniae*. However, the antimicrobial activities of the essential oil from the rosin on the cones of *A. Cilicica* were comparable to those obtain-

ed from the leaves of the same species (Bagci and Digrak, 1996).

It was also reported that α-pinene (68.19%), β-pinene (11.91%), myrcene (8.62%) and limonene (1.88%) were the major components of the essential oil from the rosin of cones of *Abies cilicica* (Ant. Et Kotschy.) subsp. *cilicia* Carr. grown in Turkey.

The MICs of several model components from the oil were presented in Table 2. The limonene was the most effective constituent on the microbial activities, followed by α-pinene, myrcene, and β-pinene (Table 2). The most effective antifungal activities were occurred for myrcene (8.62%) (Alma et al., 2005), which was available in the essential oil studied. The major compounds studied had lower antimicrobial activities as compared to the whole essential oils. This phenomenon can be attributed to some minor effective compounds such as camphene and Δ^3-carene (Alma et al., 2003).

Conclusions

In this study, the antimicrobial activities of essential oil obtained the rosin of *A. cilicica* subsp. *cilicia* carr. cones grown in Turkey were investigated. The results indicated that the antimicrobial activities of the oil remarkably relied on the oil concentration and its bioactive compounds. The growths of the bacteria and yeasts studied except for *E. coli* were inhibited by oil concentration of 4 µl/disc. *S. cerevisiae*, *K. pneumoniae* and *M. smegmatis* were the most sensitive microorganisms to the essential oil due to their low MIC values of 0.5 µg/ml that the results revealed that limonene was the most effective constituent on the microbial activities, followed by α-pinene, myrcene and β-pinene. The most effective antifungal activities were also determined for myrcene.

ACKNOWLEDGMENT

We appriciate financial support given to the project by the University of Kahramanmaras Sutcu Imam, Turkey.

REFERENCES

Alma MH, Mavi M, Yildirim A, Digrak M, Hirata T (2003). Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* l. growing in Turkey. Biol. Pharm. Bull. 26: 1725-1729.

Bagci E, Digrak M (1994). Antimicrobial activities of *Abies nordmanniana* spp. *nordmanniana* ve *A. nordmanniana equi-trojani*. Proceedings of XII. National Biology Congresss, 6-8 July 1994, Edirne, Turkey, pp. 227-231.

Bagci E, Digrak M (1996). Antimicrobial activity of essential oils of some *Abies* (Fir) species from Turkey. Flav. Frag. J. 11: 251-256.

Bagci E, Baser KHC, Kurkcuoglu M, Babacioglu MT, Celik S (1999). Study of the essential oil composition of two subspecies of *Abies cilicica* (Ant. et Kotschy) Carr. from Turkey. Flav. Frag. J. 14: 47-49.

Baytop A (1999). Treatment with Medicinal Plants in Turkey, 2nd Ed. Nobel Publishing Houses, Istanbul, Turkey.

Collins CH, Lyne PM, Grange JM (1989). Microbiological Methods, Butterworths & Co. Ltd, London, UK.

Dang MN, Takacsova M, Nguyen DV, Kristianova K (2001). Antioxidant activity of essential oils from various spices. Nahrung. 45: 64-66.

Davis PH (1967). Flora of Turkey and The Esat Aegean Islands, Vol 9, Edinburg University Press, Edinburg, p. 87.

Digrak M, Alma MH, Ilcim A (1999). Antibacterial and antifungal effects of various commercial plant extracts. Pharm. Biol. 37: 216-220.

Grassmann J, Hippeli S, Dornisch K, Rohrent U, Beuscher N, Elstner EF (2000). Antioxidant activity of essential oils. Possible explanations for their anti-flammatory effects. Arziennltelforschung. 50: 135-139.

Kizil M, Kizil G, Yavuz M, Aytekin C (2002). Antimicrobial activity of rosins obtained from the roots and stems of *Cedrus libani* and *Abies cilicia*. Appl. Biochem. Microbiol. 38: 144-146.

Kusmenoglu S, Baser KHC, Ozek T (1999). Constituents of the essential oil from the hulls of *Pistacia vera* L. J. Essent. Oil. Res. 7: 441-442.

NCCLS (National Committee for Clinical Laboratory Standards) (1999). Performance standards for antimicrobial susceptibility testing. The 9th International Supplement., M100-S9, Villanova, PA, p. 93.

Pattnaik S, Subramanyam VR, Bapaji M, Kole, CR (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios. 89: 39-46.

Tylor VE (1994). Herbs of Choice, Pharmaceutical Products Press, Binghamton, p. 45.