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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (21): 3923-3926, 2007
ISSN 1028-8880

Antibacterial Activity and Chemical Constitutions of Essential Oils of *Thymus persicus and Thymus eriocalyx* from West of Iran

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Abstract: The essential oils of *Thymus persicus* and *Thymus eriocalyx were* collected in Lorestan province, west of Iran and were examined by GC/MS and bacteriological tests. Twenty seven compounds representing 92.095% of *T. persicus* and 99.77% of *Thymus eriocalyx* essential oils were identified. The major constituents of *T. persicus* were thymol (10.71%), carvacrol (25.71%), γ-terpinene (5.63%), α-pinene (1.14%), β-pinene (1.02%), limonene (11.65%) trans-sabinene hydrate (7.78%) and l-borneol (4.07%) and the major compounds of *T. eriocalyx*. were 1, 8-cinole (3.07%), L-linalool (1.01%), thymol (66.34%), caryophyllene oxide (2.96%) and carvacrol (7.5%). The oils also were examined for antibacterial activities against 6 standard bacteria by the broth microdilution and disc diffusion methods. They exhibited significant antibacterial activities against *Staphylococcus aureus* (MIC =1 : 235, MBC =1:20), *Escherichia coli* (MIC = 1:320, MBC =1:80) and *Pseudomonas aeroginosa* (MIC = MBC = 1: 1280). The results were compared with control antibiotics.

Key words: *Thymus persicus*, *Thymus eriocalyx*, essential oils, antibacterial

INTRODUCTION

There are eleven species of *Thymus* (Labiatae) grow wild in Lorestan province, west of Iran. The Persian name for this plant is Aveshin which has long been used as spice and medicine (Zargari, 1990). In fact it is now a very popular spice for topping the pizza in Iran. In old Persian medicine, Aveshin used for treatment of cough, skin and intestinal illnesses besides being used against helmentic parasites (Zargari, 1990; Mozaffarian, 1998). In modern medicine, however, *Thymus* essential oils have been used as flavor, food preservatives, antiseptic, antispasmodic, digestive and expectorant in cough and cold remedies (Burt *et al*., 2003; Stahl-Biskup and Seaz, 2002). Recent studies have shown that *Thymus* species has strong antibacterial, antifungal, antiparasit and antioxidant activities (Stahl-Biskup and Seaz, 2002). These applications have made the genus very popular. Thus considerable efforts have been made to identify the chemical composition and biological activities of essential oils obtained from different species and subspecies of this rather useful plant (reviewed by Sthal-Biskup and Seaz, 2002). Most of the early studies have been reported from west and north of Mediterranean region. In this study, we have examined *Thymus persicus* and *Thymus eriocalyx* essential oils for chemical composition and antibacterial activities. To our knowledge, there has been no report on the chemical and biological properties of the *T. persicus* and *Thymus eriocalyx* which grow wild at 1470 m altitude in Zagross mounts, west of Iran. It has been acknowledged that many factors can affect the compositions and subsequent antibacterial activities of essential oils from a given species. These are including soil compositions, altitude (De Feo *et al*., 2003), genotype (Shu and Lawrence, 1997) harvesting seasons, geographical source (Arras and Greela, 1992; Faleiro *et al*., 2002), part of plant used (Delaquis *et al*., 2002) and method of extraction (Sefidkon and Fand Dabiri, 1999). In fact some different result we have found may have highlighted some of the effects above mentioned.

MATERIALS AND METHODS

Plant materials: The fresh leaves of *Thymus persicu* and *Thymus eriocalyx* were collected at 1470 m altitude from Zagross Mountain in April 2005. The fresh leaves of *Thymus eriocalyx* (Ronniger) Jalas (Family: Labiatae) were collected from 1800 m of Zagros Mountain in the Lorestan state, west of Iran, in July 2005. The plants were identified

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and authenticated by Dr. H. Amiri at the Department of Biology of the University of Lorestan. The research then carried out in The Lorestan University of Medical Sciences in October 2005.

Isolation of the essential oils: The fresh leaves of the plants (43 and 54 g) were separately hydro distilled in a Clevenger-type apparatus for 2 h. The oils were dried over anhydrous sodium sulfate and immediately injected into GC/MS.

Analysis of the oils: GC analyses were carried out on a Shimutzu 17A gas chromatograph equipped with a FID and a BP-5 capillary column (30 m×0.25 mm; 0.25 μm film thickness) in the Lorestan University. The oven temperature was held at 60°C for 3 min then programmed at 5°C/min to 300°C. Other operating conditions were as follows: Carrier gas, He with a flow rate of 5 mL min^{-1}; injector temperature, 230; detector temperature, 300°C; split ratio, 1:8. GC/MS analyses were performed on a Shimutsu 17A GC coupled with Shimutsu QC5050 Mass system and a BP-5 capillary column (30×0.25 mm; 0.25 μm film thickness). The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. Mass range was from m/z 50-500 amu. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oils were identified by comparison of their mass spectra and retention indices with those published in the literature (Adams, 1995) and presented in the MS computer library (Shimutzu, Japan).

Microorganisms: Antibacterial evaluations were carried out against standard bacteria in the Microbiology Research Laboratory of The Lorestan University of Medical Sciences. The tested bacteria were *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeroginosa* ATCC 27853. The bacteria were obtained from the Microbiology Reference Laboratory (BoAli Hospital, Tehran). A *Bacillus cereus* strain originally isolated from rice in the Foodstuff Laboratory of the Department of Health (Khoramabad) was used. The bacteria were grown on the Muller Hinton broth or agar (Merck, Germany).

Antibacterial testing: The plant samples were filter-sterilized and used for disc diffusion and broth microdillution technique (Mahon and Manoselis, 2000). Paper discs (Θ 6.5 mm) were impregnated with 40 μL of the samples and the solvent was evaporated under a safety cabinet at room temperature. Bacterial suspension's turbidity were compared and equalized with the Mac Farland 0.5 standard. The suspension then spread over a Muller Hinton agar plate a by sterile swab Gentamycin and Ciprfloxacine were used as positive controls. The plates were incubated at 35°C overnight and the inhibition zone was measured. Minimum Inhibition Concentration (MIC) was determined in a 96 well flat-bottom sterile plates (Nunc, Denmark). The bacteria inoculums were grown in Muller-Hinton broth to the lag phase and then adjusted to the turbidity of Mc Farland 0.5 standard. The plant materials were serially diluted with medium in the wells and then 100 μL of bacterial suspensions was added to obtain a final concentration of 5×10^5 cfu mL^{-1} (Mahon and Manoselis, 2000). A growth control well, uninoculated and antibiotic controls were included on each plate. The plates were incubated at 35°C and the turbidity was observed on a tray-reading stand. Samples from clear wells were cultured on nutrient agar (Merck, Germany) for determination of the MBC. The MIC is defined as the lowest concentration of the test which inhibits bacterial growth and the lowest concentration that did not grow on nutrient agar plate was taken as the MBC. All experiments were repeated three times and average values were presented as the result.

RESULTS AND DISCUSSION

The hydro distillation of the leaves of *T. persicus* and *T. eriocalyx* gave pale green oils with a yield of 3.1%±0.1 (v/w) and 1.01% (v/w) on dry weight basis. The general chemical profiles of the tested oils, the percentage content of the individual components and retention indices are given in Table 1. There were 31 components which represent 92.095 and 99.77% of the total detected components. The major constituents of the oil of *T. persicus* leaves were thymol (10.71%), carvacrol (25.71%), γ-terpinene (5.63%), α-pinene (1.14%), β-pinene (1.02%), limonene (11.65%) trans-sabinene hydrate (7.78%) and l-borneol (4.07%) and the major compounds of *T. eriocalyx* were 1,8-cinole (3.07%), L-linalool (1.01%), thymol (66.34%), caryophyllene oxide (2.96%) and carvacrol (7.5%) (Table 1). There was a strong antibacterial of activity of *T. persicus* oils against *Pseudomonas aeroginosa* since the dilution of 1 in 1280 inhibited bacterial growth (MIC) and the same dilution was bactericidal too (MBC =1280) (Table 2). However effects of the oils on the other tested bacteria were insignificant at the tested concentration. There was no zone of inhibition against tested bacteria except 11 mm against *Pseudomonas aeroginosa*. The control Gentamicin (10 μg) disc produced 20 mm zone of inhibition against this bacteria. De Feo *et al.* (2003) have showed that soil

Table 1: Chemical composition of *Thymus persicus* and *Thymus eriocalyx* leaves

Compound	RI	*T. persicus* (re1%)	*T. eriocalyx* (re1%)
a-pinene	937	1.14	1.331-
Camphene	950	1.33	1.241
B-pinene	971	1.02	0.96
a-phellandrene	995	0.86	-
(+)-3-carene	1005	1.04	1.022
Limonene	1008	11.62	1.831
a-terpinene	1011	2.54	2..454
1.Limonene	1021	0.10	0.551
Cis-ocimenol	1025	0.87	0.601
Rans-sabinene hydrate	1028	0.59	0.56
Beta-ocimene	1048	5.63	2.011
y-terpinene	1050	7.78	0.98
1,8-cineol	1084	5.24	-
L-Linalool	1088	1.01	0.681
L-camphor	1112	3.61	-
1-sabinene	1115	0.56	7.552
1-borneol	1138	4.07	5.562
Terpineol	1159	0.83	13.788
Thymol	1162	66.34	0.231
a-terpinoleno	1185	1.05	0.581
Cis-sabinene hydrate	1204	1.05	22.029
Carvcrol methyl ether	1228	1.11	13.977
Carvacrol	1238	25.71	5.658
Trans-Caryophyllene	1414	0.05	0.709
Ethyl cinamate	1432	2.05	-
Beta-Bisabolene	1485	0.88	2.96
Cis-bisabolene	1509	1.65	0.67
Caryophyllene oxide	1548	2.56	-
Sapthulenol	1577	1.59	1.283
Juniper camphor	1596	0.97	-
Cis-Bisabolene	1661	1.75	-

Table 2: The MIC and MBC (reciprocal of dilution) of *Thymus persicus* and *Thymus eriocalyx* essential oils against some standard bacteria

	Essential oils (dilution^{-1})				Ciprofloxacine (g mL^{-1})	
	T. persicus		*T. eriocalyx*			
Bacteria	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	100	100	235	20	1.0	5.0
P. aeroginosa	1280	1280	>100	>100	2.0*	2.0*
B. cereus	>100	>100	>100	>100	1.0	1.0
S. aureus	>100	>100	>320	80	0.5	2.5
S. epidermidis	>100	>100	>100	>100	4.0	12.0
E. faecalis	>100	>100	20	>100	5.0	2.0

*Gentomycin was used

chemical properties and altitude are important factors in determining the composition and subsequent antibacterial properties of essential oils from *T. spinulosus*. They have reported low percentage of phenols but high level of its precursors, terpinen and p-cymene in the oils. While the oils were least effective on *Pseudomonas aeroginosa* compare to other examined bacteria (De Feo *et al*., 2003). Rassoli and Mirmostafa (2003) have reported that essential oils of *T. persicus* collected from Mount Damavand, center of Iran, contain Carvacrol (27.01%), thymol (11.86%), ρ-cymene (10.16%), a-terpineol (9.51%), nerol (9.41%), γ-terpinene (6.51%) and thymol acetate (5.3%) as the major component at flowering stage. Their report has also indicated that the oils were active against most tested bacteria except for *Pseudomonas aeroginosa*.

We have identified nine components in the report to be identical to our findings (Table 1). These including, 1,8-Cineol, Linalool, 1-borneol, thymol, carvacrol methyl ether, carvacrol, cis-bisabolene, aryophyllene oxide and Sapthulenol while the P-cymene was absent. The pattern of components we have found may suggests a new chemotype in the *Thymus* genus and explain different antibacterial activities although the soil and altitude may have had effects on the oils as explained earlier. Strong anti-pseudomonas activities found in this study may raise a hope for further application of *T. persicus* essential oils against this resistant and problem producing bacterium in medicine and food preservatives. In conclusion, essential oils of *T. eriocalyx* and *T. Persicus* showed strong activities against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeroginosa*. Although the starting dilutions (1:100 equal to 0.5 μL mL^{-1}) were generally low, it is expected that the oils would probably show more activities against other species of bacteria when tried at higher concentration.

ACKNOWLEDGMENTS

This research was supported by the research grants from The Lorestan University of Medical Sciences. Help and technical assistant of The Lorestan University in the GC/MS analysis are greatly appreciated. Special thanks to Miss Zahra Moosavi for her technical work on the microbiological assessment and to Dr. Amiri for identification of the plants.

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