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Chemical Composition and Antimicrobial Activity of Essential Oil of Thyme (*Thymus vulgaris*) from Eastern Morocco

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ABSTRACT

The essential oil from flowering Thyme (*Thymis vulgaris* L.) an aromatic member of the Lamiaceae family, from Morocco, obtained by hydrodistillation, was analysed by GC/FID and GC/MS. The constituents were identified by their mass spectra and Kovats' indices. Forty three compounds consisting 97.85% of the total components were identified from the oil obtained with 1% yield. Among those, camphor (38.54%), camphene (17.19%), α-pinene (9.35%), 1, 8-cineole (5.44%), borneol (4.91%) and β-pinene (3.90%) were the major oil components. Essential oil of Thyme was evaluated for its antibacterial activities against six Gram-positive and Gram negative pathogenic bacteria: *Staphylococcus aureus*, *S. epidermidis, Streptococcus* sp*., Pantoa* sp*. and Escherichia coli*.

Key Words: Essential oil composition; GC/FID; GC/MS antibacterial activities

INTRODUCTION

Thymus vulgaris L. (thyme), locally known"zaatar" or "zaitra', a member of the family Lamiaceae, is widely used in Morocco folk medicine for its expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative and diuretic properties. The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants all over the world. *Thymus* species are commonly used as herbal tea, flavoring agents (condiment & spice) and medicinal plants (Stahl-Biskup & Saez, 2002). The published results reveal that major volatile constituents obtained from the aerial parts of the plant are geranial, linalool, γ - terpineol, carvacrol, thymol and *trans*-thujan-4-ol/terpinen-4-ol (Piccaglia *et al.*, 1993).

Plant essential oils and extracts have been used for many thousands of years, especially in food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin & Deans, 1997). It has long been acknowledged that some plant essential oils exhibit antimicrobial properties (Finnemore, 1926) and it is necessary to investigate those plants scientifically, which have been used in traditional medicine to improve the quality of healthcare. Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens (Prabuseenivasan *et al*., 2006).

Recent studies have shown that *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities (Stahl-Biskup & Saez, 2002). Aim of the present work was to determine the essential oil composition of eastern Moroccan thyme oil composition. These results will allow deduction of which components are likely to contribute to the antimicrobial activity and determination of any relationships between the components and their antibacterial activity.

MATERIALS AND METHODS

Plant material. Samples of thyme were collected during the flowering period in eastern Morocco (Taforalt) in May, 2007. Identification of the species was confirmed and a voucher specimen was preserved in scientific institute in Rabat. The dried aerial parts were submitted to Hydro distillation for 3 h using Clevenger type apparatus, according to the European Pharmacopoeia (1996). The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C until used.

Gas chromatography. Essential oil samples (0.1 µL) were injected neat into an HP 6890 gas chromatography equipped with a flame ionisation detector (FID) and a 30 m x 0.25 mm HP-5 (cross-linked Phynel-Methyl Siloxane) column with 0.25 µm film thickness (Agilent), was used for the

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study. Helium was used as carrier gas, the flow through the column was 1, 4 mL min^{-1} and the splitless mode was used. The column was maintained at 40°C for 5 min, increased to 230°C at rate of 10°C min^{-1} and finally raised from 230 to 280 at rate of 30°C min^{-1}.

Mass spectrometry analysis. The oil was analysed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 6890 mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph. The MS operating parameters were as follows: ionisation potential, 70 eV; ionisation current, 2 A; ion source temperature, 200°C, resolution, 1000. Mass unit were monitored from 30 to 450 m/z. Identification of components in the oil was based on retention indices relatives to n-alkanes and computer matching with the WILLEY 275. L library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature (Adams, 1995). The chromatographic conditions were identical to those used for GC analysis.

Antimicrobial activity. The essential oils were individually tested against Gram positive and Gram negative bacteria. Some bacterial strains used in this study were obtained from American type culture collection (ATCC), National Institute of Healthy (NIH), USA. All bacteria were stored in trypticase soy (Sanofi Diagnostic Pasteur, France) broth containing 25% (v/v) glycerol (Sigma- Aldrich) at -20°C. Prior to use, the cultures were propagated twice in the appropriate media as mentioned above to make them physiologically active.

Microplate bioassay. The microplate bioassay (micro dilution) was used for the determination of minimum inhibitory concentration (MIC) (NCCLS, 1999). The MIC was defined as lowest concentration of thyme oil inhibiting visible bacterial growth after incubation for 20h at 37°C. Into each well 100 µL of Brain Heart Infusion broth (BHI, Difco Laboratories, Detroit, MI, USA) inoculated with the bacteria inoculum prior to the essay. An aliquot (100 µL) of the essential oil was added in first well. Geometric dilutions ranging from 0,041 to 21 mg mL^{-1} of the essential oils were prepared in a 96 well micro titre plate, including one growth control (BHI+Tween 80)and one sterility control (BHI+Tween 80+test oil). The contents of the wells were mixed and micro plates were incubated at 37°C for 24 h. The MIC was determined by quantitative tetrazolium based colorimetric method. Ten microliters of 4mg mL^{-1} solution of 3-(4,5- dimethylthiazo-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Biochemika, Fluca) in distilled water were added to each well. Plates were incubated at 37°C. After a few minutes at room temperature; the plates were read. A colour change from blue to mauve was indicative of bacterial growth.

RESULTS

Chemical composition of the essential oil. Essential oil yield was 1.0%. Freshly isolated essential oil was a yellow liquid with intensive, narcotic odour. The components of essential oil were separated into five classes, which were monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and others (Table I). Based on GC and GC–MS analysis of the essential oil of thyme 41 components were identified, which represented 97.85% of the total detected constituents. The major constituents of the oil were camphor (39.39%), camphene (17.57%), α-pinene (9.55%), 1,8- cineole (5.57), borneol (5.03%), β-pinene (4.32%) . Other components were presents in amounts less than 2% (Table I).

The essential oil from thyme contained camphene, α-pinene, β-pinene, myrcene the most important monoterpenes hydrocarbons.In particular, oxygenated monoterpenes were the most abundant compound group of the oil (54.82%).

Antimicrobial activity. As can be seen in Table II, essential oil obtained from thyme found to have antimicrobial activity against all microorganisms tested, with a range of MIC values from 0.33 to 2.67 mg mL^{-1}. The maximum activity of the essential oil was observed against Gram negatives bacteria *Pantoa sp* (0.66 mg mL^{-1})*, Escherichia coli* (1) (0.33 mg mL^{-1} and *E. coli* (2) but this oil has poor activity on the growth of Gram posives bacteria such as s.aureus and *S. epidermidis with* MIC of 1.33 mg mL^{-1} and *Streptococcus sp* with MIC of 2.67 mg mL^{-1}.

DISCUSSION

In our study, Camphor, a ketone component, described the chemotype of thyme essential oil of eastern Morocco. The comparison of our result with literature shows important qualitative and quantitative differences in compositions. The genus *Thymus* has numerous species and varieties and their essential oil composition has been studied earlier (Guillen & Manzanos, 1998, Jordán *et al*., 2003; Sotomayor *et al*., 2004). From thyme grown in in other countries (Piccaglia *et al*., 1993), six chemotypes has also been reported, geraniol, linalool, *γ*- terpineol, carvacrol, thymol and *trans*-thujan-4-ol/terpinen-4-ol. Our results differ from those obtained by Özcan *et al.* (2004) who studied the oil composition of thyme of the same specie sample in Turkey, in which thymol (46.2%), alpha terpinene (14.1%), p-cymene (9.9%) alphapinene (3.0%) was revealed to be dominant. For the Spanish thyme essential oil, the major components quantified were 1,8-cineole, followed by terpenyl acetate, borneol, linalool, beta - pinene, alpha-terpineol and camphor (Jordán *et al.,* 2006). On the contrary carvacrol, which were not detected in our sample were found to be the main componenet in the previose report (Hudaib *et al.*, 2002). Thymol, which was present at low concentration (0.24%) in our sample, represented 44.4-58.1% of the total oil in the previous report (Branauskiene *et al.,* 2003). Comparison between our results and the results of other reports showed differences, probably due to the plant varieties or sites, as well as the time of harvesting.

Table I. Components of the Thyme oil from eastern Morocco. The analysis was made as composite samples

K.I	PIC	Composé	R.T.	%
n.d.	1	Tricyclene	8.16	0.64
n.d.	2	Alpha-thyjene	8.34	0.46
929	3	Alpha pinene	8.48	9.35
945	4	Camphene	8.844	17.19
972	5	Beta pinene	9.46	4.23
989	7	Myrcene	9.83	3.21
1011	8	Alpha terpinen	10.32	0.27
1020	9	Para cymene	10.49	1.19
1026	10	1,8-cineole	10.60	5.45
1045	11	Trans Beta ocimene	10.96	0.09
1055	12	Gama terpinene	11.14	0.55
1064	13	Cis-sabinene hydrate	11.31	0.46
1079	14	Camphenilone	11.60	0.31
1084	15	Alpha-terpinolene	11.68	0.11
1097	16	Linalol	11.93	0.14
1113	17	Alpha-thyjone	12.19	0.29
1123	18	Unknown	12.36	1.07
1126	20	Neoalloocimene	12.42	0.53
1146	22	Camphor	12.75	38.54
1164	25	Borneol	13.05	4.92
1174	26	Terpinene-4-ol	13.22	2.21
1181	27	Para-cymen-8-ol	13.33	0.28
1186	28	Alpha terpineol	13.41	0.57
1204	31	Verbenone	13.71	0.13
1215	32	Carveol 1	13.86	0.22
1223	33	Unknown	13.98	0.11
1239	34	Carvarcrol metyl ethyl	14.20	0.53
1271		Unknown	14.66	0.11
1282	35	Bornyl acetate	14.82	0.40
1301	35	Thymol	15.08	0.24
1375	39	Alpha-copaene	16.10	0.30
1385	40	Beta-bourbonene	16.24	0.12
1410	41	Alpha-Gurjunene	16.57	0.66
1421	42	Beta-caryophyllene	16.71	0.09
1464	48	Aromadendrene	17.25	0.11
1479	49	Alpha-elemene	17.44	0.22
1483	50	Germacrene-d	17.49	0.23
1498	51	Bicyclogermacrene	17.68	0.13
1523	52	Delta-cadinene	17.98	-
1583	53	Spathulenol	18.69	0.73
1589	54	Caryophellene oxide	18.77	0.56
1598	55	Viridiflorol	18.87	-
1611	56	Beta-oplepenone	19.05	0.07

Table II. Minimal inhibitory concentration (MIC) of essential oil from Thyme

Bacterial species	Gram type	MIC (mg mL^{-1})
Staphylococcus aureus ATCC 25923	+	1.33
S. epidermidis ATCC12228	+	1.33
Streptococcus sp.	+	2.67
Pantoa sp.	-	0.66
Escherichia coli (1)	-	0.33
E. coli (2)	-	1.33

It is known that genetic constitution and environmental conditions influence the yield and composition of volatile oil produced by thyme plants. Correlations between chemotype polymorphism, sexual polymorphism and the environment have been detected (Gouyon *et al*., 1986). The antifungal and antibacterial activity exhibited by *Thymus* genus essential oil has been demonstrated by several researchers (Cruz *et al*., 1989; Karaman *et al*., 2001; Rasooli & Mirmostafa, 2003; Rota *et al*., 2004 & 07). Most studies reporting the action of essential oils against food spoiling organisms and food borne pathogens agree that essential oils are relatively more active against Gram+ve than Gram−ve bacteria (Lambert *et al*., 2001). Deans and Ritchie (1987) and Deans *et al.* (1995) observed that the susceptibility of Gram-positive and Gram-negative bacteria to plant volatile oils had a little influence on growth inhibition. However, some oils appeared more active with respect to Gram reaction, exerting a greater inhibitory activity against Gram-positive bacteria. It was often reported that Gram-negative bacteria were more resistant to the essential oils present in plants (Smith-Palmer *et al*., 1998). The cell wall structure of Gram-negative bacteria is constituted essentially with Lipopolysaccharides (LPS). This constituent avoids the accumulation of the oils on the cell membrane (Bezić *et al*., 2003).

The results obtained in our study showed that the Gram-negative bacteria were more sensitive to the essential oil of thyme. For that reason, our results (Table II) did not show any selectivity towards Gram +ve bacteria. Biological activity of essential oils depends on their chemical composition, which is determined by the genotype and influenced by environmental and agronomic conditions (Marotti *et al*., 1992). Much of the antimicrobial activity in essential oils from *Thymus* genus appears to be associated with phenolic compounds (thymol & carvacrol) (Consentino *et al*., 1999; Davidson & Naidu, 2000; Skocibusic *et al*., 2006; Rota *et al*., 2007). According to Jalsenjak *et al*. (1987), Sivropoulou *et al.* (1997) and Sur *et al*. (1991), camphor and 1, 8-cineole are mainly responsible for the antimicrobial activity of the plant oils, which contain them. Terpinen-4-ol (2.21%), although a minor constituent in the oil under study, is known to have very efficient antibacterial properties (Carson & Riley, 1995).

In our study, the antimicrobial activity of the oil could be due to borneol, alpha-pinene and beta -pinene. Borneol has been reported to have significant antimicrobial activity (Tabanca *et al.,* 2001; Vardar *et al.,* 2003). Pinene-type monoterpene hydrocarbons (alpha-Pinene & beta -Pinene) are well-known chemicals having antimicrobial potentials (Dorman & Deans, 2000). The antimicrobial activity of the essential oil of thyme is apparently related to its terpenes type components such as, myrcene, camphene and p-cymene (Table I), since there is a relationship between the chemical structures of the most abundant oils and their antimicrobial activities. Although the mechanism of action of terpenes is not fully understood, it is thought to involve membrane disruption by the lipophilic compounds (Cowan, 1999). The essential oils containing terpenes are also reported to possess antimicrobial activity (Dorman & Deans, 2000), which are consistent with our present studies. The synergistic effects of these active chemicals with other constituents of the essential oils should be taken into consideration for the antimicrobial activity.

CONCLUSION

The essential oil of thyme showed significant antimicrobial activity. Analysis by GC– MS and GC/FID demonstrated that camphor is ketone component that described the chemotype of thyme essential oil of eastern Morocco. However, it is still necessary to investigate cytotoxicity and toxicity of the oil.

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