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Essential Oil Composition and Antibacterial Activity of Some Plant Species

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Abstract

Essential oils obtained by steam distillation from *Thymus, Veronica* and *Agrimonia* plant species were subjected to GC-MS analysis. The essential oil of *Thymus* sp. have revealed the presence of more than ten components. The in-vitro micro-biological activity of these oils were investigated against some pathogenic bacteria, and the results showed that the essential oil of *Thymus* sp. have significant antibacterial activities towards the Gram-positive bacteria and less inhibition effect on the Gram-negative test bacteria. However, the essential oil of both *Veronica* and *Agrimonia* species has showed no activity on the entire test bacteria used.

Keywords: Essential oil, Antimicrobial activity, *Thymus* sp., *Veronica* sp., *Agrimonia* sp., Gc/Ms

INTRODUCTION

Essential oils are valuable natural products consisting of many compounds that are used as raw materials in spices, food, perfumes, cosmetics, aromatherapy and phytotherapy. This has attracted the attention of many scientists and encouraged screen plants in order to study the biological activities of their oils or chemical constituents. Each of the constituents contributes to the benefit or adverse effects of these oils [1]. The recent upsurge in antibiotic-resistant infections has increased the popularity of the essential oils as naturally occurring antimicrobial agents and therefore, plant essential oils may offer a great potential alternative for the treatment of many infectious diseases that could not be cured by current drugs [1, 2]. The antimicrobial activity of essential oils is assigned to a number of small terpenoids and phenolic compounds such as thymol, carvacrol, and eugenol, which demonstrate high antibacterial activity in their pure form too [3, 4]. However, there are often large differences in the reported antimicrobial effects of oils obtained from the same plant which can be attributed to the different geographical sources, the harvesting seasons, the genotype, the climate, the drying procedure and distillation technique of the plant. All of these factors also influence the chemical composition and the relative concentration of each constituent in the essential oil [Panizzi et al., 1993]. One of the most popular methods of studying essential oil composition is gas chromatography–mass spectrometry (Gc/Ms), which allows the identification by comparing their relative retention times/indices and their mass spectra [6, 7, 8, 9, 10].

In the present study, the *in-vitro* antibacterial activity of the essential oils obtained from the three aromatic plants namely; *Thymus* sp., *Veronica* sp. and *Agrimonia* sp. of Konya- Turkey was investigated against five Gram positive bacteria and, six Gram negative bacteria. The levels and composition of these essential oil extract were characterized by Gc/Ms analysis.

MATERIALS AND METHODS

Plant Material

Aerial parts of three different plants (*Thymus* sp., *Veronica* sp. and *Agrimonia* sp. from Konya-Turkey were chosen for this study.

Essential oil extraction

The air dried plants (*Thymus*, *Veronica* and *Agrimonia*) were ground and steam distilled for 10 h. The essential oil was extracted with ether, dried over anhydrous magnesium sulfate and the composition of these extracts was subjected to Gc/Ms studies.

GC/MS analysis

Finnigan DSQ and a HP 60m x 0.32 mm ID x 0.25 mm DB-5 capillary column were used. Column temperature was programmed from 40-280 °C. Column temperature was kept constant at 40 °C for the first 1 min. then was programmed at a rate of 6 °C / min. The temperature was kept constant again at 280 °C for another 15 mins. Injection type was (1:10) and dichloromethane was used as a solvent. Gc/Ms analysis has revealed the presence of each o-Cymene (5.04 %), (M^+ 135), 119, (100 %), 91, 77, 65, 51 39); γ-terpinen (1.84 % (M^+136), 130, 93 (100 %), 77, 65, 39), linalool (27.25 % (M^+(154), 139, 136, 121, 93, 71 (100 %), 55, 43), 4-terpineol (2.68 %),

(M^+154), 190, 121, 111, 93, 71, (100 %), 41; β-fenchyl alcohol (1.19 %), (M^+139), 136, 121, 93, 59 (!00 %); thymol (36.26 %), (M^+150), 135, (100 %), 121, 91, 77); carvacrol (1.50 %), (M^+150), 135 (100 %), 91, 77, 39) and the sesquiterpene caryophyllene (M^+204), 189, 161, 133, 93, (100 %), 91, 79, 69, 67, 41.

Test Bacteria and Media

The essential oils were individually used against a range of bacteria, namely; S*taphylococcus aureus* ATCC 25923*, Shigella sonnei* RSKK 877*, Escherichia coli* ATCC 35218*, Bacillus megaterium* RSKK 5117*, Bacillus subtilis* RSKK 244*, Bacillus cereus* RSKK 863*, Pseudomonas aeruginosa* ATCC 29212*, Pseudomonas aeruginosa* ATCC 27853, *Streptococcus mutans* CNCT 8177*, Yersinia enterocolitica* ATCC 1501*, Salmonella* 21.3. Bacterial strains were cultured overnight at 37°C in Mueller Hinton Agar (MHA) for antibacterial tests.

Susceptibility tests

Susceptibility of the bacterial strains to the essential oils was investigated using the disk diffusion method [11]. The culture suspensions were prepared and adjusted against 1 Mc Farland turbidity standard tubes. MHA medium (15 ml) was poured into each sterile petri dish. Then the surface of the agar medium dish was inoculated with 100 µl cultures. Oils were sterilized by filtration through a 0.45µm membrane filters. Empty sterilized discs of 6 mm were each impregnated with 20 ml essential oil. Discs were placed on agar plates, and the plates were incubated at 37 °C for 24 h. The inhibition zones formed on the medium were evaluated in mm. On each plate an appropriate reference antibiotic disc, depending on the test bacteria was applied for comparison. All of the experiments were performed in duplicate

The susceptibility of the strains to the essential oils was further evaluated by agar dilution method; MHA medium plates containing different concentrations of essential oil The minimal inhibitory concentration (MIC) was defined as the lowest concentrations at which no bacterial growth was observed after incubation at 37°C for 24 h. Determination of MIC's by the agar dilution method was performed, fallowing the National Committee of Clinical Laboratory Standard Guidelines [12]. The cultures were adjusted to 1 McFarland turbidity standard tubes. Serial concentrations of essential oils from different samples containing MHA were prepared as 0.1%, 0.2 %, 0.4%, 0.8%, 1.75%, 3.5 % and 7 % v/v. All tests were performed in MHA supplemented with Tween 80 detergent at a final concentration of 0.5%. Each test in this study was carried out at twice.

RESULTS AND DISCUSSION

In this study, the antimicrobial properties and the compositions of the essential oil of *Thymus* sp*., Veronica* sp. and *Agrimonia* sp. have been investigated by Gc/Ms. *The components were resolved and identified by comparing the recorded mass spectra with the Wiley library of mass spectra.* The essential oil from *Thymus* sp., which mainly contained; O-cymene (5.04%), γ-terpinen (1.84%), linalool (27.25%), 4-terpineol (2.68%), β-fenchyl alcohol (1.19%), thymol (36.26%), carvacrol (1.50%), and the sesquiterpenes caryophyllene (0.61%) has showed significant inhibition effect (36, 30, 29, 26 mm) against the Gram positive bacteria, namely; *B. subtilis*, *B. megaterium*, *S. aureus* and *S. mutans*, respectively. Since these bacteria had an appreciable inhibition zones, MIC value (≤0.8 % (v/v)) for each bacteria was determined. However, these results showed that *Thymus* essential oil was less active (MIC, 0.8 %) against *S. mutans* while it was most active (MIC, 0.2 %) towards *B. subtilis*. The essential oil of *Thymus* has also showed a moderate activity against the test bacteria *B. cereus*, *Y. enterocolitica*, *E. coli* and *S. sonnei* when compared with the standard antibiotics (Table 1). These results indicated that the antimicrobial activity of the essential oil extracted from *Thymus* sp. is comparable to the ones observed in some other thyme oils [5, 13, 14, 15]. It is interesting to note that the essential oils obtained from each *Veronica* sp. that contained mostly linalool (4.18%) and carvacrol (7.28%) and which was also characterized by significantly lower levels of monoterpene phenols and *Agrimonia sp.* that have possessed the lowest % content of carvacrol (1.04%) have exhibited no activity against these test bacteria therefore, from these results we could conclude that both linalool and carvacrol have either very small or no activity at all. However, previous studies of the root and seed extracts of *Agrimonia* species reports a small antibacterial activity on B. *cereus,* and *B. subtils* and *S. aureus* as well as having some anti inflammatory and anti diarrhetic activity [16, 17, 18]. Furthermore there were no reports on the anti microbial activity of the essential oils of *Veronica* species

Table 1. Antibacterial activity of the *Thymus* sp. essential oil against the test bacteria.

Test bacteria	Inhibition zone (mm)	MIC (%)	Antibiotics[a]			
B. subtilis RSKK 244	36	0.2	TE 24	OFX 26	PEN 24	AM 24
B. megaterium RSKK 5117	30	0.4	TE 24	OFX 23	PEN 28	AM 27
S. aureus ATCC 25923	29	0.4	TE 18	VAN 20	PEN 20	CN 14
S. mutans CNCT 8177	26	0.8	FEP 10	VAN 15	OFX 17	ERY 19
B. cereus RSKK 863	24	SZ	TE 24	OFX 22	PEN 27	AM 20
Y. enterocolitica ATCC 1501	24	SZ	FEP -	AK 18	CN 17	AM 21
E. coli ATCC 35218	20	SZ	FEP -	AK 17	CN 15	AM 27
S.sonnei RSKK 877	17	SZ	FEP 10	AK 11	CN 11	AM 22
P. aeruginosa ATCC 29212	-	SZ	FEP 31	TOB 20	CN 17	AK 25
P. aeruginosa ATCC 27853	-	SZ	FEP 24	TOB 21	CN 20	AK 21
Salmonella 21.3	-	SZ	FEP 9	AK 10	CN 10	AM 23

[a] TE: Tetracycline (30 µg); FEP: Cefepime (30 µg); TOB: Tobramycin (10 µg); VAN: Vancomycin (30 µg); OFX: Oflaxacin (5 µg); ERY: Erytromycin (15 µg); AM: Ampicillin (10 µg); AK: Amikacin (30 µg); CN: Gentamisin (10 µg); PEN: Penicilin (10 µg)
SZ: Small zone
-: Not determined

Table 1 has also indicated that *Thymus* sp. oil had significant antibacterial activities towards the Gram-positive bacteria and had moderate inhibition effect on the Gram-negative test bacteria (*S. sonnei, E. coli* and *Y. enterocolitica*) while exhibiting no effect on *Shigella* and *P. aeruginosa* which was reported by Sivropoulou et al (1996) to be resistant to many antimicrobial agents, including thymol and carvacrol [19]. The cell wall structure of the Gram-negative bacteria is constructed essentially with LPS that avoids the accumulation of the oils on the cell membrane [20, 21, 22]. This agrees very well with the previous reports about the Gram-negative bacteria being more resistant to the essential oils present in plants [2, 23, 24].

As thymol and linalool are the major components of the essential oils of *Thymus* sp., we assume that the antibacterial action is probably due to the effect of these two compounds. However, a synergistic activity of carvacrol and thymol against some bacteria has been reported [25]. This may explain the very small or no activity of carvacrol on the tested bacteria therefore; in order to explore the efficiency of each of these components further phytochemical work may be required.

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