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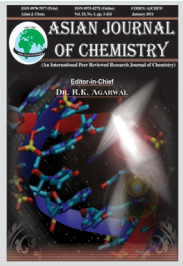

Antimicrobial Activity of Essential Oil of *Thymus kotschyanus* subsp. glabrescens

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This study was designed to examine *in vitro* antimicrobial activities of essential oil of *Thymus kotschyanus* subsp. *glabrescens* from Turkey (Kahramanmaras city). The antimicrobial activity of this plant was evaluated by the disc diffusion and minimum inhibitory concentration (MIC) methods with *Micrococcus luteus* LA 2971, *Bacillus cereus* EÜ, *Enterococcus faecalis* ATCC 15753, *Pseudomonas aeruginosa* ATCC 27853, *Mycobacterium smegmatis* RUT, *Escherichia coli* DM, *Yersinia enterocolitica* AÜ 19, *Bacillus brevis* FMC 3, *Klebsiella oxytocica* DC 113, *Rhodotorula rubra* DC 86, *Saccharomyces cerevisiae* WET 136 and *Candida albicans* DC 146. When the results of this study were compared with vancomycin, erythromycin and nystatin standards, it was found that *Thymus kotschyanus* subsp. *glabrescens* essential oil was particularly found to possess stronger antimicrobial activity and antifungal activities.

Key Words: Antimicrobial activity, Essential oil, *Thymus kotschyanus* subsp. *glabrescens*.

INTRODUCTION

Turkey is one of the richest areas in the middle latitudes in terms of plant diversity. The genus *Thymus* (Lamiaceae) is represented by 38 species (64 taxa) in Turkey and 24 of which are endemic to Turkey[1]. *Thymus* has been created with a long list of pharmacological properties[2], such as spasmolytic, antitussive, expectorant[3] and cosmetic vehicles[4] and used as folk medicine and used as tea and condiment[5]. The genus *Thymus* has numerous species and varieties and their essential oil compositions and their antimicrobial activities have been studied earlier[1,2,6-13].

Thymus kotschyanus subsp. *glabrescens* is suberect freely branching dwarf shrub lacking prostrate basal branches; flowering stems 3-10 cm, variously hairy all round. Leaves 9-13 × 4.5-6(-9) mm, incl. 1-2 mm petiole, all subequal, acutish, truncate to rounded at base, ciliate at base, oil dots numerous, usually brown-red; venation prominent beneath, with 3 pairs of lateral veins, the lowest marginal vein and often also the other two joining apically to form a marginal thickening[14].

In this study, the antimicrobial properties of essential oil from *Thymus kotschyanus* subsp. *glabrescens* from Kahramanmaras region were investigated.

EXPERIMENTAL

The Kahramanmaras City, falls within C_6 of the grid system adopted by Davis[14]. This area has different phytogeographic region, Irano-Turanian and Mediterranean, it has rich biological resources[14]. In this area, these plants are used as empiric drug, spice and herbal tea industry.

Several parts of *Thymus kotschyanus* subsp. *glabrescens* was used in this study. This plant was collected from the region Ahir Mountain (Kahramanmaras-Turkey). It was authenticated according to the conventional method[15]. This plant was identified, dried and broken into small pieces under sterile conditions.

The tested microorganisms in this study were provided from the culture collections of the Microbiology Laboratory of the Science and Art Faculty of the University of Kahramanmaras Sütçü Imam, in Kahramanmaras Turkey. *Micrococcus luteus* LA 2971, *Bacillus cereus* EÜ, *Enterococcus faecalis* ATCC 15753, *Pseudomonas aeruginosa* ATCC 27853, *Mycobacterium smegmatis* RUT, *Escherichia coli* DM, *Yersinia enterocolitica* AÜ 19, *Bacillus brevis* FMC3, *Klebsiella oxytocica* DC 113 bacteria and *Rhodotorula rubra* DC 86, *Saccharomyces cerevisiae* WET 136 and *Candida albicans* DC 146 fungi were used in this study.

Isolation and application of essential oil: The collected plant was distilled for 3 h, using a Clevenger-type apparatus according to the European Pharmacopoeia (1975)[16]. The essential oil thus obtained were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher and Shüll No:2668, Germany)[17,18]. Bioactivities were determined by measuring diameter of inhibition zones. In addition, reference antibiotic discs such as vancomycin (30 µg/disc), erythromycin

(15 µg/disc), nystatin 100 units (10 µg/disc) were used for comparision (provided by the Microbiology Division of Faculty of the Medicine Sütçü Imam University in Kahramanmaras, Turkey).

Determination of minimal inhibitory concentration (MIC): A broth microdilution susceptibility assay was used, as recommended by NCCLS, for the determination of the MIC of essential oils of *Thymus kotschyanus* subsp. *glabrescens* and some reference components[19]. 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 [sabouraud dextrose broth (SDB) + Tween 80]. Bacterial strains were cultured overnight at 37 ºC in MHB and the yeasts were cultured overnight at 30 ºC in SDB. Geometric dilutions ranging from 0.01-5.00 µg/mL 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 ºC for 24 h for bacteria and at 30 ºC for 48 h about the yeasts. The bacterial growth was indicated by the presence of a white 'pellet' on the well bottom.

Preparation of microorganism cultures: The bacteria were incubated in Nutrient Broth (NB) (Difco) at 37 ± 0.1 ºC for 24 h and the yeasts were incubated in Sabouraud Dextrose Broth (SDB) (Difco) at 25 ± 0.1 ºC for 24 h. The bacteria and yeasts (prepared as above) were injected into petri dishes (9 cm) in the amount of 0.01 mL (10^6/mL for the bacteria and 10^5/mL for the fungi)[20], 15 mL of Mueller Hinton Agar (MHA, Oxoid) and sabouraud dextrose agar (SDA) (sterilized in a flask and cooled to 45-50 ºC) were homogenously distributed onto the sterilized petri dishes[17].

Sterilized blank paper discs 6 mm in diameter were saturated with 1 and 2 µL of essential oil by micro-injector (brand name is Hamilton) per disc, then placed onto the agar plates which had previously been inoculated with the above organisms. The petri dishes were left at 4 ºC for 2 h and then the injected plates with bacteria were incubated at 37 ± 0.1 ºC for 24 h, plates inoculated with fungi were incubated at 25 ± 0.1 ºC for 48 h [13,17,18]. At the end of the period, inhibition zones were measured in millimeters (mm). These studies were performed in triplicate.

RESULTS AND DISCUSSION

As shown in Table-1, *in vitro* antibacterial and antifungal activities of essential oil of *Thymus kotschyanus* subsp. *glabrescens* and and the inhibition zones were formed by standard antibiotic discs.

The essential oil of this plant show strong antimicrobial activity against the tested bacteria *in vitro*. According to vancomycin, erythromycin, at low quantity (1 µL/disc) and high quantity (2 µL/disc), MIC (0.10-0.50 µg/mL) the essential oil of *T. kotschyanus* subsp. *glabrescens* showed inhibition zones against all bacteria and fungi but inhibition zones were different value against bacteria and fungi.

As it can clearly be seen from Table-1, the extracts of *T. kotschyanus* subsp. *glabrescens* have antibacterial and antifungal activities. At low quantity (1 µL) and high quantity (2 µL), the essential oil of *T. kotschyanus* subsp. *glabrescens* showed inhibition zones against all bacteria and fungi but inhibition zones were different value against bacteria and fungi in the experiments. In this experiment, *Bacillus cereus*, *Klebsiella oxytocica*, *Saccharomyces cerevisiae* were resistant at low concentrations (1 µL) besides other bacteria and fungi. But *Micrococcus luteus*, *Escherichia coli*, *Bacillus brevis*, *Candida albicans* were sensitive at low concentrations (1 µL) besides other bacteria and fungi. On the other hand, *Klebsiella oxytocica*, *Rhodotorula rubra* were resistant at low concentrations (2 µL) besides other bacteria and fungi. But *Enterococcus faecalis*, *Candida albicans* were sensitive at low concen-trations (2 µL) besides other bacteria and fungi.

TABLE-1
ANTIMICROBIAL ACTIVITIES OF *Thymus kotschyanus* subsp. *glabrescens* ESSENTIAL OILS

Microorganisms	Inhibition zone (mm)*					
	Thymus kotschyanus subsp. *glabrescens*			V30	E15	N10
	1 (µL/disc)	2 (µL/disc)	MIC (µg/mL)	µg/disc		
Micrococcus luteus LA 2971	20	36	0.25	21	34	NT
Bacillus cereus EÜ	12	32	0.15	16	25	NT
Enterococcus faecalis ATCC 15753	15	40	0.15	27	28	NT
Pseudomonas aeruginosa ATCC 27853	18	30	0.25	17	35	NT
Mycobacterium smegmatis RUT	18	34	0.25	22	27	NT
Escherichia coli DM	20	24	0.50	11	9	NT
Yersinia enterocolytica AÜ 19	16	26	0.10	17	28	NT
Bacillus brevis FMC 3	20	30	0.15	16	24	NT
Klebsiella oxytoca DC 113	12	20	0.15	21	18	NT
Rhodotorula rubra DC 86	20	44	0.25	NT	NT	14
Saccharomyces cerevisiae WET 136	12	50	0.25	NT	NT	18
Candida albicans DC 146	22	60	0.15	NT	NT	18

MIC: Minimum inhibitory concentration of the oil, V30: vancomycin (30 µg/disc), E15: Erytromycin (15 µg/disc), N10: nystatin 100 units (10 µg/disc), NT: not tested. *Values, including diameter of the filter paper disc (6.0 mm), are means of three replicates.

Essential oils from other *Thymus* species have also displayed high levels of antimicrobial and antifungal activities[21,22]. Recently Rasooli and Mirmostafa[12] found in a similar study that extracts of *Thymus kotschyanus* Boiss and Hohen and *Thymus persicus* L. have strongly bactericidal effects. According to Bonjar[23], the antimicrobial activity of methanolic extract of *T. kotschyanus*, was found against *Bordetella bronchiseptica*, *Micrococcus luteus* in disc diffusion method. Similarly, Nariman *et al.*[24] indicated that *T. kotschyanus* showed anti *Helicobacter pylori* activity by the disk sensitivity method.

Tepe *et al.*[25] showed that the antimicrobial activity of undiluted *T. eigii* essential oil, the strongest activity was observed against *B. catarrhalis* and *C. perfringens*, followed by *B. cereus*, *S. aureus*, *S. pneumoniae* and *M. smegmatis* in broth microdilution method. Additionally, Essawi and Srour[26]

reported that the most active antibacterial plants against both gram-positive and gram-negative bacteria were *T. vulgaris* and *T. origanium*. In previous studies, the antimicrobial activity of *Thymbra spicata* L. var. *spicata* essential oil (0.5 µL) was found against all tested bacteria and fungi in disc diffusion method[27] and the antimicrobial activity of *Thymus eigii* Jalas essential oil (0.5 µL) was found against all tested bacteria and fungi in disc diffusion and minimum inhibitory concentration methods[13]. Furthermore, Rasooli and Mirmostafa[3] mentioned that *T. pubescens* and *T. serpyllum* essential oils were strongly bactericidal even at higher dilutions with the exception of *P. aeruginosa*. In contrast, Alzoreky and Nakahara[28] found that acetone extract of *T. serpyllum* was inactive against *S. aureus* and *L. monocytogenes*.

There were several earlier studies on antifungal activity of *Thymus* spp. The lowest minimum inhibitory concentrations was 0.03 % (v/v) thyme oil against *C. albicans* and *E. coli*[29], *T. vulgaris* essential oil was fungicidal effect against *Macrophomina phaseolina* and *Bipolaris spicifera*[30] and methanol extract of *T. vulgaris* showed anticandidal activities against Clotrimazole-resistant *Candida albicans*[23].

Present study was similar to previous studies, despite the different extraction method used. The latter depends on a number of parameters of essential oils such as environmental conditions, collection period, dehydration procedure, storage condition, isolation method[31]. Generally, action of essential oils is the result of the combined effect of both their active and inactive compounds[32]. These inactive compounds might influence resorption, rate of reactions and bioavailability of the active compounds[33].

Antimicrobial properties of the essential oils and various extracts from many plants have recently been of great interest in both academician and the food industreis, because their possible use as natural additives emerged from a growing tendency to replace synthetic antimicrobials with natural ones. Owing to strong antibacterial and excellent protective features exhibited in antimicrobial activity tests, the essential oil of *Thymus kotschyanus* subsp. *glabrescens* could be considered a natural source that can be freely used in the food industry as a culinary herb.

In conclusion, essential oil of *Thymus kotschyanus* subsp. *glabrescens* can be used for protection against bacteria and, in some cases, against fungi.

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