NATURAL DRUGS

ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *THYMUS CAPITATUS* AGAINST *HELICOBACTER PYLORI*

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Abstract: *Helicobacter pylori* (*H. pylori*) is an infective agent and the main resourceful factor of chronic, active type B gastritis, peptic and duodenal ulcer, gastric carcinoma and mucosa-associated lymphoid tumors. Nowadays, pharmacological treatment of *H. pylori* infections attracts attention besides antibiotic treatment. The aim of this study is to analyse the chemical composition of the essential oils of *Thymus capitatus* and the antimicrobial activity against *H. pylori*. Aerial parts of wild-growing plant materials were collected during the flowering stage from three different locations. Essential oil composition analyses were performed simultaneously by GC-FID and GC-MS systems. The antimicrobial activity of the essential oil was tested by broth dilution method. The thymol percentage was determined as more than half of the essential oil composition of *Thymus capitatus-Yedidalga* (51.9%) and *Thymus capitatus-Yıldırım* (57.1%); however, the composition of thymol was 47.2% followed by 15.1% (*p*-cymene), 10.0% (γ -terpinene) and 5.7% (carvacrol) for *Thymus capitatus-Boğaz/Gime*. The essential oil from *Thymus capitatus-Boğaz/Gime* showed the lowest MIC and MBC values and the strongest bactericidal activity against *H. pylori* compared to the other tested essential oils. Bactericidal efficacy of essential oil of *Thymus capitatus* on *H. pylori* could have been affected due to the varied rates of major compounds. *Thymus capitatus* essential oil could be an alternative way for the treatment of *H. pylori* infections.

Keywords: *Helicobacter pylori*, *Thymus capitatus*, *in vitro*, essential oil

Helicobacter pylori (*H. pylori*) is a Gram-negative spiral bacterium identified in 1982 that is accepted as a critical causal agent in gastroduodenal diseases including chronic gastritis, peptic ulceration, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma (MALT) (1-3). The first data on the prevalence and molecular characteristics of *H. pylori* was produced by Krashias et al. in Cyprus. Krashias et al. found that the prevalence of the *H. pylori* was 39.8 % (41/103) (3). The treatment of *H. pylori* infection includes standard triple therapy with proton pump inhibitors. In addition to these therapies, studies

have indicated that adjuvant therapy such as probiotics, bovine lactoferrin and curcumin can lower the side effects of triple therapy and can increase the treatment effectiveness (4).

The genus *Thymus*, which belongs to the Lamiaceae family, is rich in essential oils (EO). thymol, carvacrol, γ -terpinene and *p*-cymene are the principal components of *Thymus* essential oils with different ratios and these components are responsible for the pharmacological effects (5, 6). In general, the ranges among the percentages of the major compounds in the EO composition are represented as chemotypes in nature. If the quantity of a com-

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pound is more than half of the EO, this chemotype is called “pure”; however, if there are multiple dominant compounds, this chemotype is called “mixed”. In the literature, various chemotypes of the *Thymus* genus have been reported around the World (7-12). This genus is represented by two species in Cyprus: *Thymus capitatus* (L.) Hoffmanns. & Link and *Thymus integer* Griseb. Ethnobotanical surveys in Cyprus have shown that *T. capitatus* is one of the most widely known and used aromatic plants for medicinal purposes for the preservation of health, as well as for the treatment of various diseases (1, 13). The aerial parts of this species are used in Cyprus to treat some respiratory tract infections, gastrointestinal and cardiovascular system disorders in the form of tea or by chewing (14-17). Additionally, for the treatment of mouth ulcers and oral cancers, the young leaves of *T. capitatus* are used by chewing in Northern Cyprus folk medicine (18). The antimicrobial activity of the EO (19, 20) and the extracts (21, 22) of this genus against *H. pylori* has been previously reported.

To the best of the author’s knowledge, no work has been conducted on the antimicrobial activity of the essential oil of *T. capitatus* against *H. pylori*. In the present study, it is aimed to analyze the chemical composition of the essential oils of *T. capitatus* collected from three different regions and the antimicrobial activity of these oils against *H. pylori*.

EXPERIMENTAL

Plant materials

The aerial parts of the plant materials growing wild in Northern Cyprus were collected during the flowering stages. Samples were gathered from three different locations in Northern Cyprus, designated as the western (Yedidalga) (Tc1), eastern (Yıldırım) (Tc2), and northern (Boğaz-Girne) (Tc3) regions. Voucher specimens were kept at the Herbarium of Near East University (NEUN). The Herbarium numbers of Tc1, Tc2 and Tc3 are NEUN 6882, 6881 and 6883, respectively.

Isolation of essential oil

One hundred grams of air-dried aerial parts of the plant material was hydrodistilled with 1 L of water for 3 h in a Clevenger-type apparatus. The EO were stored at 4°C until analysis. The yields were calculated as w/w by using a pycnometer. Stock *T. capitatus* EO was prepared in dimethylsulfoxide (DMSO, Sigma-Aldrich) and dissolved in 10% DMSO. The concentration rate of EO was 90%.

GC/MS analysis

The GC-MS analysis was conducted with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 mm film thickness) was used with helium as the carrier gas (0.8 mL/min). The GC oven temperature was kept at 60°C for 10 min and the programmed to 220°C at a rate of 4°C/min, kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The split ratio was adjusted at 40 : 1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

GC analysis

The GC analysis was conducted using an Agilent 6890N GC system. The FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of components

Identification of the EO components was conducted by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 3 Library) (23, 24) and in-house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data (25, 26) was used for the identification.

H. pylori strain

H. pylori ATCC 43504 was obtained from the American Type Culture Collection (Rockville, MD, USA) and then used in this study.

Preparation of stock clarithromycin solution

Clarithromycin solution was prepared by dissolving 25 mg clarithromycin in 25 mL methanol. 9 mL of phosphate-buffered saline was added into 1 mL of clarithromycin solution and the stock solution was obtained as 100 µg/mL (w/v). The stock solution was sterilized with 0.22 µm sterile syringe filter and 2-fold serial dilutions were made in phosphate-buffered saline.

Antimicrobial activity of EO

The broth microdilution method was performed as recommended by Lesjak et al. (27) and

Table 1. The essential oil compositions of the three localities of *T. capitatus* from Northern Cyprus.

RRI	Compound	1%	2%	3%
1032	α -Pinene	1.0	0.8	1.1
1035	α -Thujene	0.5	0.6	0.9
1076	Camphene	0.7	0.5	0.9
1118	β -Pinene	0.2	0.2	0.3
1159	δ -Car-3-en	-	0.1	0.1
1174	Myrcene	1.7	1.8	2.2
1176	α -Phellandrene	0.2	0.3	0.4
1188	α -Terpinene	1.5	2.0	2.3
1203	Limonene	1.3	0.9	1.0
1218	-Phellandrene	0.5	0.5	0.6
1255	γ -Terpinene	4.9	6.9	10.0
1280	<i>p</i> -Cymene	13.6	12.3	15.1
1290	Terpinolene	0.5	0.6	0.5
1452	Oct-1-en-3-ol	0.9	0.7	1.0
1474	<i>trans</i> -Sabinene hydrate	0.1	0.1	0.2
1553	Linalool	0.6	0.2	0.5
1604	Thymol methyl ether	0.2	0.1	0.2
1611	Terpinen-4-ol	0.6	0.4	0.4
1612	β -Caryophyllene	2.0	2.1	2.2
1694	Neral	0.4	0.3	0.2
1706	α -Terpineol	0.5	0.3	0.3
1708	Ledene	tr	0.7	-
1719	Borneol	3.7	1.1	3.0
1740	Geranial	0.5	0.4	0.4
1741	β -Bisabolene	0.1	-	0.2
1751	Carvone	0.1	tr	tr
1784	(<i>E</i>)- α -Bisabolene	0.1	-	0.2
1808	Nerol	0.2	0.2	0.3
1857	Geraniol	0.1	0.2	0.1
1864	<i>p</i> -Cymen-8-ol	0.2		0.2
2008	Caryophyllene oxide	0.6	0.4	0.5
2144	Spathulenol	0.3	0.2	0.2
2181	Isothymol	0.3	0.2	0.3
2186	Eugenol	0.3	0.3	0.3
2198	Thymol	51.9	57.1	47.2
2221	Isocarvacrol	1.2	1.0	1.0
2239	Carvacrol	8.5	6.5	5.7
	Total	100.0	100.0	100.0

1: *Thymus capitatus* - Yedidalga, 2: *Thymus capitatus* - Yildirim, 3 *Thymus capitatus* - Bogaz/Girne, RRI Relative retention indices calculated against n-alkanes, % calculated from FID data, tr Trace (< 0.1%)

clarithromycin was used as the positive control. The bacterial suspensions were adjusted to 2×10^8 CFU/mL⁻¹ standard turbidity. The prepared EO were placed in wells and inoculated brain heart infusion broth was added. The microplates were incubated for 48 h with an aeropack system. After the incubation period, 100 µL aliquots of the medium were plated on *H. pylori* agar plates. Colonies were counted after incubation for 5 days. DMSO was used to enhance oil solubility, therefore it was accepted that DMSO had no bacterial effect against *H. pylori* and was tested as a negative control.

The minimal inhibitory concentration (MIC) was determined as the lowest concentration of the sample that prevented visible growth and the minimum bactericidal concentration (MBC) was determined by the lowest concentration resigned negative subcultures or only one colony.

RESULTS

The chemical composition of the EO from three different locations

The EO of Tc1, Tc2 and Tc3 were analyzed by GC-FID and GC-MS systems simultaneously. The composition of EO obtained from three locations was identified in percentages (Table 1). The number of compounds identified from Tc1 (Yedidalga), Tc2 (Yıldırım) and Tc3 (Boğaz-Girne) was 36, 34 and 36, respectively. The thymol percentage was determined as more than half of the EO composition of Tc1 (Yedidalga (51.9%) and Tc2 (Yıldırım)) (57.1%); however, the composition of thymol was 47.2% followed by 15.1% (*p*-cymene), 10.0% (γ -terpinene) and 5.7% (carvacrol) for Tc3 (Boğaz-Girne).

Antimicrobial activity against *H. pylori*

Tc1 (Yedidalga), Tc2 (Yıldırım) and Tc3 (Boğaz-Girne) MIC values were < 0.5 µg/mL, < 0.5 µg/mL and < 0.25 µg/mL, respectively. The EO from Tc3 (Boğaz-Girne) showed the lowest MIC

and MBC values and the strongest bactericidal activity against *H. pylori* compared to the other tested EO. The MIC and MBC values are given in Table 2.

DISCUSSION AND CONCLUSION

At the present time, there are problems with treatments against *H. pylori* infection including multidrug resistance, high expenses, drug interventions, poor satisfaction, side effects and their brunt on the normal intestinal flora (28). In the last decade, interest in EOs has increased on their bactericidal, virucidal, fungicidal and antiparasitic effects (29) due to the fact that interest in studying the antimicrobial activities of the EO has been increasing. Additionally, the traditional uses of medicinal plants could lead to improvement in the treatment of infections such as gastroduodenal diseases.

According to the results of chemical composition and antimicrobial activity against *H. pylori* of the essential oils, Tc3 (Boğaz-Girne) was determined the most active in all three tested EOs. Furthermore, the rate of thymol/carvacrol decreased, while the rate of γ -terpinene/*p*-cymene increased for Tc3 (Boğaz-Girne) in comparison with Tc1 (Yedidalga) and Tc2 (Yıldırım)). The results of the chemical contents of the essential oils of *T. capitatus* shows that Tc1 (Yedidalga) and Tc2 (Yıldırım)) is found as pure thymol chemotypes due to the rate of thymol, while Tc3 (Boğaz-Girne) is found as a mixed chemotype. In the literature, it is found that carvacrol chemotypes (8, 11, 13) of *T. capitatus* are common while thymol chemotypes are rare (14, 30, 31). Lesjak et al. reported that *Satureja hortensis*, *Origanum vulgare* subsp. *vulgare* and *O. vulgare* subsp. *hirtum* EOs demonstrate the highest activity (MIC = 2 µg /mL) against *H. pylori* (27). Eftekhari et al. reported that the antimicrobial activity of the EO of *T. caramanicus* against *H. pylori* was higher than the clarithromycin (19) and these results were supported in this study's

Table 2. Minimum Inhibitory Concentrations (MICs) and Minimum bactericidal concentration (MBC) of tested essential oils against *H. pylori*.

Essential Oil	Location	MIC (µg/mL)	MBC (µg/mL)
<i>Thymus capitatus</i>	Tc1	< 0.5	1.00
	Tc3	< 0.25	0.50
	Tc2	< 0.5	1.00
Positive control	Clarithromycin	< 1	1.00
Negative control	DMSO	--	--

results. In addition, the antimicrobial effect against *H. pylori* of the EO of *Thymus sp.* was found to be more effective than pure compounds such as carvacrol and thymol (19). We had limited studies with *Thymus spp* anti-*H. pylori* activity. Dandlen SA. et. al studied with different species of the *Thymus spp.* (*T. caespitatus*) EO from Planalto Central and its main component, carvacrol is an effective inhibitor of *H. pylori* 26695 intracellular growth (32). In the present study, the highest thymol value was found in Tc2 (Yıldırım) (57.1%); however, Tc3 (Boğaz-Girne) was found as the most active against *H. pylori*. Therefore, it is possible to mention that the rates of the compounds in EO have shown a synergistic effect against *H. pylori*. However, we need further study to test pure components of thymol and carvacrol. In addition, according to study results, the use of carvacrol rich EO or its main components seems to have the potential to be used in combination with treatments potentiating a total eradication (32, 33).

According to the results of the present study, it is suggested that the *T. capitatus* EO could be an alternative for the treatment of *H. pylori* infections. Therefore, further investigations are needed in order to determine the *in vivo* antimicrobial activity against *H. pylori* of the EO obtained from *T. capitatus* growing wild in Northern Cyprus. Also, the limitation of our studies was limited studies with *Thymus spp.* anti-*H. pylori* activity and further studies should be done with pure components of thymol and carvacrol.

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Conflict of interests

The authors declare no conflict of interest.

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