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Chemical composition and antimicrobial effects of *Thymus daenensis* on *Helicobacter pylori*

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ABSTRACT

High occurrence of resistance in the *Helicobacter pylori* strains of human clinical samples caused medical practitioners to found a good alternative therapeutic choice. High phenolic contents of the *Thymus daenensis* essential oil covered its high antimicrobial effects. The present investigation was done to study the chemical composition and anti-*H. pylori* effects of *T. daenensis* essential oil. Aerial parts of the *T. daenensis* were collected from the Yasuj city and transferred to the laboratory. Essential oil was extracted using the Clevenger apparatus. Chemical components of *T. daenensis* was identified using the GC-mass analyzer. Anti-*H. pylori* effects of *T. daenensis* was determined using the disk diffusion method. Twelve (99.24%) chemical components were totally identified in the essential oil of the *T. daenensis*. Thymol (42.81%), gamma-Terpinene (20.39%) and para-Cymene (9.72%) were the most commonly identified chemical components. Distribution of beta-Myrcene, D-Limonene, beta-Pinene and Terpinen-4-ol were moderate. *H. pylori* strains harbored the highest levels of sensitivity against tetracycline and ampicillin antibiotics, while those of resistance were seen for the erythromycin, clarithromycin and metronidazole antibiotics. Inhibition zone diameter increased from 0 to 18.6±0.5 mm which represented dose depended anti-*H. pylori* effects of *T. daenensis* essential oil. Extensive production of *T. daenensis* full from thymol, gamma-Terpinene and para-Cymene will help researchers to formulate an effective antibiotic agent for treatment of the cases of *H. pylori*-infection.

KEY WORDS: *THYMUS DAENENSIS*, CHEMICAL COMPONENTS, ANTIMICROBIAL EFFECTS, *HELICOBACTER PYLORI*

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is an extracellular gram-negative, microaerophilic and spiral bacterium which is known as the causative agent of various types of gastrointestinal diseases and disorders such as gastric adenocarcinoma, gastric ulcer, gastritis, and lymphoma (Shrestha *et al*., 2012). It has been estimated that 30 to 90 percent of hospitalized patients with such gastrointestinal diseases and disorders were infected with *H. pylori* (Shrestha *et al*., 2012; Mastromarino *et al*., 2005; Vu and Ng, 2000). Prevalence of *H. pylori* in in developed and developing countries is about 40% and 90%, respectively (Suerbaum and Michetti, 2002). Gastrointestinal diseases caused by *H. pylori* often treated with antimicrobial agents. Prescription of ampicillin, amoxicillin, clarithromycin, metronidazole and tetracycline antibiotics is the most common way for treatment of the cases of *H. pylori* (Bytzer *et al*., 2011; Rimbara *et al*., 2011; Yang *et al*., 2014). Nevertheless, occurrence of extreme levels of antibiotic resistance in the *H. pylori* strains caused several concerns regarding their treatment (Bytzer *et al*., 2011; Rimbara *et al*., 2011; Yang *et al*., 2014; Ghotaslou *et al*., 2015).

Therefore, medical practitioners and also pharmacological companies have a tendency to found a suitable alternative treatment for *H. pylori*. Medicinal plants may be a powerful sources of antimicrobial agents which can be used for treatment of infectious diseases. The genus Thymus L. goes to the major family (Lamiaceae), and included approximately 215 species in the world. Totally, 14 species of Thymus have been described in the zone of Iran (Stahl-Biskup and Saez, 2002; Rechinger, 1982; Mojab *et al*., 2008; Fachini-Queiro *et al*., 2012). *T. daenensis*, *T. carmanicus*, *T. daenensis*, *T. persicus*, *T. vulgaricus* and *T. trautvetteri* are the most commonly endemic species in Iran (Stahl-Biskup and Saez, 2002; Rechinger, 1982; Mojab *et al*., 2008; Fachini-Queiro *et al*., 2012). *T. daenensis* subsp. daenensis is an endemic subspecies of Iran and especially high altitudes of Zagros mountains (Stahl-Biskup and Saez, 2002; Rechinger, 1982; Mojab *et al*., 2008; Fachini-Queiro *et al*., 2012). Areal parts and volatile constituents of *T. daenensis* are used in Iranian traditional medicine.

Thymus species and especially *T. daenensis* are usually applied as tasty and flavoring agents in tea and also spices and medicinal drives including antimicrobial, anti-fungal, antispasmodic, and anti-inflammatory agent (Stahl-Biskup and Saez, 2002; Rechinger, 1982; Mojab *et al*., 2008; Fachini-Queiro *et al*., 2012; Gautam *et al*., 2014). Previous investigations showed high anti-parasites, antibacterial, antiviral, antifungal, spasmolytic and antioxidant effects of Thymus species (Stahl-Biskup and Saez, 2002; Rechinger, 1982; Mojab *et al*., 2008; Fachini-Queiro *et al*., 2012; Gautam *et al*., 2014).

There were no previously published data on the antimicrobial effects of *Thymus daenensis* on the *H. pylori*. Therefore, the present investigation was carried out to study the chemical composition and antimicrobial effects of the essential oil extracted from the *Thymus daenensis* on ten clinical isolates of *H. pylori*.

MATERIAL AND METHODS

PLANT AND ESSENTIAL OIL EXTRACTION

From July to August 2013, aerial parts of *T. daenensis* at the flowering stage were collected from the plains of the Dena mountain, Yasuj, South-west of Iran. The samples of the plant were identified and a voucher specimen was deposited at the Herbarium of Research Center of Agricultural of Shahrekord city, Iran. Essential oil of the collected *T. daenensis* was extracted by water distillation using the Clevenger apparatus. The essential oil was dried over anhydrous sodium sulfate and stored at 17°C until further analysis.

IDENTIFICATION OF THE CHEMICAL COMPONENTS

Samples were studied by the Gas Chromatography (GC, Agilent 7890 A) and Mass Spectrometry (MS, Agilent 5975 C). Identification of the chemical components was done according to the method described previously by Borugă *et al*. (2014) (Borugă *et al*., 2014). Conditions of the GC stage were as follow: temperature range of 50 to 250°C at 40°C/min with a solvent delay of 5 min. Injector of the device was kept at 250°C. Helium at a flow of 1.0 mL/min was the inert gas, and the injected volume in the splitless mode was 1 µL. The MS conditions were as follow: ionization energy of 70 eV, quadrupole temperature of 100°C, scanning velocity of 1.6 scan/s and weight range of 40-500 amu.

BACTERIAL STRAINS

Ten rough *H. pylori* strains were obtained from the clinical cases of gastrointestinal disorders of Alzahra Hospital, Tehran, Iran. *H. pylori* strains were regularly cultured on Brucella agar (Merck, Germany) supplemented with 5–7% sheep blood, amphotericin (2mg/l), polymixin-B (8mg/l), and vancomycin (6mg/l) and were incubated at 37°C for 3–7 days on the microaerophilic conditions (10% CO2 and 95% humidity). Then, *H. pylori* strains were approved using the morphological, Gram staining and biochemical tests (rapid urease, oxidase and nitrate) and PCR amplification of *16S rRNA*

Table 1. Standard values for determination of sensitivity and resistance of bacteria against antibiotic agents.

Antibiotic agents	Diameter of inhibition (mm)		
	Sensitive	Intermediate	Resistant
Ampicillin (10 µg)	≥17	14-17	≤13
Metronidazole (5 µg)	≥15	12-15	<12
Erythromycin (5 µg)	≥18	14-17	≤12
Clarithromycin (2 µg)	≥18	14-17	≤13
Tetracycline (30 µg)	≥19	15-18	≤14

gene (Forward: 5'-CTGGAGAGACTAAGCCCTCC-3' and Reverse: 5'-ATTACTGACGCTGATTGTGC-3') (110 bp) (Ho *et al*., 1991).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Suspensions of the fresh cultures were made in saline and turbidity was adjusted to 1×10^8 bacteria/ml (corresponding to turbidity with OD 0.8 at 600 nm). Two-hundred microliters of microbial suspension were placed on 50-ml Mueller Hinton agar plates containing 10% fetal calf serum (Sigma, UK) and incubated for 2–5 days at 37°C under microaerophilic conditions. *H. pylori* ATCC 26695 were used as quality control. Tests were done three times. Table 1 shows the standard values for susceptibility determination (CLSI, 2012). The antimicrobial effects of *T. daenensis* essential oil were performed by the disc diffusion method. For this purpose, sterile blank disks (6 mm) were inoculated with concentrations of 0.6, 1.25, 2.5, 5 and 10 (%v/v) essential oil and were placed on dense cultures of bacteria and incubated for 2–5 days at 37°C under microaerophilic conditions. Susceptibility of *H. pylori* strains were also determined against ampicillin (10 µg), metronidazole (5 µg), erythromycin (5 µg), clarithromycin (2 µg) and tetracycline (30 µg) antibiotic agents (Oxoid, UK) (CLSI, 2012).

STATISTICAL ANALYSIS

SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) and one-way analysis of variance (ANOVA) test were used for statistical analysis. P <0.05 was considered as significant difference.

RESULTS AND DISCUSSION

Table 2 represents the chemical components of the essential oil of the *T. daenensis*. Totally, 12 chemical components were identified in the essential oil of the *T. daenensis* (99.24%). Thymol (42.81%), gamma-Terpinene (20.39%) and para-Cymene (9.72%) were the most commonly identified chemical components in the essential oil of the *T. daenensis*. Frequency of beta-Myrcene, D-Limonene, beta-Pinene and Terpinen-4-ol were 3.71%, 3.56%, 3.82% and 3.45%, respectively.

Table 3 represents the inhibition zone diameter for each of the ten clinical isolates of *H. pylori* against antibiotic agents. Results showed that *H. pylori* strains had the highest levels of sensitivity against tetracycline and ampicillin antibiotics. Most resistance was seen for the erythromycin, clarithromycin and metronidazole antibiotics. Statistically significant differences (P <0.05) were seen between the zone of inhibition and types of antibiotics.

Table 2. Chemical components of the essential oil of the *T. daenensis*.

Chemical components	Retention Time (RT) (min)	Frequency (%)
beta-Phellandrene	8.26	1.92
beta-Myrcene	6.97	3.71
alpha-Thujene	5.39	2.94
alpha-Phellandrene	7.53	2.07
D-Limonene	8.04	3.56
alpha-Pinene	5.63	2.12
beta-Pinene	6.89	3.82
Terpinen-4-ol	12.55	3.45
Caryophyllene	17.32	2.71
para-Cymene	8.46	9.74
gamma-Terpinene	8.96	20.39
Thymol	16.17	42.81
Total		99.24

Table 3. Inhibition zone diameter for each of the ten clinical isolates of *H. pylori* against antibiotic agents.

Antibiotic agents	Diameter of the zone of inhibition for H. pylori strains (mm)									
	HP1	HP2	HP3	HP4	HP5	HP6	HP7	HP8	HP9	HP10
Metronidazole (5 µg)	0	0	0	0	0	0	16±0.4	15.4±0.2	0	0
Tetracycline (30 µg)	15.7±0.5	17.5±0.1	17±0.6	17.9±0.5	16±0.6	16.8±0.7	16.5±0.3	14.2±0.1	0	0
Ampicillin (10 µg)	12.1±0.6	0	11.3±0.8	9.1±0.7	0	19.8±0.5	16.2±0.8	0	34.8±0.8	11.7±0.6
Erythromycin (5 µg)	0	0	0	0	0	0	18.3±0.5	0	0	0
Clarithromycin (2 µg)	0	0	0	0	0	0	23.2±0.4	0	0	0

Table 4 represents the inhibition zone diameter of the various concentrations of the *T. daenensis* essential oil against *H. pylori* strains. We found that increase in the concentration of essential oil cause its higher antimicrobial effects on the *H. pylori* strains isolated from the clinical cases of gastrointestinal disorders (P <0.05). Inhibition zone diameter increased from 0 to 18.6±0.5 mm which showed that the anti-*H. pylori* effects of *T. daenensis* essential oil is dose depended.

Failure of majority of therapeutic options and especially ampicillin, amoxicillin, clarithromycin, metronidazole and tetracycline antibiotics caused us to study the antimicrobial effects of *H. pylori* strains against *T. daenensis* essential oil. The results of the present investigation showed that the *T. daenensis* essential oil had a high antimicrobial effects on the *H. pylori* strains isolated from clinical cases of gastrointestinal disorders. Several studies have been approved the anti-*H. pylori* effects of some medicinal plants. Falsafı et al. (2014) (Falsafı *et al*., 2014) reported that *Satureja bachtiarica* essential oil showed strong antibacterial activity against clinical isolates of *H. pylori* (17.6 ± 1.1 mm and 0.035 ± 0.13 µl/ml). They showed that anti-*H.* pylori effects of *S. bachtiarica* is due to its high carvacrol content. Esmaeili et al. (2012) (Esmaeili *et al*., 2012) reported that Shoya powder and essential oils of *Thymus vulgaris* and *Eucalyptus globulus* had significant effects on the *H. pylori*. They showed that Minimum Inhibitory Concentration (MIC) of the *T. vulgaris* and *E. globulus* were 10.8 and 46.4 (µg/ml) respectively.

The antimicrobial effects of the *T. daenensis* essential oil is due to the presence of some active constituents with their hydrophobicity which enables them for rupturing cell membranes and intrastructures. We found that Thymol (42.81%), gamma-Terpinene (20.39%) and para-Cymene (9.72%) were the most commonly identified chemical components in the essential oil of the *T. daenensis*. Previous study in Romania (Grigore *et al*., 2010) showed that the most commonly detected components in the essential oil of the Thymus were p-cymene, γ-terpinene and thymol which was similar to our findings, while those of Morocco (Imelouane *et al*., 2009) and Spain (Ballester-Costa *et al*., 2013) are entirely different with us. P-cymene, γ-terpinene and thymol were the most commonly detected components in previous studies conducted on Iran (Pirbalouti *et al*., 2013), Poland (Kowalski and Wawrzykowski, 2009), Italy (De Lisi *et al*., 2011) and Spain (Rota *et al*., 2008). Probably types of samples, method of sampling, genus of plants, and differences in the climate and weather of various

Table 4. Inhibition zone diameter of the various concentrations of the *T. daenensis* essential oil against *H. pylori*.

Essential oil concentrations	Diameter of the zone of inhibition for H. pylori strains (mm)									
	HP1	HP2	HP3	HP4	HP5	HP6	HP7	HP8	HP9	HP10
0.6%	0	5.8±0.4	6±0.5	0	5.3±0.8	0	0	4.1±0.2	11.2±0.6	5.5±0.2
1.25%	0	5.3±0.9	7.8±0.1	7±0.0	6.7±0.3	0	0	5.2±0.8	14.3±0.4	5.2±0.5
2.5%	4.6±0.3	8.3±0.0	9.7±0.4	10.2±0.5	9.1±0.1	11.4±0.5	8.8±0.2	7.5±0.5	15.5±0.1	8.4±0.3
5%	5.3±0.5	13.1±0.5	13.2±0.1	12.8±0.6	11.6±0.2	12±0.9	14.1±0.1	10.2±0.4	16.7±0.2	11.6±0.4
10%	6.2±0.7	15.7±0.2	13.6±0.2	14.3±0.4	14±0.5	17.1±0.2	16±0.3	12.3±0.4	18.6±0.5	13.2±0.8

geographical regions are the main factors which may affected on the chemical components of Thymus plants of different studies.

Antimicrobial effects of *T. daenensis* essential oil is depends on its chemical compositions. It seems that high content of the phenolic compounds (thymol) and terpene hydrocarbons (γ-terpinene) is the main factor for the high anti-*H. pylori* effects of *T. daenensis*. Previous investigations showed that para-cymene does not display any antimicrobial activities alone (Dorman and Deans, 2000), while it has a strong antibacterial effects in relation with thymol and γ-terpinene (Delgado *et al*., 2004; Gallucci *et al*., 2009).

CONCLUSION

In conclusion, we found that *T. daenensis* essential oil has a high antimicrobial effects on the H. pylori strains of human clinical infections. As it showed, Thymol, gamma-Terpinene and para-Cymene were the most commonly identified chemical components in the essential oil of the *T. daenensis*. In compare with the findings of other researchers, Preparation of good conditions for growth of *T. daenensis* full from these chemical components will help us to present an efficient antibiotic agent for treatment of the cases of *H. pylori*-infection.

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REFERENCES

Ballester-Costa, C., Sendra, E., Fernández-López, J., Pérez-Álvarez, J.A. and Viuda-Martos, M. (2013). Chemical composition and in vitro antibacterial properties of essential oils of four Thymus species from organic growth. Industrial Crops and Products, Vol. 50, pp. 304–311.

Borugă, O., Jianu, C., Mişcă, C., Goleţ, I., Gruia, A.T. and Horhat, F.G. (2014). Thymus vulgaris essential oil: chemical composition and antimicrobial activity. Journal of Medicice and Life, Vol. 7, No. 3, pp. 56-60.

Bytzer, P., Dahlerup, J.F., Eriksen, J.R., Jarbøl, D.E., Rosenstock, S. and Wildt, S. (2011). Diagnosis and treatment of Helicobacter pylori infection. Danish Medical Bulletin, Vol. 58, No. 4, pp. C4271.

Clinical and Laboratory Standards Institute (CLSI). (2012). Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement M100-S21, Wayne Pa.

De Lisi, A., Tedone, L., Montesano, V., Sarli, G. and Negro, D. (2011). Chemical characterization of *Thymus* populations belonging from Southern Italy. Food Chemistry, Vol. 125, No. 4, pp. 1284–1286.

Delgado, B., Fernández, P.S., Palop, A. and Periago, P.M. (2004). Effect of thymol and cymene on *Bacillus cereus* vegetative cells evaluated through the use of frequency distributions. Food Microbiology, Vol. 21, No. 3, pp. 327–334.

Dorman, H.J.D. and Deans, S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, Vol. 88, No. 2, pp. 308–316.

Esmaeili, D., Mohabati Mobarez, A. and Tohidpour, A. (2012). Anti-Helicobacter Pylori Activities of Shoya Powder and Essential Oils of *Thymus vulgaris and Eucalyptus globus*. Oepn Microbiology Journal, Vol. 6, pp. 65-69.

Fachini-Queiroz, F.C., Kummer, R., Estevão-Silva, C.F., Carvalho, M.D., Cunha, J.M., Grespan, R., Bersani-Amado, C.A. and Cuman, R.K. (2012). Effects of Thymol and Carvacrol, Constituents of *Thymus vulgaris* L. Essential Oil, on the Inflammatory Response. Evidence Based Complementary and Alternative Medicine, Vol. 2012, pp. 657026.

Falsafı, T., Moradi, P., Mahboubi, M., Rahimi, E., Momtaz, H., Hamedi, B. (2014). Chemical composition and anti-Helicobacter pylori effects of *Satureja bachtiarica* bunge essential oil. Phytomedicine, Vol. 22, No. 1, pp. 173–177.

Gallucci, M.N., Oliva, M., Casero, C., Dambolena, J., Luna, A., Zygadlo, J. and Demo, M. (2009). Antimicrobial combined action of terpenes against the food-borne microorganisms *Escherichia coli, Staphylococcus aureus and Bacillus cereus*. Flavour and Fragrance Journal, Vol. 24, No. 6, pp. 348–354.

Gautam, N., Mantha, A.K. and Mittal, S. (2014). Essential oils and their constituents as anticancer agents: a mechanistic view. Biomed Research International, Vol. 2014, p. 154106.

Ghotaslou, R., Leylabadlo, H.E. and Asl, Y.M. (2015). Prevalence of antibiotic resistance in *Helicobacter pylori*: A recent literature review. World Journal of Methodology, Vol. 5, No. 3, pp. 164-74.

Grigore, A., Paraschiv, I., Colceru-Mihul, S., Bubueanu, C., Draghici, E. and Ichim, M. (2010). Chemical composition and antioxidant activity of Thymus vulgaris L. volatile oil obtained by two different methods. Romanian Biotechnological Letters, Vol. 15, No. 3, pp. 5436–5443.

Ho, S.A., Hoyle, J.A. and Lewis, F.A. (1991). Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. Journal of Clinical Microbiology, Vol. 29, No. 11, pp. 2543-2549.

Imelouane, B., Amhamdi, H., Wathelet, J.P., Ankit, M., Khedid, K. and El Bachiri, A. (2009). Chemical Composition and Antimicrobial Activity of Essential Oil of Thyme (*Thymus vulgaris*) from Eastern Morocco. International Journal of Agricultural Biology, Vol. 11, No. 2, pp. 205–208.

Kowalski, R. and Wawrzykowski, J. (2009). Essential oils analysis in dried materials and granulates obtained from *Thymus vulgaris L., Salvia officinalis L., Mentha piperita L. and Cham-*

omilla recutita L. Flavour and Fragrance Journal, Vol. 24, No. 1, pp. 31–35.

Mastromarino, P., Conti, C., Donato, K., Strappini, P.M., Cattaruzza, M.S. and Orsi, G.B. (2005). Does hospital work constitute a risk factor for *Helicobacter pylori* infection. Journal of Hospital Infections, Vol. 60, No. 3, pp. 261-268.

Mojab, F., Poursaeed, M., Mehrgan, H. and Pakdaman, S. (2008). Antibacterial activity of *Thymus daenensis* methanolic extract. Pakistan Journal of Pharmaceutical Science, Vol. 21, No. 3, pp. 210-3.

Pirbalouti, A.G., Hashemi, M. and Ghahfarokhi, F.T. (2013). Essential oil and chemical compositions of wild and cultivated *Thymus daenensis Celak and Thymus vulgaris* L. Industrial Crops and Products, Vol. 48, pp. 43–48.

Rechinger, K.H. (1982). Flora Iranica. Eriocaulaceae. Lfg. No. 89, Vol 152. Austria, Graz, Akademische Druck und Verlagsanstalt.

Rimbara, E., Fischbach, L.A. and Graham, D.Y. (2011). Optimal therapy for *Helicobacter pylori* infections. Nature Review Gastroenterology and Hepatology, Vol. 8, No. 2, pp. 79-88.

Rota, M.C., Herrera, A., Martínez, R.M., Sotomayor, J.A. and Jordán, M.J. (2008). Antimicrobial activity and chemical composition of Thymus vulgaris, *Thymus zygis and Thymus hyemalis* essential oils. Food Control, Vol. 19, No. 7, pp. 681–687.

Shrestha, S., Paudel, P., Pradhan, G.B., Shrestha, L. and Bhattachan, C.L. (2012). Prevalence study of *H. pylori* infection in dyspeptic patients coming to Nepal Medical College Teaching Hospital, Jorpati, Kathmandu. Nepal Medical Collection Journal, Vol. 14, No. 3, pp. 229-233.

Stahl-Biskup, E. and Saez, F. (2002). Thyme, The genus Thymus, Taylor and Francis, p. 331

Suerbaum, S. and Michetti, P. (2002). Helicobacter pylori infection. New England Journal of Medicine, Vol. 347, pp. 1175-86.

Vu, C. and Ng, Y.Y. (2000). Prevalence of *Helicobacter pylori* in peptic ulcer disease in a Singapore hospital. Singapore Medical Journal, Vol. 41, No. 10, pp. 478-481.

Yang, J.C., Lu, C.W. and Lin, C.J. (2014). Treatment of *Helicobacter pylori* infection: current status and future concepts. World J Gastroenterology, Vol. 20, No. 18, pp. 5283-93.