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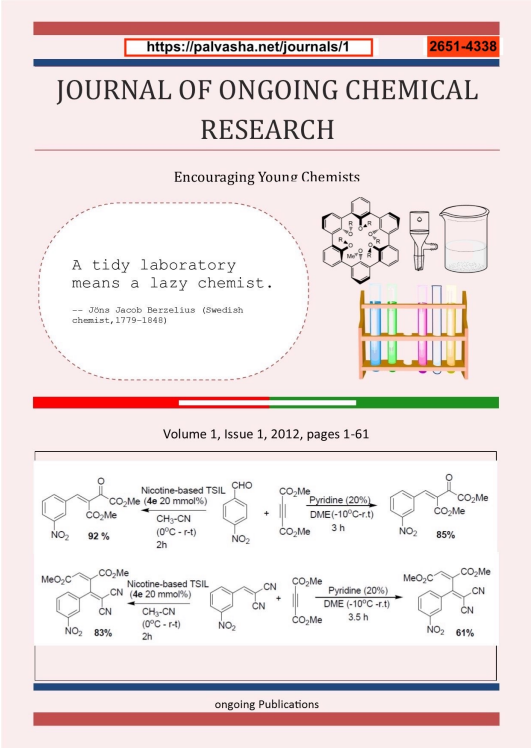

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Research Article

Antimicrobial And Urease Inhibition Activity Of Volatile Oil Obtained From Aerial Parts Of Thyme (*Thymus Vulgaris* L.)

Elif Ayşe Erdoğan Eliuz*, Yusuf Sıcak,
For affiliations and correspondence, see the last page.

Abstract

The demand for *T. vulgaris* L. (Thyme) and its derivates has became popular since it has many biologically active components such as mainly antimicrobial, antiinflammatory, antioxidant characteristics. Especially, there were many researches on the antimicrobial performance of *T. vulgaris* L. essential oil. In this study, we analyzed the content of the essential oil of *T. vulgaris* aerial parts by using GCMS, its antimicrobial activity to some pathogens and urease inhibition activity. The major components of the oil in thyme were thymol (48.80%), *γ*-terpinene (15.26%), *p*-cymene (10.35%), linalol (3.87%), *β*-myrcene (3.20%). Then, The minimum inhibitory concentration (MIC) were determined against *Escherichia coli* (ATCC 25293), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25925), *Pseudomonas aureginosa*, *Candida albicans* and *Candida parapsilosis* by using spectrophotometric microbroth technique. We showed that microoganisms were inhibited by the essential oil extracted thyme aerial parts at the end of the 24 hours incubation. Highest inhibition were reported against *S. aureus* (MIC=7.5 µg/mL), while lowest inhibition were found to be *C. albicans* (MIC=105.3 µg/mL). Also, the antiurease activities were tested against urease enzymes such as thiourea, spectrophotometrically. The essential oil (27.40±0.46%) of thyme showed higher urease inhibition activity than thiourea (23.08±0.19%). Consequently, it clearly suggested that the essential oil from *T. vulgaris* aerial parts are a potential source of antimicrobial and urease inhibitory ingredients for the drug and food industry.

Keywords: Chemical composition, Antimicrobial activity, Thymus vulgaris, Urease inhibition activity, Volatile oil,

INTRODUCTION

The thyme plant, which has been used in the treatment of infectious diseases and pain since the ancient centuries, maintains its importance in the same way today. In our country, *Thymus*, *Origanum*, *Satureja*, *Thymbra* and *Coridothymus* species belonging to the family of Lamiaceae are called thyme, depending on the components of timol/karvakrol in the content. *T. vulgaris* L., which we evaluate in our study, is a species of *Thymus*, one of the most important genus of Lamiaceae family [Baser et al, 1993; Reddy et al, 1998; Panizzi et al, 1993]. Preferably, it is an ornamental plant growing in lime soil and spread to Mediterranean countries. It is a plant with intense aroma that opens its flowers in different colors ranging from pink to purple in June to August. Many studies on biological activities antiseptic, antispasmodic, antimicrobial and antioxidant of *T. vulgaris* were reported [Marino et al, 1999; PinaVaz et al, 2004]. Therefore, it is found among the best known medicinal plants and especially, its essential oil have important substances of industrial food, pharmaceutical and cosmetic industry [Nikolic et al, 2014].

In the present study, it is aimed to report antimicrobial and antiurease activity of the essential oil from aerial parts of thyme and compare with the similar studies.

RESULTS AND DISCUSSION

Chemical Composition

The results of the chemical composition of the essential oil of *T. vulgaris* were presented in Table 1. In the present study, thymol (48.80%), γ-terpinene (15.26%), *p*-cymene (10.35%) were the main component in the essential oil of aerial parts of thyme, followed by linalol (3.87%), *β*-myrcene (3.20%), α-thujene (2.87%), α-terpinene (2.77%), α-pinene (2.63%), carvacrol (2.14%) less amounts than 2.0% with camphene, β-pinene, sabinene, 3-carene, α-phellandrene, 1,8 cineole, limonene, terpinen-4-ol, verbenol, *cis*-sabinene, α-terpineol, β-caryophyllene, β-copaene and α-cadinene.

Table 1. Essential oil composition of *T. vulgaris*

RT (min)	Component	Quantity (%)	RT (min)	Component	Quantity (%)
8.576	*α*-Pinene	2.63	27.364	1,8-Cineole	1.25
9.715	*α*-Thujene	2.87	28.421	Limonene	1.11
9.128	Camphene	1.05	30.055	Linalool	3.87
10.369	*β*-Pinene	0.70	31.283	Terpinen-4-ol	0.80
12.403	Sabinene	0.55	33.617	Verbenol	0.41
13.054	3-Carene	1.90	33.976	*cis*-sabinene	0.93
15.831	*β*- Myrcene	3.20	36.066	*α*-Terpineol	0.36
17.609	*α*-Terpinene	2.77	36.846	Thymol	48.80
21.168	*α*-Phellandrene	0.88	37.162	Carvacrol	2.14
22.645	*γ*-Terpinene	15.26	38.040	*β*-Caryophyllene	1.47
24.190	*p*-Cymene	10.35	39.252	*β*-Copaene	0.42
25.314	Terpinolene	0.87	40.326	*α*-Candinene	0.33

RT: Retention Time

Many studies about the major elements of essential oil of *T. vulgaris* were reported earlier. For instance, Piccaglia et al, 1991[], were noted that main components in oil of thyme were linalool, thymol, geraniol, γ- terpineol, carvacrol, trans-thujan-4-ol/terpinen-4-ol. In another study, thymol, alpha terpinene, p-cymene were abundant in oil [Ozcan et al, 2004]. The major components determined were 1,8-cineole, terpenyl acetate, borneol, linalool, beta pinene, alphaterpineol and camphor [Jordan et al, 2006]. Many factors such as morphological, ecological and genetic diversity may be cause these differences in chemical composition of oil Li et al, 2015; Figueiredo et al, 2008].

Imelouene et al. (2009) reported that the main components of the essential oil extracted from aerial parts of *T. vulgare* were camphor (38.54%), camphene (17.19%), α-pinene (9.35%), 1, 8-cineole (5.44%), borneol (4.91%) and *β*-pinene (3.90%) [Imelouane et al, 2009]. In contrast, we determined the main components of the oil were to be thymol (48.80%), γ-terpinene (15.26%), *p*-cymene (10.35%). Although our study is different from the work of Imelouene and coworkers, it is similar to the work of the Sartoratto and coworkers. They were noted thymol (79.15%), *p*-cymene (3.27%), γ-terpinene (2.57%) were the major component of the oil [Sartoratto et al, 2004]. Furthermore, Hudaib and coworkers (2002) defined that thyme collected in June and July (young plant) have the highest % content of the thymol (51.2%) and carvacrol (4%) [Hudaib et al, 2002].

Antimicrobial Activity

The antimicrobial activity of essential oil of *T. vulgaris* were showed in Figure 1. Ampiciline and fluconazole antibiotics were performed as positive reference standards for bacteria and yeasts, respectively. These standards inhibited microorganisms at all concentration more than 0.48 µl/ml. The thyme volatile oil showed stronger activity against Gram-positive (*S. aureus* and *B. subtilis*) than Gram-negative bacteria (*P. aureginosa* and *E. coli*) and yeasts (*C. albicans* and *C. parapsilosis*). The high MIC values were found to be 7.5 µg/mL for *S. aureus* and 10.01 µg/mL for *B. subtilis*. MIC values of thyme oil were also exhibited to be 33.6 µg/mL for *E. coli,* 64.1 µg/mL for *P. aureginosa*, 22.5 µg/ml for *C. parapsilosis*, 105.3 µg/mL for *C.albicans.*

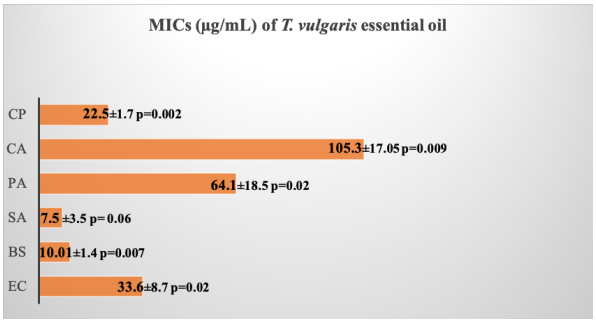

Figure 1: Statistical analysis of MICs value of *T. vulgaris*against *E. coli*, *B. subtilis*,*S. aureus*(ATCC 25925), *P. aureginosa*,*C. albicans*and *C.parapsilosis*for 24 hours. The average MIC (µg/mL)values were expressed with the standard deviation (SD±) and significance level (p).

Imelouene and coworkers (2009), MICs of essential oil of thyme were calculated between 0.33 and 1.33 mg/mL against *E. coli* and *S. aureus* [Imelouane et al, 2009]. Sartorotto and coworkers (2004) noted that *S. aureus, B. subtilis* and *C. albicans* were sensitive to *T. vulgaris* essential oil at MIC of 1.00 mg/mL, 0.49 mg/mL and 2.00 mg/mL [Sartoratto et al, 2004]. *E. coli* were rather sensitive (100%) to methanol extract of the thyme aerial parts [Alanis et al, 2005]. *S. aureus* and *E. coli* showed moderate susceptibility towards the essential oil of *T. vulgaris* with MIC values of 31.2 and 62.5 µg/mL, respectively [AlBayati, 2008]. Studies have confirmed that *T. vulgaris* inhibits microorganisms in moderately and that they are a more powerful antimicrobial agent of the subspecies whose content is rich in carvacrol and thymol [Dorman et al, 2000,Cosentino et al, 1999].

Urease Inhibition Activity

The available study was noted to determine urease inhibition properties of essential oil from *T. vulgaris* aerial parts. IC_{50} values of urease inhibition of the oil were found as 27.40 ± 0.46 µg/mL while IC_{50} values of anti-urease of thiourea which is standard of urease inhibition were detected as 23.08 ± 0.19 µg/mL.

Table 2. Urease inhibitory activity of essential oil extracted from *T. vulgaris* aerial parts.

Compound	**Urease Inhibitory Activity**
	IC_{50} (µg/mL)
***T. vulgaris* essential oil**	27.40±0.46
***Thiourea*[b]**	23.08±0.19

[a]Value represent the means ± standard deviation of three parallel measurements (p<0.05)

[b]Reference compound

There is little study about urease inhibition activity of *T. vulgaris* essential oil. Esmaeili and coworkers [Esmaeili et al, 2012] were researched that antimicrobial activity on *Helicobacter pylori* than EO from *T. vulgaris* by rapid rrease broth test. They obtained that *T. vulgaris* significantly inhibited *H. pylori* with MIC of 42.4 µg/mL. In another study, urease inhibitory activity of *Thymus kotschyanus* extract at concentration of 10 mg/ml were found to be 17.94% on *H. pylori* [Nabati et al, 2012].

In sum, we researched the antimicrobial activity and, for the first time urease inhibitory activity of essential oil extracted from *T. vulgaris* aerial parts. The oil, rich in thymol, *γ*-terpinene and *p*-cymene, were detected to have high antimicrobial performance against

pathogens. It was reported remarkably urease inhibitory activity. Hence, further research is needed on *T. vulgaris* urease inhibitory activity.

EXPERIMENTAL

Chemicals and spectral measurements:

All chemicals and solvents obtained from E. Merck (Darmstadt, Germany), FlukaChemie (FlukaChemie GmbH, Sternheim, Germany, Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany).

Plant material: *T. vulgaris* were collected from Köyceğiz region of Muğla, Turkey, during June-July 2017, identified at the Herbarium of Biology, Faculty of Science, Muğla Sıtkı Koçman University, Turkey. The plant sample was confirmed by comparing it with the specimen voucher located at the stated herbarium.

Preparation of the extraction of essential oil: Approximately 200 g of *T. vulgaris* samples were used for the essential oil extraction process. Extraction was performed by vapor distillation for 2 hours. The mixture added to water. After liqiud-liqiud extraction, the aqua in organic phase was dried over anhydrous Na_2SO_4. Organic phase was then concentrated under vacuum. Obtained essential oil was kept in desiccator. It was protected from sunlight until analysis.

Antimicrobial screening

Antimicrobial activity of the volatile oils of thyme aerial parts were researched on several pathogens, known as *E. coli* (ATCC 25293), *P. aureginosa*, *B. subtilis* (ATCC 6633), *S. aureus* (ATCC 25925), *C. albicans* and *C. parapsilosis* using modified spectrophotometric microdilution technique. Firstly, the bacterium inoculum was prepared in 4 mL-Triptic Soy Broth medium and incubated at 37°C, overnight. After 24 hours, the cultures in MHB (Mueller Hinton Broth) were adjusted to 0.5 McFarland Standard Turbidity (~ 104) and stored at +4°C until use.

The 50 µL (37.5 mg) of *T. vulgaris* oil were dissolved in dimethyl sulfoxide (10% DMSO, 10 ml). The experiments were performed on 96-well microtiter plates and firstly 50 µL of MHB medium were added into all wells. Two-fold serial dilutions of 50 µL oil was made on all x-axis along of elisa plate. Columns 11 and 12 were used as negative and positive controls (ampiciline for bacteria and fluconazole for yeast), respectively. Finally, 10 µL culture of microorganisms was inoculated on all wells except medium control wells. The plate was incubated at 37°C for 24 hours, the growth (turbidity) was measured at 600 nm and 415 nm for bacteria and yeasts, respectively. For MIC analysis, the optical density was read both before (T0) and after 24 hours-incubation (T24). The OD (Optical density) for each replicate at T0 was subtracted from the OD for each replicate at T24. For each microorganism were calculated using the following formula:

The Percent growth (Cell viability) = (ODtest /OD control)x100.

Percent Inhibition = 1-(OD test well/OD of corresponding control well)x100.

The dose-response curves obtained from plotting the linear of the concentration of the oils against the resulting percent inhibition of microbial growth were obtained with the regression analysis, giving an R^2 value. MIC (the lowest concentration of test material which results in 99.9% inhibition of growth) were calculated using the R^2 formula on inhibition curve [Patton et al, 2006; McFarland, 1987].

Urease Inhibition Activity Assay

Solutions of essential oil of *T. vulgaris* were prepared at four different concentrations as 250-125-62,5-31,25 µg/ml for urease inhibitory assay in EtOH. EtOH was used as a control, while thiourea were used as urease standard for comparison of the activity tests. The results were given as 50% concentration (IC_{50}) for urease inhibitory and tyrosinase inhibitory activities assay.

The spectrophotometric analysis of urease inhibitory activity were performed according to the literature procedures as follows: by measuring ammonia production using the indophenol method as described by [Khatib et al, 2005].

Statistical analysis

All data on biological activity assay studies were the averages of triplicate analyses. All biological activity assays were carried out at four concentrations, and the results are presented as 50% concentration (IC_{50}) (%). Data were recorded as mean ± SEM (standard error of the mean). Significant differences between means were determined by Student's-*t* test and *p* values <0.05 were regarded as significant.

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Corresponding: Elif Ayşe Erdoğan Eliuz
Email: eliferdogan81@mersin.edu.tr
Phone: +90 364 361 6772
Fax:

Elif Ayşe Erdoğan Eliuz: Department of Food Technology, Technical Sciences Vocational School, Mersin University, Mersin-33343, MERSİN Turkey

Yusuf Sıcak: Department of Aromatic and Medicinal Plants, Köyceyiz Vocational School, Muğla Sıtkı Koçman University, Muğla-48000, MUĞLA Turkey