Sakarya University Journal of Science 26(4), 820-828, 2022

An Investigation of the Correlation of Antibacterial Activity of Thyme (*Thymus vulgaris* L.) with its Nutrient Elements

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Abstract

The present study aimed to examine the correlation of plant nutrient elements with the antibacterial activity of methanol extract of *Thymus vulgaris* L. grown on soil from the experimental field without application of fertilizers or pesticides on two Gram-positive and two Gram-negative bacteria. The extract of thyme was obtained by maceration of aerial parts of the plant using methanol as the solvent. The contents of macro (P, K, Ca, and Mg) and micro (Fe, Cu, Zn, Mn, and B) nutrients were detected by ICP-OES. The results showed that the macro nutrients were compatible with herbaceous perennial plants, while Fe, Mn and B micro nutrients were high. The methanol extract was significantly effective on inhibiting *P. aeruginosa*, *S. aureus* and *E. faecalis*, while did not inhibit *E. coli*. The correlation of K and Mg was significantly positive with the growth inhibition of *P. aeruginosa* suggesting that higher K and Mg contents of the plant would result in higher antibacterial activity against P. aeruginosa, most probably up to a point. On the other hand, significant positive correlation of B was found with the inhibition of both Gram-positive bacteria. In addition, the positive correlation of Ca with the growth inhibition of *S. aureus* was highly significant. The importance of nutrient contents in medicinal aromatic plants such as thyme was shown with this study. The results therefore imply the conscious and sustainable agriculture of medicinal aromatic plants is crucial for the antibacterial activity of thyme.

Keywords: *Thymus vulgaris* L., antibacterial activity, macro and micro nutrient elements, correlation

1. INTRODUCTION

Around one third of the flora in Turkey consists of medicinal aromatic plants [1]. Among them the third largest plant family with 550 species is the *Lamiacaea* family [2]. *Thymus vulgaris* L, known as thyme, is a flowering plant species of this family. Although this particular species is not naturally found in the flora of Turkey, it has been cultivated intensively in Turkey, so that Turkey is the leading country for thyme production and export. Thyme production in Turkey is increasing year by year, with an average quinquennial

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production of around 19 thousand ton/year [3]. There was a light decrease in 2021 compared to the previous year most probably due to decrease in demand in the market as a result of the pandemics.

The plant has a high drought tolerant and it can be cultivated in almost all soil types [4]. Thyme has a wide range of area of utilization. Pharmaceutical industry takes advantage of this medicinal aromatic plant in many applications due to its antimicrobial, uretic, blood glucose regulator, anti-inflammatory aspects. It is also a source for cosmetics such as soaps, detergents, creams, lotions, tooth pastes and mouth washes [5]. Thyme extracts have even been studied for its anti-anxiety effects through oral applications in rats [6]. Recently, thyme has been the focus of interest in agricultural applications. Thyme extract was tried on tomato wilt causing bacteria and promising results were obtained in order to use this herbal extract for field applications [7]. Similarly, thyme extract was shown to be utilized for crop protection of zucchini an alternative to chemical pesticides. The field trials revealed that thyme extract could be used as phyto-stimulant and plant strengthening hence increased crop yield [8].

The active phytochemicals in the essential oil of *Thymus vulgaris* L. are responsible for the medicinal aspects. The essential oil amount in *Thymus vulgaris* L. is 1.09-2.67%, which increases under drought as opposed to the crop yield [5]. The main components of the essential oil are thymol (40-70%), p-cymene, γ-terpinene, carvacrol, β-caryophyllene and α-terpinene, as the most abundant thymol is responsible for the characteristic smell of the plant [9].

Organic and clean agriculture is crucial for sustainable production of thyme, which is a very economically important plant for Turkey [5]. Moreover, all around the world, this plant matters as a medicinal aromatic plant, especially due to practical use in daily life for its antimicrobial activity. The nutrient contents of the agricultural crops depend on the agricultural implementations [10, 11]. The macro and micro nutrient elements are vital for growth, development and metabolic reaction of plants. The secondary metabolites are also dependent on the nutrient element contents. The nutrient change may considerably affect the antimicrobial activities of medicinal aromatic plants [12]. To the best of our knowledge, there is lack of information on the correlation of nutrient content and microbial inhibitory effect of *Thymus vulgaris* L. In order to sustain more conscious cultivation of plants with high medicinal and economical importance, information on medicinal aspects of such plants and their cultivation should be gathered and integrated. That is why, we have investigated the correlation of antibacterial activity of aerial parts of thyme with its nutrient elements in this study.

2. MATERIALS AND METHODS

The plant material used in this study was *Thymus vulgaris* L. grown under controlled lab conditions. The seeds purchased from a local seed supplier were germinated in peat (Klasmann Deilmann, Potground H). The specifications of the peat were provided elsewhere [13]. The seedlings were transferred to a 5L pot filled with soil acquired from the trial fields of Faculty of Agriculture, Namık Kemal University. Three seedlings were planted in the pot. The soil characteristics were as follows: The pH of the soil was 6.45, the content of available P_2O_5 was 36.2 mg/kg and exchangeable K_2O 225.18 mg/kg. Moreover, the organic matter concentration was 1.91% [12, 14]. No fertilizers and pesticides were used during the experiment. The plants were regularly watered and harvested after two months of period before the plants started to flower, the aerial parts were sequentially air dried and kept at 65 °C for 48 h in drying oven. The dried plant material was grinded and used to analyze the elemental content and the herbal extraction. The elemental content analyses were carried out in Namık Kemal University Central Lab (NABILTEM) on the dried plant material using ICP-OES (Inductively coupled plasma atomic emission spectroscopy).

In order to obtain herbal extraction, maceration was carried out with methanol as the solvent at room temperature. Grinded aerial parts of thyme were mixed with methanol (99.9%) (Sigma Aldrich) as 1:20 w/v. The mixture was kept at

room temperature for 3 days, filtered through filter paper, and the solvent was evaporated. The residual material after evaporation was dissolved in dimethyl sulfoxide DSMO (Thermo Fisher) and used for antibacterial activity test, as DMSO is known to have no antibacterial activity on the tested bacteria.

Agar well diffusion method was adopted to investigate the antibacterial activity of herbal extracts of *Thymus vulgaris* L. Mueller Hinton agar plates were prepared by autoclaving the agar medium at 121 ℃ for 20 minutes. All the equipment were also sterilized with autoclaving and the experiments were carried out under aseptic conditions. Two Gram positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212) and two Gram negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC27853) were tested for the antibacterial activity of the thyme in this study. They were first grown on Columbia blood agar containing 5% sheep blood (Biomerieux). Next, for each bacterium, bacterial solution of 0.5 McFarland was prepared using sterile saline solution. The bacterial solutions were inoculated on the surface of Mueller Hinton agar plates (Himedia) with the help of sterile swab. Wells of 6 mm diameter were formed using sterile pipette tips and 50 µL of thyme extract was added to the wells. The plates were kept at 37 ℃ for 24 hours, after which the clearance around the wells were measured in mm and compared with the inhibition zone of 10 µg Gentamicin sulfate (Sigma Aldrich), as a positive control. The experiment was conducted with three replicates.

The basic statistics of the data were calculated using Minitab 13® and analyzed with Pearson Correlation.

3. RESULTS AND DISCUSSION

We studied the correlation of some nutrient elements of aerial parts of lab-grown thyme (*Thymus vulgaris* L.) with the antibacterial activity of its methanol extract on 4 different bacteria. The aboveground parts of the plant were dried as a whole and grinded to be used for macro and micro nutrient element determination and to obtain herbal extraction. The amounts of some of the macro and micro nutrient elements determined in thyme used in this study are given in Table 1.

Whole plants were dried and grinded properly, and some micro and macro nutrient elemental contents were determined with ICP-OES. The amounts of the micro and macro elements determined in basil plants used in this study is given in Table 1.

Table 1
Some macro and micro nutrient elements of *Thymus vulgaris* L.

	Nutrient Elements	**Amount**	**Limit values** (Mills and Jones 1996)**.**
Macro nutrient elements	P (%)	0.323 ± 0.003	0.25-0.29
	K (%)	2.515 ± 0.021	2.17-3.15
	Ca (%)	1.482 ± 0.306	0.50-1.25
	Mg (%)	0.414 ± 0.0872	0.29-0.40
Micro nutrient elements	Fe (mg kg^{-1})	585.00 ± 6.80	85-118
	Cu (mg kg^{-1})	1.27 ± 0.0153	6-9
	Zn (mg kg^{-1})	23.80 ± 0.377	68-99
	Mn (mg kg^{-1})	117.73 ± 2.15	38-98

B (mg kg^{-1})	34.63± 0.240	17-28

elements for the plants [18]The higher Mn and B levels in plant can be explained by this situation.

Table 2
Correlation between macro and micro nutrient elements of *Thymus vulgaris* L.

	P	**K**	**Ca**	**Mg**	**Fe**	**Cu**	**Zn**	**Mn**	**B**
P	1								
K	.992*	1							
Ca	.971	.934	1						
Mg	.978	.996*	.900	1					
Fe	1.00**	.990*	.975	.975	1				
Cu	.984	.954	.998**	.925	.986	1			
Zn	.910	.954	.785	.977	.904	.821	1		
Mn	1.00**	.995*	.965	.983	.999**	.979	.920	1	
B	.881	.816	.968	.763	.888	.952	.606	.870	1

The macro nutrient elements of the thyme grown in lab conditions on the soil obtained from the experimental fields of the university were very closed to the expected values given by Mills and Jones [15]. However, the situation for micro nutrient elements differs. While Cu is lower than the expected, iron is very high than the limit values. Iron is a very abundant element found in the earth crust, but not available for plants in alkaline and neutral soils [16]. The soil in Thrace Region of Turkey, where the university resides, is, however, usually acidic soil [17]. The soil in this study was also slightly acidic with pH 6.4 [14]. The acidity in soil increases the plant availability of the micro nutrient elements mentioned in this study.

Moreover, the concentrations of micro nutrient elements affect the availability of other nutrient

Moreover, a higher B level might be expectable since Turkey has the greatest reserves for boron, high boron concentrations in soil might be expected all around soil in Turkey [19]. The significant positive correlations of Fe and Mn, which are highly abundant in thyme in this study, with the other macro and micro elements can also be explained by this phenomenon (Table 2).

The methanol extract of thyme obtained by maceration was tested on two Gram-negative and two Gram-positive bacteria with agar well diffusion method (Figure 1). The cell wall differences between Gram-positive and Gram-negative bacteria may result in varying reactions of bacteria to different agents [20]. The results of this study were given in Table 3. When compared with the activity of a standard antibiotic

(Gentamicin), the extracts were most effective on the Gram- positive *E. faecalis* and the Gram-negative *P. aeruginosa*.

Figure 1 Examples from antibacterial activity testing agar

Table 3
Antibacterial effect of *Thymus vulgaris* L. on different bacterial strains

Bacterium	Inhibition zone (mm)	Zone of Gentamicin	Inhibition degree
S. aureus	11.67±1.53	23	++
E. faecalis	12.67±0.58	19	+++
P. aeruginosa	11.34±1.53	16	+++
E. coli	6.0 ± 0.00	18	+

++++ Excellent activity (100% inhibition), +++ Good activity (60-70% inhibition), ++ Significant activity (30-50% inhibition), + Negligible activity (10-20% inhibition), - no activity (<10% inhibition), Gentamicin: standard antibiotic

As a generalized result, a higher antibacterial activity of thyme extract on Gram-positive bacteria might be mentioned. The activity of the herbal extract on *E. coli* was negligible in this study. The two-layered cell wall of Gram-negative bacteria are usually responsible more resistance against antibacterial agents [21, 22]. However, a study on antibacterial activities of different plant extracts showed that E. coli was susceptible to herbal extracts [23]. Our previous results with another medicinal aromatic plant basil revealed a higher antibacterial activity against Gram-positive bacteria [12, 13]. Other studies also showed that thyme extract was highly effective on a Gram-positive bacterium *Clavibacter michiganensis* subsp. *michiganensis* which was the factor causing tomato wilt disease [7].

Methanol was found to be the one of the two best solvents among 7 other solvents for herbal extraction of thyme [24]. In the mentioned study, the researchers investigated the antibacterial activity of extracts with different solvents on a Gram-positive bacterium *Bacillus subtilis* and suggested to use either methanol or water + Tween 20 to obtain herbal extract. Methanol extraction was also chosen for basil and shown to degrade cell wall of bacteria, therefore can act better on Gram-positive bacteria whose cell wall consist of only peptidoglycan layer [25].

Table 4
Pearson's correlation coefficients between the antimicrobial activity and macro and micro nutrient elements of *Thymus vulgaris* L.

	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B
S. aureus	.984	.954	.998**	.925	.986	1.000	.821	.979	0.99**
E. faecalis	.871	.804	.963	.749	.878	.945	.589	.859	1.00**
P. aeruginosa	.980	.997**	.904	1.000***	.997	.929	.974	.984	.769
E. coli	-	-	-	-	-	-	-	-	-

* = P < 0.1; ** = P < 0.05; ***= P < 0.01

The correlations of plant macro and micro nutrient elements with the antibacterial activity of the methanol extract were given in Table 4. The values used for antibacterial activity are inhibition zone diameters. Therefore, the higher the inhibition zone, the stronger the antibacterial effect of the extract. The antibacterial activity on *E. coli* was negligible, therefore, the correlation was alike. The significant positive correlation of Ca and B on Gram-positive bacteria are remarkable. The cell wall of these bacteria has high affinity for Ca and Mg, which destabilize the cell wall and thus kill the bacteria [26, 27]. Therefore, the plants grown in soils with similar

characteristics to the one used in this study might be suggested for higher antibacterial activity against especially *S. aureus*, which is very crucial by being a member of normal flora of human, but gained importance due to the methicillin resistance, which resulted in spreading of nosocomial infections [28]. Besides Ca, B correlation with antibacterial activity was very significantly positive. It is known that boron compounds damage bacteria cells by harming the enzymes in protein synthesis and therefore they are considered to be antibiotics and disinfectants [29, 30]. Boron compounds are increasingly used in detergents and disinfectants in Turkey as being the largest boron reserve of the world.

The highly significant positive correlation of Mg and K with inhibition of only *P. aeruginosa* growth is also remarkable. Studies showed that when Mg was limited in the growth environment, the bacterium showed a tendency to form a biofilm, and therefore persist in the environment, and also exerted its virulence and became pathogenic to the organism [31, 32]. Therefore, the plant extract being rich in Mg can be considered better for its antibacterial activity against this bacterium, which is and opportunistic human pathogen [33]. The molecular basis of the effect of extracellular K concentration on growth inhibition of *P. aeruginosa* was shown by Lindestam Arlehamn et al. (2010) [34]. Therefore, in order to fight with *P. aeruginosa* and *S. aureus* with thyme extracts, it would be better for the plant be rich in especially Ca, K, Mg, and B.

4. CONCLUSION

The present study aimed to examine the correlation of plant nutrient elements with the antibacterial activity of methanol extract of *Thymus vulgaris* L. grown on soil from the experimental field without application of fertilizers or pesticides. The plant was sufficient in terms of macro elements, while some micro elements such as Fe, Mn and B. The herbal extract was successful in inhibiting the growth of two Gram-positive and one Gram-negative bacteria, which are opportunistic human pathogens. The results revealed that the macro and micro nutrient elements were of importance for antibacterial activity of the herbal extract obtained from the aerial parts. Although, the extract of thyme was successful in inhibiting the growth of *S. aureus*, *E. faecalis* and *P. aeruginosa*, the correlation analysis of the nutrient elements showed which elements are more crucial in the fight with each bacterium. The results also suggested that higher K and Mg contents of the plant would result in higher antibacterial activity against *P. aeruginosa*, most probably up to a point. Similarly, Ca and B contents are important to fight against Gram-positive bacteria. Further studies might be carried out focusing on the maximum levels of macro and micro nutrients, especially resulting from fertilizer and pesticide use, in relation to the biological activities required for use of such medicinal plants in traditional medicine and pharmaceutical industry.

Funding

The authors have not received any financial support for the research, authorship or publication of this study.

The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

MG: Data collection, statistical analyses, Literature research, Manuscript Preparation

SA: Data collection, Manuscript Preparation

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of

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