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# Black thyme (*Thymus vul*garis), white thyme (*Thymus serpyllum*) and lemon (Thymus *citriodorus*): Antioxidant and Antimicrobial Effects

Incilay Gokbulut [1*], Seval Cing Yildirim [2]

[1] *Food Engineering Department / Engineering Faculty / Inonu University, Turkey*
[2] *Biology Department / Arts and Science Faculty, Inonu University, Turkey*

*(seval.cing@inonu.edu.tr) Email of the corresponding author*

***Abstract*** – Thyme plants have many different health-beneficial properties, including antimicrobial, antioxidant, anti-inflammatory, cardioprotective, neuroprotective, anticarcinogenic and hypoglycemic activities. In this study, the antimicrobial and antioxidant properties of three different thyme extracts: black thyme (*Thymus vulgaris*), white thyme (*Thymus serpyllum*) and lemon (*Thymus citriodorus*) were determined.
The antioxidant properties of different thyme species extracts were examined using 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) methods, while total phenolic compounds Folin- It was examined using the Ciocalteu method. Accordingly, the highest amount of TPC was determined in the Thymus citriodorus species as 4306.45 mg GA/g. Total phenolic concentrations were found to vary between 3606.32 and 4306.45 mg GAE / kg, ABTS values between 247.30 and 286.36 mmol Trolox / g, and DPPH inhibition percentage between 97.99% and 78.54%. The DPPH inhibition of black thymus (*Thymus vulgaris*) had higher DPPH inhibitions (97.99 5) than white thymus and lemon thymus respectly (86 % and 78%).
The antimicrobial effect of these thyme extracts against *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* was determined by the agar diffusion method. Thyme extracts showed different levels of antimicrobial activity against the tested microorganisms. The methanol extract of black thyme (*Thymus vulgaris*) showed the highest inhibitory activity (18 mm) against *Salmonella typhimurium.* All the tested thyme extracts exhibited considerable magnitude of antibacterial activity against the tested panel of microorganisms.

*Keywords – Thymus Vulgaris, Thymus Serpyllum, Thymus Citriodorus, Antioxidant, Antimicrobial*

## INTRODUCTION

The health benefits of plants have been studied and evaluated for a long time. Spices and herbs, in particular, attract attention with their antioxidant and antimicrobial effects due to the active compounds they contain. Black thyme (*Thymus vulgaris)* is a plant species native to the Mediterranean region and is used as a spice, especially in kitchens. Black thyme has strong antioxidant properties due to the phenolic compounds it contains. For example, compounds such as thymol and carvacrol support the antioxidant effects of black thyme. These antioxidant effects help reduce oxidative stress caused by free radicals and prevent cell damage. At the same time, the antimicrobial effects of black thyme are also noteworthy. Studies have shown that the antimicrobial properties of black thyme are particularly effective on certain types of bacteria and fungi. Lemon thyme (*Thymus citriodorus*) is a herb known primarily for its pleasant scent and citrus flavor. Lemon thyme has a strong antioxidant potential thanks to the essential oils and phenolic compounds it contains. Especially compounds such as thymol and geraniol in lemon thyme support their antioxidant effects. By reducing the damage caused by free radicals in the body, antioxidants can slow aging and reduce the risk of certain diseases. Also, the antimicrobial properties of lemon thyme are essential. Studies have shown that lemon thyme inhibits some types of bacteria and fungi. White Thyme (*Thymus serpyllum*) is an herb commonly found, especially in Europe and Asia. White thyme has antioxidant properties thanks to the phenolic compounds and flavonoids it contains. These compounds may protect against the harmful effects of free radicals. Its antioxidant effects show that white thyme positively affects health by reducing oxidative stress and preventing cell damage. The antimicrobial effects of white thyme are also noteworthy. Studies show that white thyme has antimicrobial effects on some bacteria and fungi.

Thyme plants are claimed to be endowed of distinct health beneficial properties, including antimicrobial, antioxidant, anti-inflammatory, cardioprotective, neuroprotective, anticarcinogenic and hypoglycemic activities (Kindl et al. 2015; Iauk et al., 2015; Ozkan et al., 2016; Afonso et al., 2020). Although these benefits have been mostly associated with essential oils (Kubatka et al., 201; Grulova et al., 2015), *Thymus* phenolic-rich extracts are an attractive target for the screening of bioactive compounds for possible industrial applications in distinct fields, including food, cosmetics or pharmaceutical industries (Brewer, 2011). Among them, counteraction of oxidative stress events is a central issue underlying the valorization of these plants.

In this study, the antimicrobial and antioxidant properties of three different thyme extracts: black thyme (*Thymus vulgaris*), white thyme (*Thymus serpyllum*) and lemon (*Thymus citriodorus*) were determined.

## MATERIAL AND METHOD

### Plant material and experimental studies.

Thyme (*Thymus vulgaris, Thymus citriodorus, Thymus serpyllum*) plants were obtained from Milas/Muğla. The plant sample was identified, and the voucher specimen was deposited at the Department of Biology. The thyme plants were harvested by hand and dried in the shade at room temperature at an average of 25°C. The plant were extracted by methanol: water (80:20, v/v) at room temperature for 72 h. The hydro alcoholic solutions were filtered with filter paper (Whatman no:1), evaporated by a rotary evaporator and the remaining water was removed by lyophilization. The final residual material was resolved in methanol at known concentrations before antimicrobial and antioxidant activity tests.

### Determination of Total Phenolics

The total phenolic content of methanolic extracts was determined spectrophotometrically according to the Folin & Ciocalteu procedure based on the procedures described by Velioglu et al. (1998) with some modifications. In this assay, the methanolic extract (20 µL) was oxidized by 1 mL Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), reaction mixtures were neutralized with saturated sodium carbonate (1 mL) after 3 min, and final volume of the mixture was adjusted to 3 mL with distilled water. The tubes were then mixed and allowed to stand for 2 h. The absorbance was measured using a Shimadzu (Tokyo, Japon) UV/1700 spectrophotometer at 725 nm and compared to gallic acid (SigmaAldrich, Steinheim, Germany) calibration curve. The total phenolic content was expressed as µg gallic acid equivalents (GAEs) /g DW. All determinations were carried out in triplicate

### Antioxidant Activity

### DPPH Assay

The radical scavenging activities of the extracts against DPPH (Fluka, Steinheim, Germany) were established spectrophotometrically. The DPPH assay was carried out according to a modified version of the method described by Brand Williams et.al. (1995). In this assay, the methanolic extract at known concentrations was used as a stock solution. Aliquots (100 µL) of the plant extract were mixed with 1.9 mL of a methanol solution of DPPH (25 mg/L). After 30 min incubation at room temperature, the absorbance was measured at 520 nm against methanol. Results were expressed as µg TEAC/g DW. Each assay was performed in triplicate. The antiradical activity of samples was calculated according to following equation;

$$\% \text{Inhibition of } DPPH = ((Absorbance Control) - Absorbance\ Sample)) / (Absorbance\ Control) * 100$$

### ABTS Assay

In this experiment, the stock solution of ABTS radical cation (ABTS+ ) solution was produced by reacting 7.0 mM ABTS stock solution with 2.45 mM (final concentration) potassium persulfate in the dark for 16 h. The stock solution was diluted with methanol by adjusting the absorbance to 0.700 ± 0.020 at 734 nm. The methanolic extract of the each of sample at known concen trations was used in ABTS assay as a stock solution. A diluted ABTS solution (1.9 mL) was added to 100 μL of methanolic solution and the absorbance was measured after 6 min at 734 nm. The same procedure was performed for Trolox and the antioxidant activity value was recorded as Trolox equivalent (mmol Trolox eq/g dried weight). All determinations were carried out in triplicate

### Antimicrobial tests

The antibacterial activities of Thyme samples on Gram positive and negative bacteria was determined by agar well diffusion method (NCCLS, 1999). Methanol extracts of tymes were sterilized by filtration through 0.45 μm membrane filter. All of the microorganisms were incubated at 37±0.1 °C for 24 h by inoculation into Mueller Hinton Broth (Oxoid). After incubation, small amounts of bacterial colony was removed from fresh bacteria cultures and the density of bacterial suspensions was adjusted with sterile physiological solution against 0.5 Mc-Farland standard tubes. 100 μL of prepared culture was spread on the Mueller-Hinton Agar and then 100 μL of extract solution was impregnated into the wells of agar plates directly. The inoculated plates were incubated for 24 h at 37°C. Following incubation, the diameter of inhibition zone was determined in mm. As positive controls, Ampicillin (10 μg) and Tetracycline (30 μg) discs were used. DMSO and methanol solvents were also used as negative controls.

## RESULTS AND DISCUSSION

In the study, antioxidant activity values and total phenolic substance contents were determined in three different thyme species. The results of the total phenolics content and antioxidant capacities of Different thymes were shown in Table 1.

Table 1. Total phenolic compounds and antioxidant capacities of *Thymus* extracts

| | %inhibition of DPPH | ABTS mmol Trolox/g | TPC mgGA/kg dw |
|---|---|---|---|
| *Black (Thymus vulgaris)* | 97.99 | 278.34 | 4162.63 |
| *White (Thymus serpyllum)* | 84.98 | 247.30 | 3606.32 |
| *Lemon (Thymus citriodorus)* | 78.54 | 286.36 | 4306.45 |

Accordingly, the highest amount of TPC was determined as 4306.45 mg GA /g in the *Thymus citriodorus* species. In their study, where Anvar et al. (2022) examined the antioxidant and antibacterial effects of different extracts of thyme (*Thymus vulgaris*), the total phenolic content (TPC) value of 80% methanolic extract of thyme was found to be 1236.0 ± 1.51 mg/g. In our study, the TPC value of *Thymus vulgaris* extract was determined as 4162.63 ± 1.51 GAE mg/g.. Mihailovic-Stanojevic et al. (2013) determined the total phenol content in *T. serpyllum* as 2008.33 ± 10.6 mg/g GAE. These values are lower than the value we found in our study.

Although there are many methods for determining the antioxidant activity of extracts, the DPPH is the most widely used method for the quantification of free radical scavenging activity. This method is depend on the spectrophotometric measurement of the reduction in the characteristic purple color of 2.2-diphenyl picrylhydrazyl (DPPH) as a stable free radical. The reason for this decrease in color is the sweeping of DPPH by these antioxidants in the presence of antioxidants that give electrons and hydrogen atoms (Brand-Williams et al. 1995). The percentage inhibitions for DPPH assay are ranged from 97.99 to 78.54. *Thymus vulgaris* extract had the highest DPPH inhibition and this situation could be due to high content of phenolic components such as carvacrol and thymol content, these phenolic components with known antioxidant activity (Tural and Turhan, 2017). When the DPPH results of exctracts were compared with the literature, thyme, showed higher DPPH inhibition than the value determined by Embuscado (2015) as 52, %. These values may have changed depending on the thyme types, environmental conditions, solvent type and extraction methods.

In addition to DPPH assays, ABTS analyses are commonly used for measuring antioxidant capacities of thyme extracts. The ABTS method is depend on the generation of a blue/green ABTS·+ that can be decreased by antioxidants (Floegel et al., 2011). ABTS values of thymes changed from 247.30 to 286.36 mmol Trolox/g dw. The methanol extract of lemon thyme showed high both ABTS values and total phenolic compounds. Phenolic substances may be the main compounds responsible for antioxidant activities of thyme. The strong positive correlation between antioxidant capacity and total phenolic component of herbs and spices has been previously reported (Aliakbarlu, 2014; Chan et al., 2016). Differences of these investigations could be due to different extraction procedure. ABTS activity values detected were 286.36, 278.34 and 247.30 mmol Trolox/g, respectively. The highest ABTS activity value detected was lemon (*Thymus citriodorus)* thyme.

DPPH free radical scavenging potential is directly related with the contents of phenolic compounds present in the tested plant extract.
The phenolic compounds are mainly present in the leaves, flowers, roots and fruits of medicinal plants. The biological properties of different plant materials may differ from each other due to varying nature and structural features of the bioactive constituents.

The antioxidant activities of phenolic compounds are affected by their concentrations as well as their chemical structure. In general, flavonoids consist of structural elements that contribute to antioxidant activity (an ortho-dihydroxy structure (3',4'-OH) in the B ring, a 2,3-double bond combined with a 4-oxo function in the C ring, and hydroxyl groups at positions 3 and 5. show higher antioxidant activity than phenolic acids due to the presence of one or more of them (Lou, Xu, et al., 2020). It is understood that plants with high total phenolic content do not always present high antioxidant activity values and that the antioxidant activity in plants is not directly affected by the total phenolic content. Various environmental factors affect the concentration of phenolic compounds in plants. In this context, higher growth temperatures and CO2 levels increase the flavonoid content and concentrations of phenolic compounds (Wang ve diğ, 2003). The results of the antimicrobial activity of *Thymus* extracts were shown in Table 2.

Table 2. Antimicrobial activity* of T*hymus* extracts

| Concentration (40mg /ml) | Inhibiton zone diameter (mm) | | | | |
|---|---|---|---|---|---|
| | *B. cereus* | *E. faecalis* | *E. coli* | *S. typhimurium* | *P. aeruginosa* |
| *Black (Thymus vulgaris)* | 12.00 ±0.97 | 8.50 ±0.32 | 16.00 ±0.29 | 18.00 ±0.22 | 14.50 ±0.34 |
| *White (Thymus serpyllum)* | 8.70±0.40 | 8.72±0.42 | 14.11±0.16 | 11.33±0.47 | 14.40±0.10 |
| *Lemon (Thymus citriodorus)* | 5.00 ±0.40 | 7.34±0.29 | 12.67±0.20 | 12.01±0.41 | 12.66±0.32 |

* Diameter of the inhibition zone formed around the film discs; (mm)

In general, black thyme has a higher antimicrobial efficacy than other thymes. This situation may be due to high content of phenolic components such as thymol and carvacrol. This is in accordance with, previous studies which showed that carvacrol and thymol had strong antibacterial activity (Cetin et al., 2011; Du et al., 2015). These components may inactivate the essential enzymes, disturb energy production and structural component synthesis and the genetic material functionally or react with the cell membrane activity (Celikel and Kavas, 200

The mechanism of antimicrobial activity involves several cellular processes in which the permeability of cell membrane increases and as a result ion leakage from the cell occurs that leads towards the inhibition of cell growth.

## CONCLUSIONS

In conclusion, the study investigated the antioxidant and antimicrobial properties of three different thyme extracts: black thyme (*Thymus vulgaris*), white thyme (*Thymus serpyllum*), and lemon thyme (*Thymus citriodorus*). The results indicated significant variations in total phenolic content (TPC), DPPH inhibition, and antimicrobial activity among the thyme species.

The highest TPC was observed in *Thymus citriodorus*, reaching 4306.45 mg GA/g, emphasizing its rich phenolic composition. The DPPH inhibition percentages ranged from 97.99% in black thyme to 78.54% in lemon thyme, highlighting the potent antioxidant capabilities of these thyme extracts. Black thyme, specifically *Thymus vulgaris*, exhibited the highest DPPH inhibition, potentially attributed to its high thymol and carvacrol content.

Antimicrobial tests revealed substantial inhibitory activity against *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. Black thyme, particularly *Thymus vulgaris*, demonstrated the most pronounced antimicrobial efficacy, with the methanol extract showing the highest inhibition zone (18 mm) against *Salmonella typhimurium*.

These findings underscore the diverse health-promoting properties of thyme extracts, including their antioxidant and antimicrobial potential. The variations in bioactivity among different thyme species highlight the importance of considering plant diversity in harnessing their benefits for various industrial applications, such as in the food, cosmetic, and pharmaceutical industries. Further research and exploration of thyme phenolic-rich extracts may contribute to their potential utilization in combating oxidative stress and microbial threats in different fields.

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