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Essential oils and fatty acids of *Thymus vulgaris* seeds: chemical composition, antioxidant and antimicrobial activity

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Abstract

Medicinal plants are a natural source of many existing medical products, were used widely to enhance food taste and make a fragrance, therapeutic, and preservative additive. The essential oils (E.Os) and fatty acids (F.As) are the origins of various phytochemical substances and are commercially beneficial in food and they are several biological activities including antiviral, antibacterial, anti-parasitic, and anti-inflammatory. So, the Thymus vulgaris L. seeds (Thyme) was investigated to extract E.Os and F.As and was analyzed via mass spectrometry (GC / MS) to determine their chemical composition. The results indicated that the antioxidant properties of extracting were examined using the (DPPH) free radical-scavenging method, ability of the E.Os and F.As ranged from 86.60% to 82.60 % respectively compared to the standard Quercetin (85.32 %). However, antimicrobial activity was represented against Bacillus cereus, Staphylococcus aureus, E. coli and Proteus vulgaris. Suggesting a possible link between such essential oils/fatty acids and the elimination of harmful, or potentially lethal pathogen, at least to some extent.

Keywords: *Thymus vulgaris* seeds, essential oils, fatty acids, chemical composition, antioxidant capacity, Antimicrobial.

Introduction

Thymus vulgaris L. (Thyme) is a perennial native herb in Southern and Central Europe, Africa, and Asia [1]. It is a flowering plant related to the Lamiaceae family of mint, and it grows up to a height of 15-30 cm[2]. This plant is widely used in traditional medicine for treating various diseases [1, 2]. Thyme is an annual shrub native well in the Mediterranean and West climates. This plant's E.Os are utilized as a flavour enhancer in meals, Essential oils from this plant are used in food, pharmaceutics and cosmetics as an aroma additive. In addition, its essential oil has been reported to possess various biological activities [3]. Thyme has gained tremendous attention worldwide as both a medicinal and a therapeutic agent such as 6-gingerol, thymol and carvacrol and the herb is also unique for its potential ability to combat acne while possessing a range anti-bacterial properties that could alleviate some ailments. The most important bioactive compounds present in this herb are now present the significant biochemical effects of thyme. [4, 5]

Furthermore, depending on the harvest area, the composition are greatly varies. Thyme seeds contain many types of fatty acids and these oils have advantages in the food and pharmaceutical industries [6, 7].

The essential oils (E.Os) which are a product derived from natural sources, are considered the plant secondary metabolism ingredients, and many of these oils are used as seasonings and medical products. It is possible to describe complex mixtures of reactive, lipophilic, odoriferous, and liquid compounds. [8] They play a significant role in the defence of plants such as antiviral, antibacterial, antifungal, and even against herbivore assault. There are commercially employed in the herbal, cosmetics, farming, sanitation, food, and perfume industries. [9] E.Os can be extracted from various parts of aromatic plants, such as seeds, buds, roots, leaves, flowers, fruits, Oil glands, ducts, cysts, or glands hairs.[10, 11]

In the same manner, Free radicals play a crucial role in tissue injury, inflammation, and neurodegenerative diseases as specific pathological conditions. They are used as protection techniques by human body via antioxidants elements[12]. Recently, antioxidants are used to prevent certain diseases, such

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as kidney diseases[13], heart diseases[14] and some types of tumors [15]. The antioxidant characteristics of bioactive components in the thyme composition were identified[16]. The effect of thymol and carvacrol as antibacterial, antiviral, and aroma regulation has been well understood after compounding centuries of research and observation on the plant [17].

Antibiotic resistance is when bacteria gradually develop genetic resistance against antibiotics resulting in unchecked and rapid growth of a new strain of that bacterium, resulting in an altered and more severe form of illness due to the bacteria becoming more difficult to treat, or in some instances, effectively untreatable. It is still a major clinical issue, prompting researchers to look for new alternatives, such as using antibacterial plant extracts and essential oils to treat bacteria [18]. Since ancient times, thyme plants have been used for healing, antiseptic fumigators, food preservation, and other beneficial effects.[19] Nowadays, the pronounced antimicrobial activity of *Thymus vulgaris* EOs has been indicated[20].

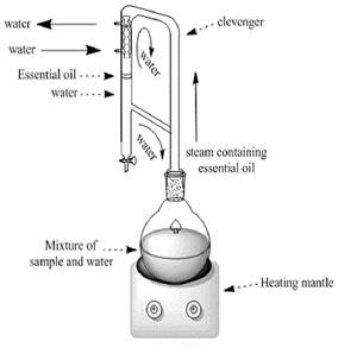
Consequently, our aim was isolation and characterization of the essential oil and fatty acids of *Thymus vulgaris* L. that grows in Iraq and investigate their effect as antioxidants and antibacterial agents.

Material AND METHODS

Plant material

In April 2019, seeds of *Thymus vulgaris* were harvested in Mosul, Iraq, and dried to a constant weight. The collected plant was taxonomically described at Biology Department, University of Mosul, Mosul, Iraq.

Extraction essential oil by Clevenger apparatus



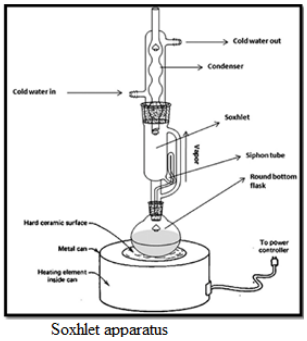
apparatus of Clevenger

The experiment was carried out in the apparatus of Clevenger.
In this process, water and seeds were mixed and boiled; water was evaporated to extract *Thyme* oil. The vapor was condensed via reflux condenser. Both water and oil flow from condenser into graded tube to easily isolate oil and distilled water was returned to the flask and so on. The oil was rested enough time to be clear for collection in a dark glass vial in a fridge until analysis.

Extraction of oil (Fatty acids) by Soxhlet apparatus

Soxhlet apparatus was used to extract oils and fats of Thyme seeds.

After grinding the plant seeds, the prepared sample within a thimble was loaded into the Soxhlet extractor's main chamber. A flask containing hexane solvent equipped with a condenser. The solvent had been refluxed. The solvent vapor went through a distillation arm and filled the thimble of solid into the chamber. The chamber that contains the solid material was filled slowly with warm solvent. When the chamber in Soxhlet was almost full, a siphon side arm automatically emptied the chamber. The solvent runs down to the distillation flask. The desired compound was concentrated in the distillation flask after several cycles. The hexane was used as solvent in our extraction. The miscella, a mixture of oil and solvent from the concentrated distillate flask, was taken off to distill. A rotary evaporator was used to remove the solvent. The residue was put in a drier position and weighed to constant value.



Soxhlet apparatus

Analysis of oils by GC-MS

E.Os analysis was carried out using the Gas Chromatography with Mass Spectrometry (GC-Ms) to determine the oil composition and the quantity of each composition.


GC-Ms on an Agilent 7890A gas chromatograph coupled with an Agilent 5975C inert Mass Selective Detector (MSD) mass spectrometer system

The extracted Thyme oils were analyzed by GC-Ms on an Agilent 7890A gas chromatograph coupled with an Agilent 5975C inert Mass Selective Detector (MSD) mass spectrometer system with triple axis detector. The gas chromatography was equipped with a fused silica capillary column AB-5MS (5 % phenylmethylpolysiloxane, 30.0 mx 0.25 mm, and film thickness 0.25µm).

Antioxidant activity DPPH assay

The antioxidant activity of the E.Os and F.As of Thyme was achieved by assessing their capability to scavenge stable radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH**)**.The assay was spectrophotometrically Performed.

- 1 mg of sample dissolved in 1 ml of ethanol.
- 20 mg of DPPH dissolved in 100 ml of ethanol.
- Absorbance value of blank and resultant solutions reported.

- Quercetin is as a positive control agent, a synthetic antioxidant.
- DPPH's disappearance was spectrophotometrically read at 517 nm.
- Essential oil and fatty acid scavenging (%) of DPPH-free radical was determined as follows:

Scavenging (%) = 100 x (A_{blank} -A_{sample}/A_{blank}).

The concentration of essential oils and fatty acids generating 50 percent scavenging (IC_{50}) was estimated from the graph-plotted percentage of scavenging against E.Os and F.As:

Inhibition (%) = 100 x (A_{blank}-A_{sample} / A_{blank})

Where A_{blank}: the absorption of control (all reagents except the test compound) and the A_{sample}: absorption of test compound.

The extract concentration with an inhibition of 50 percent (IC50) was determined from the graph inhibition percentage against extract concentration. Synthetic antioxidant Quercetin was used as the positive control, and all triplicate tests were performed.[21]

Source of microbial strains and selection

Thyme extractions were evaluated against our laboratory's stock culture of four microbial strains. *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 6538), as Gram-positive and *E. coli* (ATCC 8739) and *Proteus vulgaris* (ATCC 7829) as Gram-negative. The Microorganisms have been securely stored in freezing conditions using bead vials.

The preparation of microorganism's population (inoculums)

The microorganism's population (inoculums) were grown and prepared according to literature.[22]

Disk diffusion technique agar

The antibiotic susceptibility test was conducted using the conventional methods[23]. Triplicates of each experiment were performed.

RESULTS AND DISCUSSION

The obtained results from GC-MS indicated that essential oils of *Thymus vulgaris* were described in Table 1; 17 compounds were identified. Results showed that the main constituent of E.Os is Thymol (20.58%), then carvacrol (12.20%), Estragole (11.10%), tetradecanolide (8.68%) and carbamic acid (8.60%).

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

No	Compound	percentage	Retention index	Retention time (min)
1	**Thymol**	**20.58**	**1172**	**4.382**
2	Phenyl glycol	6.23	1298	4.478
3	Carvacrol	5.98	1262	4.581
4	Carbamic acid	8.60	1620	4.736
5	Estragole	11.10	1262	4.938
6	Eugenol	0.94	1392	6.131
7	Caryophyllene	4.73	1494	7.702
8	dibutylhydroxytoluene	1.05	1668	9.880
9	Alloaromadendrene oxide	1.02	1462	11.542
10	Anisole	0.61	1209	18.14
11	Nonanedioic acid	4.25	1917	19.16
12	Pentadecenoic acid	8.54	1859	21.951
13	Adipic acid	2.10	2145	22.361
14	*propanoic acid*	1.49	2468	24.790
15	Carvacrol	12.20	2085	24.973
16	tetradecanolide	8.68	2085	25.220
17	pentadecanolide	1.89	1400	25.748

GC-MS analyses of thyme fatty acid indicated presence of 10 compounds (only seven of them are fatty acids) of 100% total oil. The main constituent is linoleic acid (36.41 %), then oleic acid, palmitic acid, pentadecanoic acid, myristic acid, palmitoleic acid and stearic acid (31.59, 19.89, 2.60, 2.49, 2.26, 1.60 and 6.07 %, respectively), Table 2.

Table 2. Fatty acids and another natural compounds of *T. vulgaris* seeds.

No	Compound	percentage	Retention index	Retention time (min)
1	Anisole	1.85	1190	4.533
2	Palmitic acid	19.89	1262	4.751
3	Palmitoleic acid	2.26	1878	17.590

4	**Linoleic acid**	**36.41**	**1869**	**18.430**
5	Tetradecenol	0.77	1664	20.814
6	Myristic acid	2.49	2300	20.937
7	Oleic acid	31.59	2175	21.789
8	Stearic acid	1.60	1610	22.265
9	Eicosyl acetate	0.54	2375	23.039
10	Pentadecanoic acid	2.60	2788	27.974

Antioxidant capacity

DPPH method is used to get free-radical scavenging activity of *Thymus vulgaris* extract [24] to get antioxidant activity. Figure 1 shows that the maximum radical activity was 86.60%, in EOs extract compared to the standard Quercetin (85.32 %) at the same 40 µ/mL concentration.

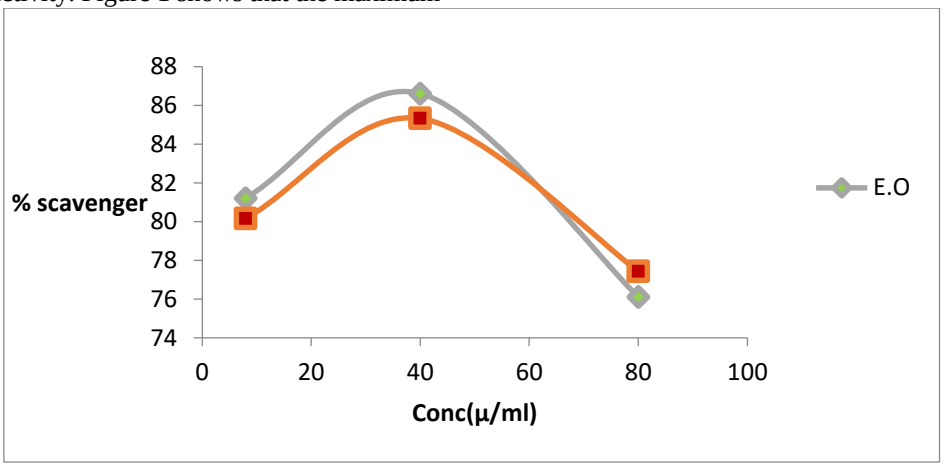


Figure 1: Antioxidant activity of Essential oils.

The total polyphenol content such as thymol and carvacrol as essential oils have high concentration with greater antioxidant activity, [25] cause the defensive function of essential oils.[26] For fatty acids, the percentages of DPPH radical inhibition were determined to check the extract's activity was 82.60 % in comparison with Quercetin (85.32 %) at the same concentration 40 µg / mL, Figure 2.

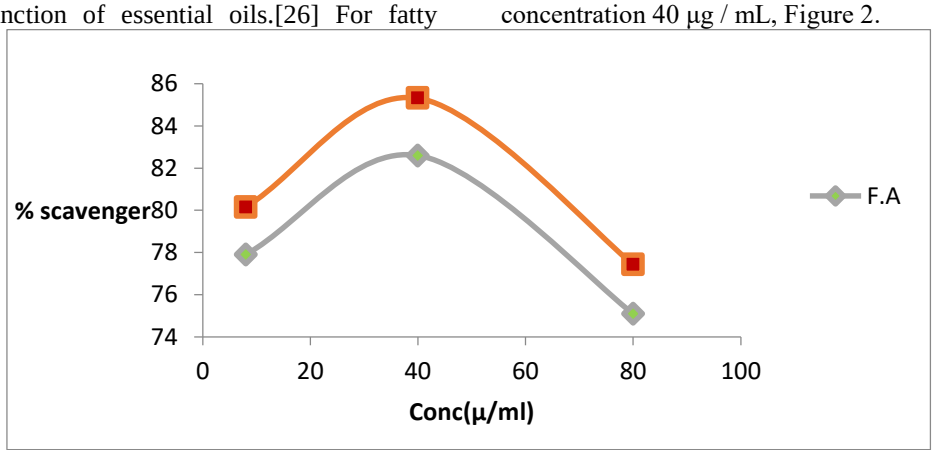


Figure 2: Antioxidant activity of Fatty acids.

Also, the proposed experiments proved that the antioxidant activity is directly related to the size of the hydrocarbon chain in the configuration of the F.As molecule.[27]

Antimicrobial activities

To investigate the antimicrobial activity of extracted oils that had been obtained from T. vulgaris seeds, we used disc diffusion method [28]against four strains of bacteria. Table 3 indicates our obtained results according to the type of bacteria and concentration.

A. Extracted essential oils

- **At concentration of 1.25 µg/ml**, the maximum effect of oils was on *P. vulgaris* (13 mm), *B. cereus* (11 mm), *E .coli* (10 mm) and *S. aureus* (7 mm).
- **At concentration of 2.5 µg/ml**, the maximum effect of oils was on *P. vulgaris* (17 mm), then *B.*

cereus (16 mm), *S. aureus* (14 mm), and *E.coli* (12 mm), respectively.

- **At concentration of 5 µg/ml,** the highest effect was equally on *S. aureus, B. cereus, and P. vulgaris* (18 mm), then *E.coli* (17 mm)
- **At concentration 10µg / ml**, the greatest effect was on *S. aureus* (22 mm), with the same effect on *B. cereus* and *E. coli* (21mm) and *P. vulgaris* was 20mm.
- Finally, **at concentration of 20µg/ml**, the higher effect was on *S. aureus* (26 mm), in comparison with Amikacin (22 mm) and Gentamycin (25 mm) as standard antibiotics.

Table 3. Antimicrobial activity of Essential oil

Essential oil	concentration µg/ml	*Zone of Inhibition (mm)*			
		S. aureus	*B. cereus*	*E. coli*	*P. vulgaris*
	1.25	7	11	10	13
	2.5	14	16	12	17
	5	18	18	17	18
	10	22	21	21	20
	20	26	24	25	25
standard antibiotics	Amikacin	22	24	25	23
	Gentamycin	25	23	22	24

B. Extracted Fatty acids

- **At 1.25 µg/ml**, *P. vulgaris* afforded the highest sensitivity to 9 mm fatty acids, with equal effect of *B. cereus* and *S. aureus* (8 mm) and no effect on *E.coli*.
- **At 2.5 µg/ml**, the higher effect of fatty acids was on *B. cereus* (15 mm) then on *P. vulgaris* (12 mm), *S. aureus* (11 mm) and *E. coli* (9 mm).
- **At 5 µg/ml**, the higher effect was 17 mm on both *S. aureus* and *B.cereus*,
- At **10 µg/ml**, the greatest effect of fatty acids was on *S. aureus* (21 mm) then on *B. cereus* and *E. coli*.
- At **20 µg/ml**, the greatest effect was on *S. aureus* (25 mm). All these results in comparison with of the antibiotic Amikacin and Gentamycin, Table 4.

Table 4. Antimicrobial activity of Fatty acids

Fatty Acids	concentration µg/ml	*Zone of Inhibition (mm)*			
		S. aureus	*B. cereus*	*E. coli*	*P. vulgaris*
	1.25	8	8	0	9
	2.5	11	15	9	12
	5	17	17	14	15
	10	21	19	19	18
	20	25	22	23	20
standard antibiotics	Amikacin	22	24	25	23
	Gentamycin	25	23	22	24

Conclusion

Thyme (*Thymus vulgaris* L.) is a popular medicinal member of family of Lamiaceae. It has many essential oils and fatty acids that have pharmacological and biological potential. Our study focused on Thyme seed oils that were extracted and identified by GC/MS. Antioxidant and antibacterial properties showed good results.

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