Journal

J. Biol. Chem. Environ. Sci., 2012, Vol. 7(1): 273-295
www.acepsag.org

CHEMICAL COMPOSITION, ANTIMICROBIAL AND BIOLOGICAL ACTIVITY OF *THYMUS VULGARIS* (THYME) ESSENTIAL OIL FROM MINIA, EGYPT

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ABSTRACT

In Egypt, many of medicinal plants are used in folk medicine for their antimicrobial activity. *Thymus vulgaris* (thyme) is a member of the family labiates. Thyme -in its crude herb form- is carminative, antibiotic, antiathematic and antitussive. It is important in the food industry for seasoning meats, soup, sauces, pickles, bakery products and ice cream. Thyme is indicated for colds, influenza, bronchitis, sore throat, low immunity, anemia and asthma. It dries and cleanses mucous secretions and soothes irritated mucous membranes. It is excellent for simultaneously building immune resistance.

The major constituents of essential oil of *T. vulgaris* were thymol (38.28%), *ρ*-cymene (28.63%) and *γ*-Terpinene (10.40%). *T. vulgaris* oil inhibited the growth of *Sarcine lutea* and *Escherichia coli* more than *Pseudomonas sp, Bacillus cereas* and *Staphylococcus aureus*. Also *T. vulgaris* oil inhibit the growth of *Penicillium expansum* more than *Aspergillus niger*.

T. vulgaris at two different doses (150 & 300mg/kg b.w) decrease body weight gain than control. *T. vulgaris* oil cause significant decline in liver weight when administered at the lower dose (150mg/kg b.w) after 30 days of treatment. But the higher dose (300mg/kg b.w) did not significantly differ from the control one. Also data showed significant reduction in kidney weight when rats were treated with *T. vulgaris* oil at two doses after 30 days of treatment. At the same time thyme did not cause significant change in the weights of spleen and testis. This was pronounced with the two tested dosed.

Thyme oil at the two doses to male albino rats caused insignificant increase in AST activity. This was pronounced with two doses in rats. Serum ALT activity was significantly reduced in male albino rats treated with *T. vulgaris* at two doses. *T. vulgaris* oil did not cause significant changes in ALP enzyme activity than normal with the two dosed.

The serum total protein and serum albumin were markedly increased by different doses of *T. vulgaris* than control. Significant decreased in serum globulin content was noticed in the rats treated with two doses of *T. vulgaris*.

T. vulgaris at the two doses did not cause any significant differences in serum urea content. This was pronounced with the two tested doses.

INTRODUCTION

Ancient Egyptians used thyme for embalming. The ancient Greeks used it in their baths and burnt it as incense in their temples, believing it was a source of courage. The spread of thyme throughout Europe was thought to be due to the Romans, as they used it to purify their rooms and to "give an aromatic flavour to cheese and liqueurs". In the European Middle Ages, the herb was placed beneath pillows to aid sleep and ward off nightmares. In this period, women would also often give knights and warriors gifts that included thyme leaves, as it was believed to bring courage to the bearer. Thyme was also used as incense and placed on coffins during funerals, as it was supposed to assure passage into the next life.

The medicinal plants, such as thyme (*Thymus vulgaris*), are a numerically large group of economically important plants. They include various species, which are used in the treatment of various diseases. These plants not only have a medicinal effect but also contain aromatic substances and essential oils used in food industries. The most important constituents in these plants are menthol and several other components that are used not only in foods, but also in most kinds of cosmetics **(Deans and Svaboda, 1990, Rinzler , 1990, Abd El-Malak *et al.,* 1995**; **Ibrahim *et al.*, 1998** and **Tollba, 2003**, **Ghazalah and Ibrahim, 1996** and **Abd El-latif *et al*., 2002**).

Thyme is indicated for colds, influenza, bronchitis, sore throat, low immunity, anemia and asthma. It dries and cleanses mucous

secretions and soothes irritated mucous membranes. It is excellent for simultaneously building immune resistance (**Rinzler, 1990**).

The yield of volatile oil of thyme ranged from 0.4 to 3.4% in common thyme and from 0.7 to 1.38% in Spanish thyme **(Blumental *et al*., 2000),** 0.5 to 2.5% in fresh flowering tops **(Dewick, 2002**), 1.2% of volatile oil, **(Evans, 2002)** 2.07% **(Hassanein, 1982)**, 2.5% **(Wagnar** and **Bladt, 2001)**, 0.92 % on dry weight basis, **(Hussein, 2005)** and 1.67 % form thyme aerial parts, **(Al-Bayati, 2008).**

The specific gravity of the essential oil of thyme was found to be 0.9662 at 15°C, 0.9723 at 25°C **(Hassanein, 1982)** and 0.9009 at 15°**C (Hussein**, **2005).** The relative density was found to be 0.915 to 0.935 **(European pharmacopeia, 2002).** The optical rotation of the essential oil of Thyme was found to be 2° 20̀ at 20°C **(Guenther, 1952)**, -5° to + 1° at 20°C (**Morton**,1977), -5° 33̀ **(Hassanein, 1982)** and -2° 9̀ **(Hussein, 2005).** The Refractive index of essential oil of thyme was found to be between 1.4919 to 1.5100 at 20°C **(Guenther, 1952; Morton, 1977**; **Hassanein, 1982; European Pharmacopeia, 2002** and **Hussein, 2005)**. The essential oil of thyme was found to be soluble at 25°C in 1 to 1.5 vol. of 80% alcohol **(Guenther, 1952),** soluble in 2.5 vol of 70% alc., 1:2 vol. of 80 % vol. **(Morton, 1977)**, soluble in 3 vol. of 70% alcohol **(Hassanein, 1982)** and soluble in ethyl alcohol 80 % (1:2 v/v) **(Hussein, 2005).** The acid value of the essential oil of thyme was found to be 1.14 **(Hassanein, 1982)** and 1.1 (**Hussein, 2005).**

Blazquez and Zafra-polo (1990), Piccaglia and Marotti (1991), **Senatore (1996)** and **Daferera *et al.* (2000)** analyzed the essential oil of *T. vulgaris* by GC-MS and reported that thyme essential oil was characterized by the presence of γ- terpinene, ρ-cymene, carvacrol and thymol. The essential oil of *T. vulgaris* contained γ-terpinene (5.0-10.0%) β-myrcene (1.0-3.0%) carvacrol (1.0-4.0%),thymol(36-55%), linalool (4-6.5 %), terpinen-4-ol (0.2-2.5 %) and *ρ*-cymene (15.0-28.0 %) **(British pharmacopoeia, 2001; Wagner and Bladt, 2001** and **Stoeva *et al.*, 2001)**. **Asllani and Toska (2003)** and **Atti-santos *et al.* (2004)** studied the essential oil of Albanian and Brazilian thyme by GC and GC/MS. They reported that thymol was the most abundant constituent followed by *ρ*-cymene. **Zambonell *et al.* (2004)** studied that the essential oil composition of *T. vulgaris* by GC. The data showed that the essential oil were rich in thymol (23-38%) and its biogenetic precursors *γ*-terpinene and *ρ*-

cymene. **Hussein (2005)** analyzed the essential oil of *T. vulgaris* by GC-MS and reported that thyme essential oil was characterized by presence of α-phellandrene and thymol as the major components. **Raal *et al.* (2005)** studied the essential oil of *T. vulgaris* and reported that the principal components in the oil were thymol (0.9-75.7), carvocrol (1.5-83.5%), *ρ*-cymene (4.3-34%). γ-terpinene (0.9-19.7%). **Rota *et al.* (2008)** analyzed the essential oil of *T. vulgaris* by GC-MS and reported that the major constituents of the oil were thymol (57.7%), p-cymene (18.7%) and carvacrol (2.8%).

Bouchberg and Allegrini (1976); **Agarwal and Mathela (1979) and Hassanein (1982),** reported that thymol had the most activity against bacteria and fungi followed by carvacrol and geraniol. The antimicrobial activity of thyme essential oil against *Bacillus subtlis*, *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Campylobacter jyuni*, *Salmonella enteritidis*, *Listeria monocytogenes*, *Penicillium digitatum, Salmonella typhimurium* and *Aspergillus flavus* was reported by **Hassanein (1982), Smith-palmer *et al.* (1998), Daferera *et al.* (2000) and Packiyasothy & Kyle (2002)**, **Burt & Reinders (2003)**, **Fabio *et al.* (2003)** and **Irag-rasooli and Abyaneh (2004)**. **Zambonell *et al.* (2004)** reported the antifungal activity of commercial *T. vulgaris* oils against *Fusarium solani, Rhizoctonia solani* and *Colletotrichum lindemuthianum*. **Hussein (2005)** reported that thyme oil had the highest antimicrobial activates against tested micro-organisms. **Ayachi *et al.* (2009)** studied and tested the methanolic, dichloro-methalonic and etheric extracts of *T. vulgaris* for antibacterial activity against seven strains of *Salmonella typhimurium*. They reported that *T. vulgaris* has the potential to provide an effective treatment for salmonellosis.

Ibrahim *et al.* (2000) reported that rabbits received thyme, as feed additives, showed significant increase in blood glucose level, total protein, GOT, GPT, urea and creatinine. Total lipids showed significant improvements. **Haroun *et al.* (2002**) studied the effect of feeding *T. vulgaris* leaves to male Wistar rats, which showed increases in serum AST activity and urea. **El-Mallah (2003)** evaluated the effect of adding thyme at 1.0% to turkey diets on performance and some metabolic functions and reported that blood plasma parameters showed an improvement in total lipids. No significant effect was detected on body weight. **Tollba (2003)** reported that ducks fed thyme oil had lower values of blood, total lipids and total cholesterol content

than control group. **Hussein (2005)** studied that the thyme oil caused a significant decrease in urea, level comparing with the others hyperchloestrolemic groups. **Shati and El-said (2009)** studied the role of water extracts of *T. vulgaris* on liver and brain of mice. The results showed very highly significant increase in nitric oxide and malondialdehyde level.

The aim of this study was to examine the composition of the essential oil of thyme and its biological activity from Minia, Egypt.

MATERIALS AND METHODS

Materials:

Plant Materials

Sample of herbs of thyme (*Thymus vulgaris*) was collected from Minia experimental farm, Faculty of Agriculture, Minia University, Minia, Egypt.

Microorganisms:

Bacteria and Fungi:

Two Gram negative bacteria (*Pseudomonas sp.* & *Escherichia coli*), three Gram positive bacteria (*Bacillus cereus*, *Staphylococcus aureus* & *Sarcina lutea*) and two molds (*Aspergillus niger* & *Penicillium expansum*), were used in this investigation. All strains were obtained from the Department of Microbiology, Faculty of Agriculture, Minia University, Egypt.

Media

Nutrient agar medium (**Shatta, 1994**) and Sabouroud's medium **(Atlas, 1997)** were used to study the effect of thyme volatile oils on the growth of bacteria and fungi, respectively**.**

Experimental animals:

Thirty male albino rats, (Sprague-Dawley strain) weighting from 170-190g were obtained and housed in the biological laboratory of Biochemistry Department, Faculty of Agriculture, Minia University.

The rats were housed in plastic cages in air conditioned room at 25±2°C (with a 12 h light / dark cycle). A commercial balanced diet and tap water *ad libitum* were provided for 2 weeks before starting the experiment. The body weight of each rat was recorded at the

beginning of the experiment and every week till the end of experiment. The rats were randomly divided into three groups. Group (1) was served as control (untreated group) and was given corn oil. Group (2) and group (3) were orally treated with essential oil of thyme in doses of 150mg and 300mg/Kg body weight for three days per week during four weeks, respectively.

Sampling of blood and organs:

At the beginning of the experiment (0 day) and at the end of the biological experiment (30 days), the rats were anesthetized by diethyl ether. Blood samples were withdrawn from the retro –orbital vein of each animal and allowed to clot and then centrifuged at 3000 rpm for 15 min, and serum kept at -20°C until used in biochemical analysis.

At the end of the experiment, animals were dissected as quickly as possible and the liver, kidney, spleen and testis were excised, wiped with filter paper and weighted.

Methods

Chemical analysis of the leaves:

Air dried samples of leaves were finely ground and subjected to chemical analysis. Moisture, crude protein, crude fiber, ash and total lipids were determined according to the method of **A.O.A.C. (1990**). All analysis was performed in triplicate and means are reported.

Extraction of essential oil:

Thyme essential oil was obtained by water distillation for 2 hours according to the procedure stated by **Guenther (1952**).

Determination of essential oil content:

The essential oil content was determined according to the method described by **The United States pharmacopoeia (1995).**

Physico-chemical properties

Specific gravity, refractive index, optical rotation, solubility and acid number:

The specific gravity, refractive index and optical rotation of the oil were determined according to the procedure reported by **Guenther (1952).** The solubility and the acid number were determined according to **British pharmacopoeia (2001).**

Determination of the chemical composition of the essential oils

GC-MS analysis was performed in the Micro-analytical Center, Faculty of Science, Cairo University. The samples were GC-MS analyzed on Shimadzu QP 2010 plus, using stabilwax columm GC-MS system.

The operating conditions of GC-MS were as fallows:

GC conditions: Column: 30mx0.25mm x0.25µm. Carrier gas: Helium at 40 kpa. Injection: 200˚C

Analytical line: Ion source temp: 200˚C. Start Time: 5.00 min. End Time: 30.00 min. ACQ Mode: Scan. Event Time: 0.50 sec. Scan speed: 2000. Start m/z: 50.00. End m/z: 1000.00. Electron voltage: 70eV. Ionization Mode: EI (electron impact).

The identification of compounds was based on a comparison of their mass spectra and their retention times with those of authentic standards and by comparison of their mass spectra with those of data in Wiley and NIST libraries.

Investigation of the antimicrobial activity for the essential oils

Disc-diffusion method:

The Disc-diffusion method was used for detection the antimicrobial activity of thyme volatile oil against seven strains of microorganisms namely *Bacillus cereus*, *Staphylococcus aureu*s, *Sarcina lutea*, *Pseudomonas sp, Escherichia coli*, *Aspergillus niger* and *Penicillium expansum* as described by **Conner and Beuchat (1984).** The results were recorded by measuring the inhibiting discs zones diameter (mm).

Biochemical Assays:-

Quantitation of serum proteins:

Determination of serum total protein concentration:

Serum total protein was determined by the biuret reagent as described by **Gornall *et.al,* (1949)**.

Determination of serum Albumin:

Serum albumin concentration was determined according to the method of **Doumas *et al*. (1971**) using reagent kits.

Liver function tests (LFTs):

Aminotransferases.

Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities according to the method described

by **Reitman and Frankel (1957)**. The concentration of ALT and AST were calculated as [u /L] by using special table subjected by the same authors.

Determination of serum alkaline phosphatase activity:

Alkaline phosphatase (ALP) was measured with colorimetric method **(Belfield and Goldberg, 1971).**

Kidney function tests.

Determination of serum Urea:

Serum urea was quantified with the urease as described by **Fawcett *et al*. (1960)**.

RESULTS AND DISCUSSION

1. Chemical composition of leaves:

Chemical analysis of the *T. vulgaris* leaves was presented in Table (1). The Ash content of the leaves under study was 7.56%, crude protein 5.12%, total lipid content 7.34%, crude fiber content 71.93%, and total carbohydrates content was 8.05 %.

Table (1): Chemical composition of thyme leaves (based on dry weight).

Chemical composition	% (dry weight)
Moisture	6.29
Ash	7.56
Crude protein	5.12
Crude lipids	7.34
Crude fiber	71.93
Total carbohydrate	8.0
Volatile oil%	2.27

2. Essential oil content:

Data in Table (1) indicated that the essential oil content of thyme essential oil was 2.27% based on dry weight basis. This result is in agreement with that of **Blumental *et al.* (2000)** who mentioned that it ranged from 0.4 to 3.4% in common thyme and lower than 0.7 to 1.38% in Spanish thyme, **Hassanein (1982**) who reported that it was

2.07% on dry weight basis **and Hussein (2005**) it was 0.96% based on dry weight basis.

3. Physico-chemical properties of volatile oil

The physico-chemical properties of volatile oils were presented in Table (2):

i- Specific gravity:

Specific gravity of thyme oil recorded 0.910. **Morton (1977**) mentioned that it ranged from 0.911 to 0.954 at 20ºC, **Hassanein (1982**) reported that it was 0.9662 at 15ºC, 0.9723 at 25ºC, **British Pharmacopeia (2001**) stated that it was 0.915 to 0.935 and **Hussein (2005**) reported that it was 0.9009.

Table (2): The physicochemical properties of the *T. vulgaris* essential oils

Physico-chemical properties	Value
Specific gravity at 25ºC	0.910
Optical rotation	-2º 19̀
Refractive index	1.502
Solubility	Soluble in 2 vol. of ethanol (80%)
Acid number	1.12

ii- Optical rotation:

The optical rotation of thyme oil was -2º 19̀. This result is in accordance with that of **Guenther (1952**), -2º 20̀, **Morton (1977)** and **Hussein (2005**) who mentioned that it was ranged from -5º to + 1º.

iii- Refractive index :

Refraction index recorded 1.502. This result in agreement with the values reported by **Morton (1977**), 1.4940 to 1.5100, **British pharmacopoeia (2001**) ranged from 1.4900 to 1.5050 and **Hussein (2005**), 1.4974.

iv- Solubility:

The oil of thyme was soluble in 2 vol. of ethyl alcohol 80% (1:2 v/v). The result was in agreement with that of **Morton (1977**) who stated that it was soluble in alcohol 70% (2:5 v/v) and in alcohol 80% (1:2 v/v), **Hassanein (1982**) who reported that it was soluble in 70%

alcohol (1:3 v/v) and **Hussein (2005)** it was soluble in 80% alcohol (1: 2 v/v).

v- Acid number:

The acid number of thyme essential oil was 1.12. The result was in agreement with that of **Hassanein (1982**) reported that it was 1.14 and **Hussein (2005**), 1.1.

4. Gas chromatography-Mass spectroscopy (GC-MS):

Essential oil components, obtained by hydro-distillation of the dried leaves of *T. vulgaris* were analysed and identified by GC-MS.

The chromatogram of the volatile constituents of the oil was represented in Table (3). Their retention times and percentage composition were also reported.

The identified components in *T. vulgaris* oil are shown in Table (3). GC-MS analysis of the essential oil resulted in identification of 10 constituents. The most prominent components were thymol (38.28 %), ρ-cymene (28.63 %) and γ-terpinene (10.40 %). They constituted about 78% of total essential oil. The mass spectrum for the thymol, ρ-cymene and γ-terpinene were shown in Figures (5,6&7), respectively.

Table (3): Percentage composition of the essential oil of *T. vulgaris*.

No.	Rt (min)	Content, %
1	6.521	3.45
2	6.550	1.79
3	8.831	28.63
4	8.879	8.48
5	9.001	1.45
6	9.792	10.40
7	14.522	0.45
8	16.262	38.28
9	16.399	5.52
10	20.087	1.48

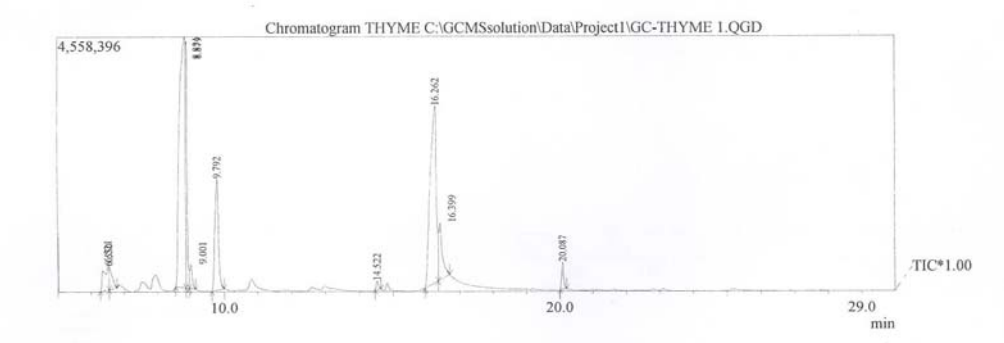
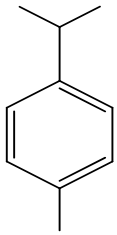


Fig (1):Gas chromatogram of Thyme oil .



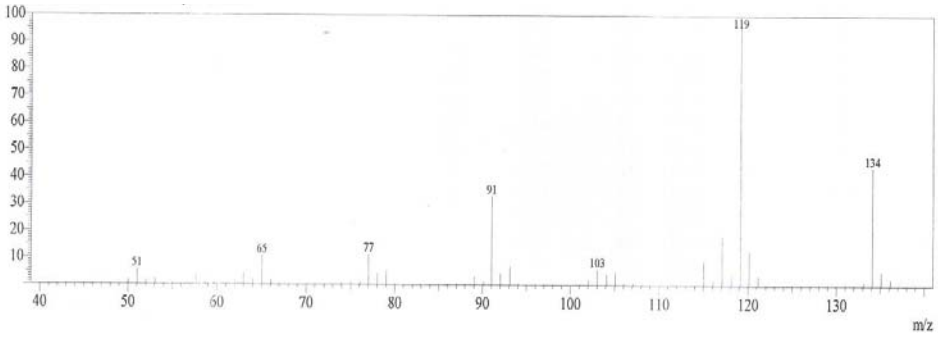
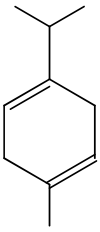


Figure (2): Mass spectrum of ρ-cymene.



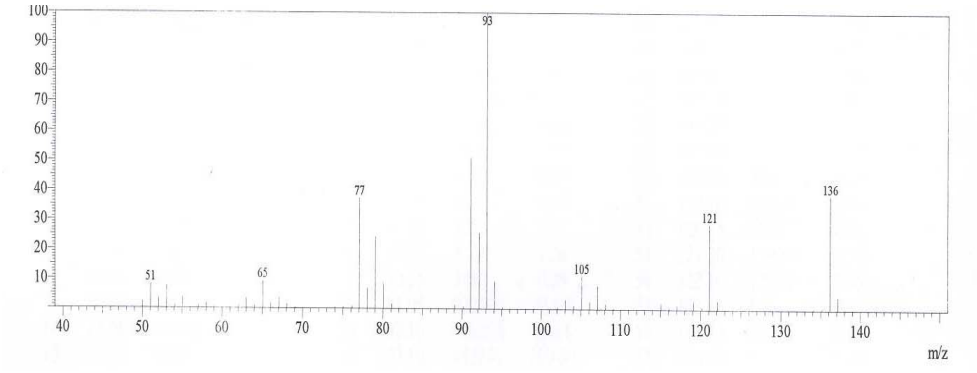
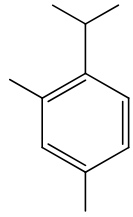


Figure (3): Mass spectrum of γ-terpinene.



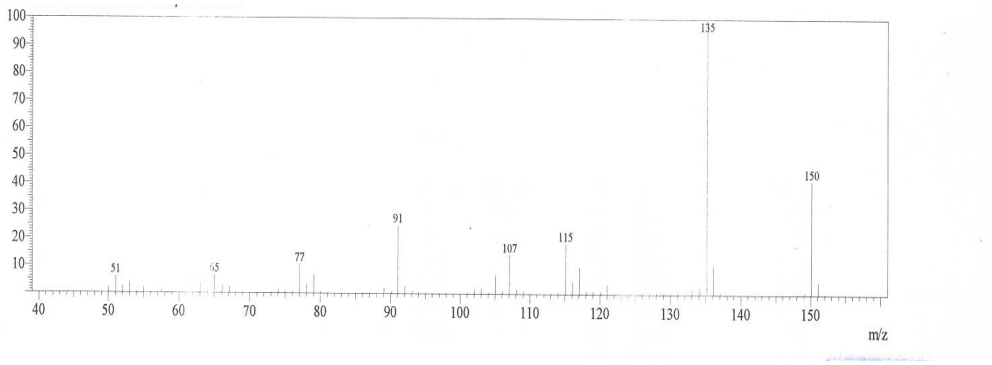


Figure (4): Mass spectrum of thymol.

The results were in accordance with those of **Wagner and Bladt (2001)**. They mentioned that the essential oil of *T. vulgaris* contained thymol (30%-70%), carvacrol (3%-15%), thymol-monomethylether (1.5%-2.5%) 1.8-cineole (2%-14%), geraniol, linalool, bornyl and linalyl acetate (1%-2.5%) and α-pinene. The essential oil of *T. vulgaris* contained γ–terpinene (5.0-10.0%), β-myrcene (1.0-3.0%), carvacrol (1.0-4.0%), thymol (36-55%), linalool (4-6.5 %) terpinen-4-ol (0.2-2.5%) and ρ-cymene (15.0-28.0) **(British pharmacopoeia, 2001)**. **Asllani and**

Toska (2003) reported that the main components of Albanian thyme identified were ρ-cymene (7.76-43.75%), γ-terpinene (4.20-27.62%), thymol (21.38-60.15%), carvacrol (1.15-3.04%) and β-caryophyllene (1.30-3.07%). **Atti-santos *et al.* (2004)** reported that the most abundant essential oils from the leaves of *Thymus vulgaris* constituent was thymol (31.5-52.4%), followed by ρ-cymene (17.1-34.4%). **Zambonell *et al.* (2004)** studied that the essential oil composition of *T. vulgaris* by was rich in thymol (23-38%) and its biogenetic precursors γ-terpinene and ρ-cymene. **Hussien (2005)** statied that the essential oil of *T. vulgaris* characterized by presence of α-phellandrene and thymol as the major components. **Raal *et al.* (2005)** reported that the principal components in the oil were thymol (0.9-75.7), carvocrol (1.5-83.5%), ρ-cymene (4.3-34%) and γ-terpinene (0.9-19.7%). **Rota *et al.* (2008)** reported that the major constituents of the *T. vulgaris* oil were thymol (57.7%), p-cymene (18.7%) and carvacrol.

5. Antimicrobial activity of Thyme essential oil:

The obtained results indicated that thyme essential oil had antimicrobial activity against the tested microorganisms. The results indicated that thyme oil inhibited the growth of *Sarcine lutea* and *Escherichia coli* more than *Pseudomonas sp*., *Bacillus cereas* and *Staphylococcus aureus*. The diameter of the inhibition zones were 22, 22, 20, 18 and 14mm, respectively. The results also showed that Thyme oil inhibited the growth of *Penicillium expansum* more than *Aspergillus niger*. The diameter of the inhibition zone were 26 and 26 mm, respectively.

Table (4): Antimicrobial activity of essential oils on the tested microorganisms expressed as inhibition zone (mm).

Tested microorganisms	Thyme essential oil conc. (v/v) EtOH		
	10%	50%	100%
Bacillus cereus	7	9	18
Staphylococcus aureus	5	10	14
Sarcina lutea	10	17	22
Pseudomonas sp.	5	12	20
Escherichia coli	8	14	22
Aspergillus niger	5	26	26
Penicillium expansum	26	26	26

The results were in agreement with those of **Agarwal** and **Mothela (1979**). They reported that thyme oil showed antimicrobial activity against 6 *Penicllium* and 5 *Fusarium* species. The activity was attributed mainly to carvacrol and thymol present in the oil. The oil of thyme has the most inhibitory effect at a concentration of 0.075 % or less against *Campylobacter jejuni, Salmonella enteritidis, Escherichia coli, Staphylococcus aureus and Listeria monocytogensis* (**Smith-Palmer *et al*,. 1998)**. **Daferea *et al.* (2000)** reported that mycelial growth was inhibited by thyme essential oil at 300µg/ml. Thyme essential oil effectively inhibited the conidial germination of *P. digitatum* completely at the concentration of 250µg/ml. **Packiyasothy and kyle (2002**) and **Fabio *et al.* (2003**) reported antimicrobial activities of the essential oil of thyme against *Salmonella typhimurium, Bacillus cereus, Escherichia coli and Aspergillus flavus*. **Hussein (2005**) reported that the thyme oil had the highest antimicrobial activities against tested microorganisms followed by lemongrass, rosemary, marjoram and fennel, respectively

6. Biological effect of Thyme oil on rats:

i. Body weight:

Results reported in Table (5), reveled that thyme at the two doses decrease body weight gain than control (76.6g). They recorded 63.00g and 69.80g for dose I and dose II, respectively.

Table 5: Effects of Thyme oil on body weight gainand daily body weight ratio of rats.

Group	Mean Initial wt(g)	Mean Final wt(g)	Body weight gain(g)	Daily body gain (g)
Control	192.8±6.80	269.4±15.4	76.60	2.532
Thyme 1	190.4±6.19	253.4±10.3	63.00	2.102
Thyme 2	180.6±11.40	250.4±10.4	69.80	2.460
Mean	187.93	257.73	69.80	2.36

Each value represents mean of 6 replication ± SE

The results were in harmony with those obtained by **El-mallah (2003**), who investigated the effect of adding thyme at 1.0% to turkey diets on performance and some metabolic functions which revealed no significant effect was detected on body weight.

ii. Internal organs weights:

Data in Table (6), indicated that thyme significantly decline liver weight when administered at the lower dose (150mg/kg bw) after 30 days of treatment. But the higher dose (300mg/kg b.w) did not significantly differ from the control one. Significant reduction in rats kidney weight was recoded, at both of the two doses, after 30 days of treatment. There is no cause significant changing in the weights of spleen and testis. This was pronounced with the two tested dosed.

Table (6): Effects of thyme and Marjoram oils on liver, kidneys, spleen, testis weight and their ratio of rats.

Groups	Liver wt		Kidney wt		Spleen wt		Testis wt	
	(g)	%	(g)	%	(g)	%	(g)	%
Control	8.88±0.77	3.29	1.93±0.07	0.72	0.92±0.12	0.34	2.64±0.22	0.98
Thyme 1	6.92±0.44	2.76	1.59±0.05	0.63	0.75±0.10	0.30	2.42± 0.17	0.97
Thyme 2	7.71±0.41	3.04	1.64±0.11	0.65	0.83±0.06	0.33	2.90±0.11	1.14
Mean	7.84		1.72		0.83		2.65	

Each value represents mean of 6 replication ± SE

Similar finding were also mentioned by **Abdo *et al***. (**2003),** who found that no significant effect was detected on live body weight, body weight gain, liver and gizzard by using medical herbal i.e. red peeper in broilers diet**.** Also**, El-mallah (2003**) summarized the effects of adding medical plants such as, *T. Vulgaris* 1.0% as natural growth promoters to turkey diets to improve growth performance. The results revealed that these medical plants improve the liver weight and body weight compared with control group but with no significant change.

iii. Effect of Thyme oil on liver function:

1. Transaminases:

Serum enzymes including alanine aminotransferase (ALT) E.C.2.6.1.2. and aspartate aminotransfrase (AST) E.C.2.6.1.1. were used to evaluate the hepatic diseases. An increase in these enzyme activities reflects liver damage, either chronic or acute. Acute inflammatory hepato cellular disorders resulted in elevated transeaminases levels **(Forstan *et al.*, 1995).**

1.1. ALT, AST and ALP activities:

Data presented in Table (7), showed that thyme oil when used at different doses to male albino rats caused insignificant increase in

AST activity. This was pronounced with two doses in rats. These finding with *thymus vulgaris* were confirmed by **El-mallah (2003**) who reported that when turkey group fed 1.0% dried *thymus vulgaris*, the values of AST activity increased.

Table (7): Effect of Thyme oil on AST, ALT and ALP activities in rats .

Group	AST U/L		ALT U/L		ALP U/L	
	0 day	30 days	0 day	30 days	0 day	30 days
Control	17.36±0.13	16.98±0.15	15.43±0.12	15.44±0.00	261.02±17.34	251.9±29.41
Thyme1	17.08±0.02	17.15±0.16	15.63±0.06	15.09±0.06	251.94±23.78	230.3±00.37
Thyme2	17.61±0.15	17.36±0.35	15.25±0.23	14.59±0.16	247.49±40.55	226.4±79.70
Mean	**17.35**	**17.16**	**15.44**	**15.04**	**253.48**	**236.20**

Each value represents mean of 6 replication ±SE

Data in Table (7) showed that serum ALT activity was significantly reduced in male albino rats treated with thyme at two doses.

On the contrary **Ibrahim *et a*l. (2000**) investigated the effect of *T. vulgaris* as feed additives on growth and metabolic change of rabbits. they reported that biochemical parameters showed significant increase in blood glucose level, total protein, liver activities (sGOT and sGPT) for rabbits received 0.50% dried leaves of *T. vulgaris* while, **El-mallah (2003**) found that sGPT decreased but not significantly with adding 1.0% *T. vulgaris* to diet turkey group.

As regards ALP, data in Table (7) indicated that Thyme oil did not cause significant changes in enzyme activity than normal with the two doses.

Total protein, Albumin (A), Globulin (G) and A/G ratio:

Data concerning the effect of *T. vulgaris* oil at different doses on serum total protein are represented in Table (8).

The data indicated that the serum total protein was markedly increased by different doses of Thyme than control (6.89), the increasing levels were 6.93 (dose I) and 7.29 (dose II) for thyme. Data show that Thyme was more effective at two doses and also show that dose II for thyme was more effective than dose I. The same table indicated that the serum albumin was markedly increased by different

doses of both Thymus than control (3.01), the increasing levels were 4.27 (dose I) and 4.46 (dose II) for Thyme and were 3.50 (dose I).

Table (8): Effect of Marjoram and Thyme on level of total protein, Albumin, Globulin (g/dl) and A/ G ratio in serum of male albino rats.

Groups	Total protein		Albumin		Globulin		A/G ratio	
	0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days
Control	6.29±0.13	6.89± 0.02	3.34±0.12	3.01±0.19	2.95±0.09	3.88±0.19	1.13	0.78
Thyme1	6.89±0.72	6.93± 0.12	3.01±0.29	4.27±0.17	3.88±0.62	2.66±0.21	0.78	1.61
Thyme2	6.76±0.39	7.29± 0.31	3.29±0.04	4.46±0.33	3.47±0.37	2.83±0.59	0.95	1.58
Mean	6.65	7.03	3.21	3.91	3.43	3.12	0.95	1.32

Each value represents mean of 6 replication ± SE

Serum globulin was calculated by the difference between total serum proteins and serum albumin. The result showed a significant decrease in serum globulin content was noticed in the rats treated with two doses of Thyme.

The results of the present study are disagree with those stated by **Ibrahim *et al.* (2000),** they reported significant increase in total protein in treated rabbits with *T. vulgaris*. Also, **El-mallah (2003)** found the same results.

Effect on kidney function, Serum urea:

Urea is the major end product of nitrogen catabolism in humans. Urea is synthesized in the liver, released to the blood and cleared (excreted) by the kidney. The changes in plasma urea level are due to alteration of renal function (diseased kidney) (**Baron, 1987**). Data presented in Table (9) revealed that the *T. vulgaris* when used at different doses did not cause any significant differences in serum urea content. This was pronounced with the two tested doses.

Table (9): Effect of Thyme on level of Urea (mg/dl) in serum of male albino rats.

Groups	Urea mg/dl	
	0 day	30 days
Control	5.89±0.36	6.17±0.04
Thyme1	6.35±0.99	6.86±2.73
Thyme2	6.25±1.42	6.94±0.53
Mean	6.16	6.66

Each value represents mean of 6 replication ±SE

These results came in contrast with the finding of **Ibrahim *et al.* (2000)** who investigated the effect of adding 0.5% dried leaves of *T. vulgaris* as feed additives on growth and metabolic change of rabbits. They recorded that biochemical parameters showed significant increased kidney functions (urea).

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التركيب الكيميائي والنشاط الحيوي والنشاط كمضاد بكتيري لزيت الزعتر من محافظة المنيا بمصر

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تم إجراء التحليل الكيميائي على الزيوت العطرية المستخلصة من العشب الكامل لنبات الزعتر نظرا لاستخدام هذه النباتات على نطاق واسع كمواد مكسبه للنكهة و الطعم و مستخدمة فى الإغراض العلاجية. تم تقدير محتوى النبات من الزيت الطيار ودراسة الخصائص الطبيعية و الكيمائية للزيت والتركيب الكيميائي لمكوناته ودراسة تأثيره علي بعض الميكروبات وعلي فئران التجارب.

أظهرت الدراسة أن محتوي الأوراق من الرماد لنبات الزعتر أعطي ٧,٥٦% إما نسبة البروتين فكانت ٥,١٢% أما الدهون فكانت ٧,٣٤% وبالنسبة للألياف فكانت نسبتها ٧١,٩٣% والكربوهيدرات الكلية كانت نسبتها ٨,٠٥%. احتواء نبات الزعتر علي ٢,٢٧% من الزيت الطيار وذلك علي أساس الوزن الجاف. الكثافة النوعية كانت ٠,٩١٠ لزيت الزعتر، الدوران الضوئي ١٩ -٢ ،معامل الانكسار ١,٥٠٢ ،الذوبان الكامل في كحول الإيثايل ٨٠% وجد أن زيت الزعتر يذوب بنسبة (١: ٢ حجم) ورقم الحموضة ١,١٢.

أوضحت الدراسة إن المكونات الرئيسية لزيت الزعتر كانت كالأتي thymol (٣٨,٢٨%) ρ-cymene(٢٨,٦٣%) γ-Terpinene (١٠,٤٠%).

أظهرت الدراسة أن زيت الزعتر كان أكثر تثبيط علي بكتريا *Sarcine lutea* و *Escherichia coli* عن بكتريا *Pseudomonas sp* و *Bacillus cereas* و *Staphylococcus aureus* وكانت أقطار التثبيط لتركيز ١٠٠% من الزيت كما يلي٢٢و٢٢و٢٠و١٨و١٤ملليمترعلي التوالي إما الفطريات فكان قطر التثبيط لفطر *Penicillium expansum* و *Aspergillus niger* ٢٦ ملليمتر لكل منهم.

في تجربة النشاط الحيوي قسمت الفئران إلي ثلاث مجموعات أعطيت المجموعة الضابطة زيت الذرة والمجموعتين الأخريتين أعطيت زيت الزعتر بجرعات ١٥٠ مليجرام/كجرام للمجموعة الأولي و٣٠٠ مليجرام/كجرام للمجموعة الثانية وأعطيت الجرعات للفئران ثلاث أيام في الأسبوع لمدة أربعة أسابيع. أوضحت الدراسة أن زيت الزعتر بمختلف جرعاته يخفض وزن الفئران عن الكنترول من ٧٦,٦ جرام للكنترول الي ٦٣ للجرعة الأولي والي ٦٩,٨٠جرام للجرعة الثانية. زيت الزعتر أدي إلي انخفاض معنوي في وزن الكبد عندما أعطيت الجرعة ١٥٠ملجرام /كجرام بعد ٣٠ يوم من المعاملة ولكن الجرعة ٣٠٠ مليجرام /كجرام لم تؤدي إلي تغيرات معنوية عن المجموعة الضابطة ،أيضا حدث انخفاض معنوي في وزن الكلي للفئران المعاملة بزيت الزعتر بالجرعتين بعد ٣٠يوم من المعاملة في نفس الوقت زيت الزعتر لم يسبب اي تغيرات معنوية في وزن البنكرياس والخصية للجرعتين. زيت الزعتر لم يسبب زيادة معنوية في نشاط إنزيم AST وذلك بالنسبة للزيوت في الجرعتين بينما نشاط ALTحدث فيه انخفاض معنوي عند المعاملة بالزيت في الجرعتين . زيت الزعتر لم يحدث أي تغيرات معنوية في نشاط الإنزيم الالكالين فوسفاتيز بالنسبة للجرعات المستخدمة. لا يوجد تغيرات معنوية في مستوي البروتين الكلي للفران عند معاملتها بالزيوت، بينما نجد أن الألبيومين حدث فيه زيادة معنوية بالنسبة للجرعة الثانية للزعتر، أما الجلوبيولين حدث فيه انخفاض معنوي عند المعاملة بزيت الزعتر في الجرعتين . زيت الزعتر لم يسبب أي فروق معنوية في مستوي اليوريا للفئران بالنسبة للتركيزات المستخدمة.