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CONTRIBUTION STUDY TO THE CHEMICAL COMPOSITION OF THE VOLATILE OIL IN THE LEAVES OF *THYMUS VULGARIS* L. AND DETERMINATION OF THE ANTI-BACTERIALL ACTIVITY AND ANTI-LEISHMANIAL VITALITY

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ABSTRACT

Thymus spp. is widely distributed plant in Syria, where it is used for its antiseptic and decongestant properties. We conducted this study to extract the essential oil from this plant and to determine the main components of this oil in addition to determine its antibacterial and anti leishmania activity compared with the antibiotic ceftriaxone. The plant material *Thymus vulgaris* L. was obtained from the local market in Damascus, where the plant samples were dried (the moisture was 4%) and the volatile oil is extracted using Clevenger apparatus (water destillation), where 100 g of thyme leaves powder steam distiled with 500 ml water). The extracted volatile oil components were analyzed with GC/ FID. The results of the GC/FID showed that the most important ingredients in the volatile oil were carvacrole and thymole (73.1%, 7.2% respectively). Antibacterial activity of the volatile oil has been determined on isolates of pathogenic identified bacteria and against the vitality of leishmania strains prepared from public health laboratories and Hospital of Dermatology of Damascus University, where it was determnined the anti bacterial activity compared with ceftriaxone and also determined the anti leishmania vitality. In this study it was found that the volatile oil of *Thymus vulgaris* has high anti-bacterial properties, and it has been found to have effectiveness against anti leishmania vitality.

KEYWORDS: *Thymus vulgaris L.*, Volatile oil, anti bacteria, anti leishmania.

INTRODUCTION

The importance of volatile plants is due to its components in its volatile oil, and the effectiveness of each volatile oil varies according to the plants that contain it.[1,2]

The volatile oils and its components vary in the plant according to several environmental and climatic factors.[1]

Volatile oils to have anti-bacterial and anti-fungal effects, some of them have anti tumor activity, and others are used in aromatherapy.[4,5]

The plant *Thymus vulgaris* L. is cultivated in the tropics and is wide spread in Syria on booth regin, highlands and slopes and is very resistant to extreme climatic factors.[1,2]

The height of the plant thyme is about 50-60 cm, with abundant branching.[1,2]

The flowers are white to pink (Fig. 1).

Fig. 1: *Thymus vulgaris* L.

Thyme contains volatile oil with a distinctive aroma rich in carvacrol and thymol. The percentage of carvacrol varies from 36.9% to 60%.

The properties of antiseptics (antimicrobial effectiveness) of thyme volatile oil are due to the

presence of thymol and carvacrol.[4,5]

The medical importance of thyme is attributed to the fact that it contains more than 44% of the phenolic substances, consisting mainly of thymol (41%), carvacrol (3.6), flavonoids and tanins.[4,5]

The leaf extract was used to relieve gastrointestinal disorders and to treat head lice and fungus when used externally. Thymol is also used as gargel in oral cavity.[9]

The Aim

1-Extract the volatile oils of thyme leaves and determination its percentage and its chemical components.

2 -Study the anti-bacterial efficacy of the volatile oil and the anti-Protozoa (anti Leishmania vitality) activities.

MATERIALS AND METHODS

1- Plant material

The plant material of *Thumus vulgaris* L. was obtained from local market (cultivation filde near the Faculty of Agriculture in Damascus) during March 2016. The collected plant material was identified, dried in the oven (temp. 50-60 degree centigrade) and powderd.

2- Extraction of the volatile oil

The volatile oil was extracted from the dried leaves *Thymus vulgaris* L.using water steam distillation method (Clevenger), so it was steam distilled 100 g of poweder dry plant material with 500 ml of water, and the distillation process lasted approximately 2 hours, where 2.0 ml volatile oil is obtained in a yellowish-red color.[5,7]

3- Antibacterial activity

3-1-Microbial strains

Antibacterial activity of the volatile oil was studied against five bacterial strains (they were provided by Damascus Health Directorate, Public Health Laboratories) and identified as:

Escherichia coli (ATCC 10536), *Bacillus subtilis* (ATCC 6633) *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* L. *Micrococcus luteus*L.

3-2- Assy of the antibacterial activity[4,10]

Antibacterial activity of the volatile oil was investigated using the disk- diffusion method.

The bacterial colonies were incubated for 18 h at 37°C in 10 mL of muller Hinton Broth. The density was adjusted using the sterile physiologic serum to obtain a density corresponding to the McFarland density of 0.5, thus obtaining bacterial suspension containing approximately 10^8 CFU / ml.

100 µl of each bacterial suspension were taked by sterile swabs and placed in the center of Muler Hinton agar medium (13 mL medium in each dishe) to obtain homogenous bacterial growth in both control and examined dishes.

Volatile oils were mixed in a mixture of dimethyl sulfoxid and water (10% Aqueous DMSO) to reach the concentration of 80% (0.5% volume / volume to facilitate the spread of the oil to from the tablets).

The sterile discs (White Man No. 1, diameter 6 mm) were loaded with about 5 µl of tablet of different concentrations of the volatile oils with a mixture in the following concentrations; 1: 1, 1: 5, 1:10, and 1:20. The discs were placed on agar surface, one disc was loaded with a 10% DMSO mixed with twin 80, and without volatile oil, and in the middle of each dishes implanted one disc; contains standard of ceftriaxone (30 µg / tablet).

The cultures were left for about 30-45 minutes at room temperature to allow the oil to spread, and incubated for about 24 hours at 37C. After incubation, the diameter of the non-growth hols was measured. All previous studies were performed three times and the mean was calculated.[11]

3-3-Anti-leishmanial activity[12]

Pathogenic leishmania and standard of Leishmania donovani strain AG 83 were collected and supplemented with 10% fetal bovine serum of pH 7.2. The logarithm phases of promastigotes (2×106 cells/ml) were incubated with or without the volatile oile along with Medium-199 at 22 °C.

The tested volatile oile was dissolved in 0.2% dimethyl sulphoxide (DMSO), and is added to the culture in graded doses. After 2 h of treatment, the tubes were centrifuged at 8000 g for about 10 min. The supernatant was decanted and the pellets were washed with 20mM phosphate buffer saline (PBS). Each pellet was dissolved in 100µl (2mg/ml) MTT solution, the tubes incubated at 22°C for 4 h and then centrifuged at 8000 g for 10 min. The resulting pellets were dissolved in 500µl 0.2% DMSO and the absorbance measured spectrophotometrically at 570nm.

% lysis = 100 – {(test – positive control)/(control – positive control)} × 100 ...(1)

3-4-Separation of the volatile oil components

Separation of the volatile oil components was done using gas chromatography connected to flame ionization detector (GC-FID).

GC-2010 SHIMADZU gas chromatograph is used with the following specifications

Column: (Precix.HB2340), length: 100 meters, diameter: 0.25 mm, inner layer thickness: 0.25 µm, carrier gas: nitrogen / air, nitrogen flow: 47 ml / min, air flow: 400 ml /min. Total: 31.8 ml /min. Flow of the gas in the column: 0.49. ml/min. Retention time: 57.8.

Detector: FID., Detector-temp: 280 C., Thermal Program: Injection Chamber Temperature: 280 C. Initial column temperature: 60 C Continue for 2 min., then rise

to 190 C at 10°C until reaching 190°C The heat is fixed at 190C for 15 min.

Chemical Standards: Carvacrol, beta-Caryophyllene,γ-terpinene, Thymol, Borneol, P-cymen, Linaloal, 1,8-Cineole .

3-5-Methods of extraction and Analysing of the volatile oil

All standards, as well as the oil samples, were extended with cyclo hexane and an equal content mix with extended standard (10 µl of each standard).

An internal Standard has been prepared as the following

90 µl of a non-flowered green oil sample is extended with cyclo hexane mixed with 10 µl of the standard.

All studied samples of the oil, in addition to the standard, and the oil mixture with the standard, were injected into GC-FID.

4- RESULT AND DISCUSSION

4-1-Separation of the volatile oil components using the GC –FID

The oil samples were analyzed using a gas chromatography (GC-FID) and the retention time of each peaks was compared with the retention time of the standards peaks. The chemical composition of the volatile oil ingredients and their proportion were determined. It was found that the basic compound consisting of *Thymus vulgaris* L. volatile oil is caravacrol 76.1% (Tab. 1).

Table 1: Chemical composition of the volatile oil in the *Thymus vulgaris* L.

terpinene	P- cymen	beta- Caryophyllene	Borneol	Thymol	Carvacrol	Component
4.1%	3.6%	4.04%	3%	5.3%	76.1%	Percentage of ingredients

4-2-Antibacterial activity

4-2-1 Antibacterial activity of the volatile oil

The results showed that the studied volatile oil samples had a counter effect against studied bacteria (Fig. 2), except *Pseudomomas aeruginosa*, which showed resistance to the volatile oil samples, whereas *Staphylococcus aureus* was showed high sensitivity (tables 2-5).

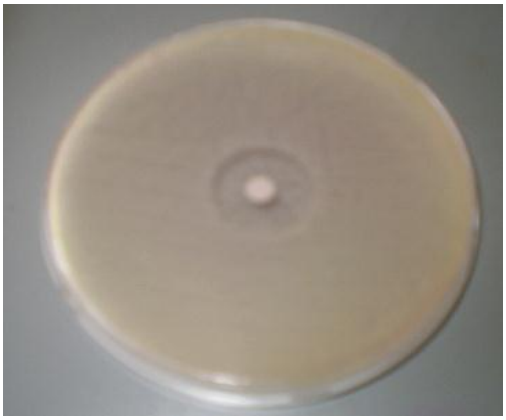

Figure 2: Non-growth hol resulting from application of essential oil to Bacillus subtillus.

Table 2: Effect of thyme volatile oil on *Escherichiacoli.*

1:20	1:10	1:5	1:1	concentration
1.4	1.6	1.9	2.6	Diameter of Growth inhipition (cm)

Table 3: Effect of thyme volatile oil on *Bacillussubtilis.*

1:20	1:10	1:5	1:1	concentration
1.5	2.1	2.1	3.5	Diameter of growth inhibition (cm)

Table 4: Effect of thyme volatile oil on *staphylococcus aureus.*

1:20	1:10	1:5	1:1	The concentration
1.6	2.5	3.2	4.3	Diameter of growth inhibition (cm)

Table 5: Effect of thyme volatile oil on *Micrococcus luteus*

1:20	1:10	1:5	1:1	The concentration
1.0	1.5	1.5	2.3	Diameter of growth inhibition (cm)

4-2-2-Effect of the Standard on bacterial strains

The Table 6 showed the anti bactrial activity of the used standard Ceftriaxone.

Table 6: Effect of Ceftriaxone on the studied bacterial strains.

pseudomonas aeruginosa	Micrococcus luteus	Staphylococcus aureus	Bacillus subtillus	Escherichia coli	bacterial strains
1.9	2.5	2.6	1.1	1.8	Diameter of growth inhibition (cm)

It was noted that the volatile oil extracted from the *Thymus vulgaris* L. has strong inhibitory effect against the growth of some gram positive and gram negative bacteria. These antibacterial properties may be related to the volatile oil of thyme mainly with its high content of phenolic substances including thymol and caravacrol which account for about 79% of previous studies on the effectiveness of these two antimicrobial compounds.

4-3-The activity of the volatile oil on the vitality of Leishmania

The IC50 dose was 3, which is evaluated by linear regression analysis using Graph Pad Prism 3 software.

In vitro anti-leishmanial activity against standard of Leishmania (strain AG83) activity has been shown from the volatile oile, (tab-7).

Table 7: Shows the volatile oile inhibition activity on the growth of Leishmania in a dose-depended manner.

Concentration of the volatile oile	Vitality %
0%	100
2%	38.58
4%	27.85
6%	17.37
10	0

5- CONCLUSION

The extracted volatile oil components were analyzed with GC/ FID. The most important ingredients in the volatile oil were carvacrole and thymole (73.1%, 7.2% respectively).

Antibacterial activity of the volatile oil has been determined on isolates prepared from pathogenic bacteria and sowie the anti-Protozoa was done using samples of leishmania prepared from public health laboratories, which idetefied and cultured in the lab.

The bacterial activity was determind using dish diffusion method and anti leishmania vitality determined using spectroscopic method.

It was found that the volatile oil of *Thymus vulgaris* has high anti-bacterial properties, which confirms the antibacterial activity of *Thymus vulgaris* volatile oil in vitro, these effects varies according to the bacterial strains and according to the basic chemical component of the extracted volatile oil, especially carvacrol.

Tha anti bacterial effect of the volatile oil is acompeined with anti- leishmanial vitality (IC50; 3).

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REFERENCES

1. Hakim W., Syrian Thyme, "Journal of Agriculture and Water" No. 24 of 2009, P. 84.
2. Hijazi T., Encyclopedia of Herbs and Plants, House of Culture, House of the Family. P.96-99.
3. Aziz R., Hamid F. and Abdullah N., Molecular Characterization and Degree of Relation between the Wild Formats of the Thymus Syriacus Using Randomized DNA (RAPD) Technology, Science Week 46 Sustainable Agriculture Development and Food Security Conference, Supreme Council for Science, 2006. Pp.: 121-123-125-122.
4. Shivalera, natural healing of medicinal plants (various diseases), Dar Radwan. P. 9.
5. Imelouane B., Amhamd H., Wathelet J.p., Ankit M., Khedid K. and El Bachiri A., Chemical composition and antimicrobial activity of essential oil of thyme (Thymus vulgaris) from Eastern Morocco." International journal of agriculture and biology "2009, the entrance date 8-7-2010.
6. Prabussenivasan S., Jayakumar M. and Ignacimuthus., In vitro antibacterial activity of some plant essential oils . "BMc comlementary and alternative medicine" 2006, the entrance date 22-7-2010.
7. Bassole I ., Ouattara A., Nebie R., Ouattara C., Kabbore Z. and Traore S., Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso ." Science direct". Phytochemistry volume 62, issue 2, 2003, the entrance date 20-3-2009.
8. Caplina J.l., Allan I .and Hanlon G.W., Enhancing the in vitro activity of thymus essential oils against staphylococcus aureus by blending oils from specific cultivars. "International journal of essential oil theraputics" 2009, the entrance date 17-7-2010.
9. Maksimovic Z., Milenkovic M., Vucicevic D. and Ristic M., Chemical composition and antimicrobial activity of thymous pannonicus all (lamiaceae) essential oil. "central European journal of biology "

volum 3, number 2, 2008, the entrance date 8-7-2010.
10. Mhd. Isam Hasan Agha, Mohammad Said Chmit, STUDY OF THE VOLATILE OIL IN LAURUS NOBILIS L. WIDE SPREAD IN SYRIA; 2018, ejpmr, 5, 4, 80-86.
11. Hmaidan A. Nasser A. Aga, Biostatistics, Damascus University Publications - Faculty of Pharmacy. Pp. 394-458-459- 460.
12. Ghosh AK, Rakshit MM, Ghosh DK. Effect of berberin Chloride on Leishmania donovani. Ind J Medicinal Res, 1983; 78: 407-411.