

The Antimicrobial Activity and the Median Lethal Dose of Thyme (*Thymus vulgaris*) Ethanolic Extract on Mice

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Abstract:

The antimicrobial activities of thyme (*thymes vulgaris*) ethanolic extract was studied by agar well diffusion technique against tested organisms (*Esherichia coli*, *Staphylococcus aureus* and *Candida albicans*). The results were showed that the extract have antibacterial and antifungal activity against tested microorganisms. The results were also showed that the median lethal dose (LD₅₀) of ethanolic extract of thyme in laboratory mice was about (4220 mg/kg B.w). The toxic signs during 24 hours after oral administration of thyme extract were rapid breathing, dullness, contraction of abdominal muscles, paralysis then death.

Key words: Thyme, Antimicrobial activity, LD₅₀

الخلاصة:

تمت دراسة الفعالية المضادة للجراثيم والمبيضات البيضاء للمستخلص الكحولي لنبات الزعتر بتقنية الانتشار بالحفر ضد جراثيم الاشيريشيا القولونية , والمكورات العنقودية الذهبية والمبيضات البيضاء. اظهرت النتائج وجود فعالية لمستخلص الزعتر الكحولي في تثبيط نمو الجراثيم والمبيضات البيضاء المستخدمة في الدراسة. كما اشارت الدراسة الى تقدير الجرعة المميتة النصفية لمستخلص الزعتر الكحولي في الفئران المختبرية وبما يقارب 4220 ملغم/كغم من وزن الجسم، حيث اظهرت الجرذان علامات تسمم سريرية خلال الاربع والعشرين ساعة بعد تجريع الجرذان لمستخلص الزعتر الكحولي عن طريق الفم وتمثلت بسرعة التنفس والخمول وتقلص عضلات البطن ثم الشلل فالموت.

مفتاح الكلمات: الزعتر، الفعالية المضادة للميكروبات، الجرعة المميتة النصفية.

Introduction:

Since ancient times, herbs and their essential oils have known for their varying degrees of atomic obialactivity(change,1995;Al-kassie, 2008). Herbs and spices are well known to exert antimicrobial actions *invitro* against important pathogens, including fungi (Ozer, *etal*, 2007). Aromatic plants are promising sources of natural antimicrobial and antioxidant compounds, the essential oil extracted from these plants have been used as alternative to antibiotics (Osman *etal*, 2005). The antimicrobial properties of essential oils from certain plants have been recognized for hundreds of years, but scientific evidence of their efficacy has been described only since the 1940s and 1950s (Deans and Ritchie, 1997). The essential oils arise from secondary metabolism of the plants leaves or stems. Plants volatile oils are isolated from non

woody plants by several methods usually distillation (Dorman and Deans, 2000).*Thymus vulgaris* (thyme) locally known (Zaatar), a member of the family lamiaceae, is a genus containing about 350 species of aromatic perennial herbs (Gruenwall *etal.*, 2004). It is aromatic plants known by antioxidant and antimicrobial activity (Babovic *etal.*, 2010). In the same field found that the essential oil of thyme has a significant rate of antifungal and antibacterial activities (Bozin, *etal*, 2006). Thyme is stated to possess carminative, antispasmodic, antitussive, expectorant, bactericidal as well as flavoring agents in food processing and many pharmacological preparations, thyme oil is still among the words top 10 most used essential oils (Stahl- Bisk up, 1991).

This study was carried out to study the antimicrobial effect of alcoholic extract

of thyme on the growth of *E. coli*, *Staph. aureus* and *C. albicans* with estimation of median lethal dose of this extract in laboratory mice.

Material and Methods:

1- The plant:

Sample of the aerial part of thyme were collected during flowering period. The taxonomic identification was determined by botany department, college of science, university of Baghdad as *Thymus vulgaris*, family Lamiaceae.

Collected plant materials were dried in the shade and the plant leaves were separated from the stems and grounded in a grinder to small particles.

2- The extraction: (Harborne, 1995)

A. Soxhlet extraction:

The finely ground sample 50 grams of thyme leaves was successively extracted with ethanol using soxhlet apparatus for 24 hours. The mixture was filtered and dried using a rotary evaporator. The final dried materials were stored in sterile bottles and kept as aliquots until it was used.

B. Steam distillation extraction: Air dried of thyme leaves were submitted for 3 hours to steam distillation using a Clevenger apparatus to produce the essential oil in a yield of 5.5% (w/w). Oil was dried over anhydrous sodium sulphate and after filtration, stored at 4°C until used.

3- Test microorganisms:

The test bacterial strains *E. coli* and *Staph. aureus* were obtained from a laboratories in Al-Yarmook hospital and *C. albicans* from the Microbiology Department in Medicine College, Babylon university.

4- The antimicrobial activity test:

The antimicrobial activity was performed by agar diffusion method (Deans and Ritchie, 1997). A tested culture of each bacterial isolates was prepared in Mueller Hinton broth to a concentration of (1×10^6) organism per ml. The inoculated agar plates were produced by mixing /ml of tested culture in 25ml of Mueller Hinton agar. After the agar plates were solidified, 4mm- diameter wells were punched into

agar, four per petridish. A volume of 100 microliter of dissolved extract and oil were inoculated into three wells and pure ethylene glycol was inoculated into the fourth well as a control. To allow the dissolved extract and oil to diffuse into agar plates, they kept at 20°C for 30 minutes before transfer to incubator. The plates were incubator at 25°C for 48 hrs. the anticandidal activity of thyme extract and oil were conducted by dispensing 15 ml of sterile Sabouraud dextrose agar. The inocula were prepared by addition of 1 ml overnight candida culture to 9 ml of Mueller- Hinton broth to yield 10^4 colony forming unit (CFU) per (μ l) of inoculum. One hundred (μ l) of dissolved thyme extract and oil were inoculated into the wells as above using a Dimethyl sulfoxide as a control. Antimicrobial activity was recorded as the width (mm) of the clear zone of inhibition surrounding the agar well. The results were reported as positive (+) if there is inhibition of growth and negative (-) if there is no growth inhibition. Triplicates sets of plates were prepared, the mean of three reading was calculated.

5- Estimation of median lethal dose (LD50) of extract:

A. Experimental animals:

Adult Swiss albino mice (25–30gm) body weight (B.W) have been used to estimate the orally administered lethal dose of ethanolic extract of thyme. The animals were kept in well air condition room at the animal house of Physiology and pharmacology Department, College of Medicine, University of Baghdad, given pellets of balanced specially prepared animal feed and water.

B. Determination of (LD₅₀):

a. Pilot study: four mice have been used for determination of the ranges of lethal doses that used in acute toxicity study, two mice for each selected dose of extract. The selected doses were 4000 mg/kg B.W, and 4800 mg/kg B.W according to the lethal outcome (death one or two mice) the range of acute toxicity doses has been selected.

b. Estimation of (LD₅₀):

Thirty mice have been divide into five groups each one consist of six mice orally administered by following lethal doses of extract according to the body weight in each group:

Group 1 = 4000 mg/kg B.W

Group 2 = 4200 mg/kg B.W

Group 3 = 4400 mg/kg B.W

Group 4 = 4600 mg/kg B.W

Group 5 = 4800 mg/kg B.W

The animals have been watched for 24 hours for the development of toxicity symptoms and lethality, with drawing the log dose – probit response curve from which the (LD₅₀) has been determined according to the probit method (Katzung, 2003).

Results:

1- Plant extraction:

The weight of crude plant was 50gm and the weight of extract was 5.48gm, so the percentage of extraction was 10.96% than the extraction yield alcoholic extracts about 10.96%.

2- The antimicrobial activity of thyme extract:

(Table 1) shows the *invitro* activity of thyme extract and oil on the growth of *E. coli*, *Staph. aureus* and *C. albicans*.

The extract and oil showed growth inhibition of tested organisms. The growth inhibition zones (mm) were 18mm, 16mm and 19mm for *E. coli*, *Staph. aureus* and *C. albicans*, respectively. (Table -1) and (figure -1, 2, 3).

Table-1: The antimicrobial activity of thyme extract and oil on the growth of tested microorganisms

The extracts	Tested microorg anisms	Growth inhibition	Zone of inhibition diameters (mm) (TriPLICATE sets)	The mean of diameters (mm)
Thyme extract A	<i>E.coli</i>	+	17 18 19	18
Thyme oil B		+	20 19 18	19
Thyme extract C		+	18 18 18	18
Control D		-	- - -	-
Thyme extract A	<i>S.aureos</i>	+	15 17 16	16
Thyme oil B		+	16 18 17	17
Thyme extract C		+	17 16 16	16
Control D		-	- - -	-
Thyme extract A	<i>C.albicans</i>	+	18 19 20	19
Thyme oil B		+	20 21 19	20
Thyme extract C		+	20 19 18	19
Control D		-	- - -	-

+ = Growth inhibition
 - = No growth inhibition



Figure-1: The inhibition effect of thyme extract and oil on the growth of *E. coli*



Figure-2: The inhibition effect of thyme extract and oil on the growth of *S. aureus*.

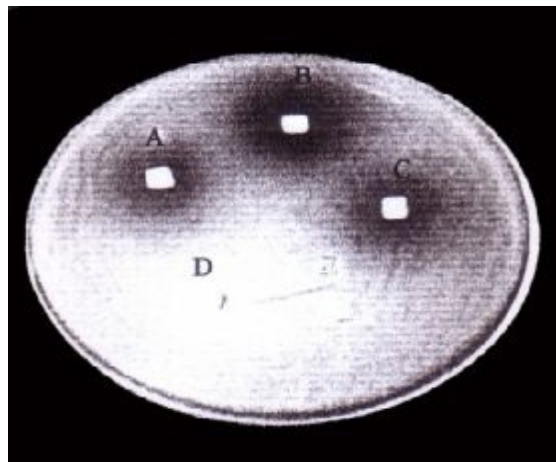


Figure-3: The inhibition effect of thyme extract and oil on the growth of *C. albicans*.

3- The median lethal dose

A- Pilot study: The result of this study revealed that the dose which caused the half of death of the mice was 4200 mg/kg B.W, so the doss which is used for the experiment ranges between 4000 and 4800 mg/kg B.W.

B- Determination of (LD50) by probit method: The mortality percent and conversion to probit number according to the acute toxic doses of thyme extract in group 1, 2, 3, 4, and 5 were listed in (table-2)

Table-2: Acute toxicity effect of different lethal doses of thyme extract in mice.

Group number	Dose mg/ml	Log dose	Total number	Number of dead animals	Mortality percent%	Probit number
1	4000	3.602	6	2	33.3	4.56
2	4200	3.623	6	3	50.0	5.00
3	4400	3.643	6	4	66.6	5.42
4	4600	3.662	6	5	83.3	5.95
5	4800	3.681	6	6	100.0	7.40

(Table-2) was showed that the dose which killed half number of animal in one group was 4200mg/ml that killed three mice in group 2. When calculate the mortality percentage and convert the dose to log dose, the median lethal dose was measured after logarithm of doses against

probit response was plotted from which (LD₅₀) was determined by vertical cross link from probity response to the log number dose, Figure (4). The calculated (LD₅₀) has been 4220 mg/kg B.W.

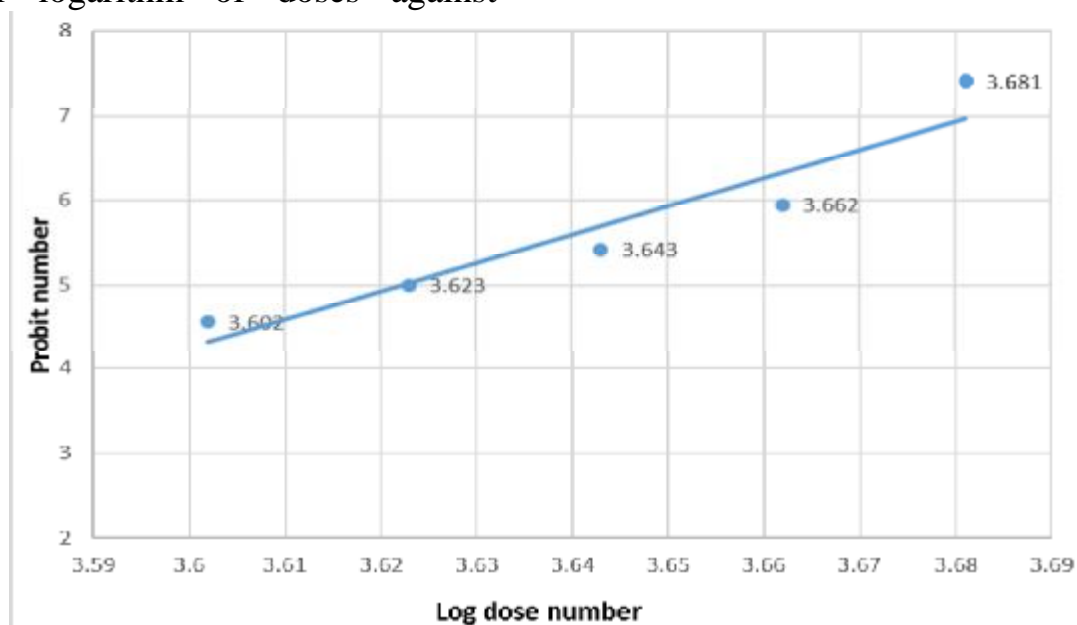


Figure (4): The median lethal dose of *Thymus vulgaris*

Discussion:

The essential oils from aromatic plants are for the most volatile parts and this lead themselves to several methods of extraction such as water and steam distillation and solvent extraction (Guenther, 1972). The specific methods of extraction depend upon the plant materials to be distilled and the desired end product. In general leaves yield crude extract were higher than steams crude extract. The

extractions of active ingredients compounds from plants depend on the type of solvent used in the extraction procedure (Majhenic *etal.* 2007). This study indicates that the most active compound in this plant are concentrated in leaves, and the ethanolic extract was the most active extracts against tested bacteria, this result were agreed with that of previous study (Ali-Shtayeh *etal.*, 1997), while another study shows that the evaluation of

antimicrobial activity of essential oils thyme is difficult because of their volatility and their water insolubility (Celiktas *et al.*, 2007). The genus thymus has numerous species and varieties and their essential oil composition has been studied earlier (Jordan, 2003, Sotomayer *et al.*, 2004). Comparison between our results and the results of other reports showed differences probably due to plant varieties or sites as well as the time of harvesting and the genetic constitution.

The antimicrobial activity of thyme extract on tested microorganisms was showed in (table-1). Biological activity of essential oils depend on their chemical composition which were determined by the genotype and influenced by environmental and agronomic condition (Marroti *et al.*, 1992). Much of antimicrobial activity in thyme extract appears to be associated with phenolic compounds thymol and carvacrol (Rota, 2007). The synergistic effects of these active chemicals with other constituents of essential oil should be taken into consideration for the antimicrobial activity. The lowest minimum inhibitory concentrations of thyme oil were 0.03% (V/V) against *C. albicans* and *E. coli* (Hammer *et al.*, 1999). Our results were agreed with previous study reported the inhibition effect of thyme essential oil on *Candida* species (Mahdavi, *et al.*, 2009), and the anticandidal activity is due to thymol (the effectiveness material of thyme) (Consentino, *et al.*, 1999).

Other studies showed that no significant differences between G^+ and G^- susceptibility after 24 hours, however after 48 hours G^- was more susceptible than G^+ bacteria which may be related to the outer membrane of G^- which give the bacterial surface strong hydrophilicity and acts as strong permeability barrier (Ultee, 1999). The (LD_{50}) of thyme extract was usually taken as one hundredth to one tenth to calculate the treatment dose. Such values provide a convenient way of comparing the potencies of drugs in experimental and clinical setting (Katzung, 2003). The study

showed that the (LD_{50}) of thyme extract was about 4220 mg/kg B.W and this considered that the extract was safe when taken orally. Feeding thyme leaves to male wistar rats at 2 or 10% of standard diet for 6 week showed that thyme leaves were not toxic to rats (Haroun *et al.*, 2002). The estimation of (LD_{50}) may differs in its value among other studies which were achieved, this was due to the difference in thyme sources and consequently difference in chemical composition, the difference in laboratory animals used, their species and number, the method of (LD_{50}) calculation and other circumstances that related to the researchers. In conclusion, thyme extract and its oil showed activities against test microorganisms, suggesting that they might be used for food preservation, and prevent candidal infections in addition to other methods such as using of different doses of ionization, radiation and temperature.

Further studies will be done to investigate the best extraction method for obtaining pure essential oil with determination of chemical analysis and antimicrobial activity against other pathogenic microorganism.

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