Study of antibacterial activity of *Thyme vulgaris* leaves water extract against different pathogenic bacteria isolated from UTI infections and antitumor activity *in vitro*

Bushra Hindi Saleh*

College of Biotechnology/Al-Nahrain University, Iraq

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**Abstract**

*Thyme vulgaris* leaves water extract were tested for antibacterial activity against different pathogenic bacteria isolated from UTI infection and antitumor activity on AMN3 cell line. *Thyme vulgaris* leaves water extract at lower concentrations (20,40) mg/ml have strong antibacterial activity against pathogenic bacteria (Staph aureus, Pseudomonas aeruginosa) while Escherichia coli were resistant to the lower concentration. Results reveals that all tested bacteria were sensitive to the higher concentration (60,80)mg/ml of thyme leaves water extract. The antitumor activity of thyme leaves water extract were tested on AMN3 cell line in which the extract have strong antitumor activity that was dose and time dependent. Thyme leaves water extract at higher concentration of (100,125) mg/ml have strong cytotoxic effects on AMN3 cell line after 72hrs.

**Citation:**

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1. Introduction

Thyme is a small leaves sprout from stem, pink or purple flowers with unique characteristic odor, a little bit hot taste growing over most moderate climate areas (Ingram et al, 2014). Thyme (*Thyme vulgaris*) have a percentage of Tannins, Saponins and volatile oils. The essential oil of common thyme (*Thyme vulgaris*), contains 20–54% thymol, 44% of phenols which mainly consists 3.6% Karvacrol, as confirmed by studies. Polyphenolic acids which are Caffeic acid, Triterpene, Rosmaric acid and Oleanic acid, while resins, gums and tannins are about 10% of the components of this plant also its contains a range of additional compounds, such as p-cymene, myrcene, borneol and nellalool that have an anti-bacterial properties (Grieve, 2008). Thymol has strong antimicrobial attributes when used alone or with other biocides. Numerous studies have demonstrated the antimicrobial effects of thymol, ranging from inducing antibiotic susceptibility in drug-resistant pathogens to powerful antioxidant properties (Agnes et al., 2012). Research demonstrates that naturally occurring biocides such as thymol reduce bacterial resistance to antibiotics through a synergistic effect, In addition, there is evidence that thymol has antitumor properties (Fayad et al., 2013).

Urinary Tract Infection (UTI) is described as the microbial invasion of any tissues of the urinary tract. Also its known as acute cystitis or a bladder infection when infection affects lower part of the urinary tract and when it affects the upper urinary tract its known as pyelonephritis. Urinary tract infections caused by different pathogenic bacteria occur in common. Rarely they may be due to viral or fungal infections (Pavam et al., 2010). Normally, the urinary tract is sterile, but infections can be caused by a variety of conditions. They can cause complicated or uncomplicated, symptomatic or asymptomatic infections (Hallin and Rosin, 2012). Anatomically UTI can be divided into upper and lower tract infections (Bagshaw and Laupland,
Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder. Although the urinary system is designed to keep out such microscopic invaders, these defenses sometimes fail. When that happens, bacteria may take hold and grow into a full-blown infection in the urinary tract (Fihn, 2003).

**Justification of Research**

Escherichia coli has been found as the most common uropathogenic bacteria in different season. The second most common pathogenic agent has been shown to be pseudomonas aeruginosa then Staphylococcus spp (Hotton, 2000).

**2. Material and Methods**

**Isolation of bacteria**

For isolation of bacteria causes UTI infection, (41) urine samples were collected from patients with UTI infections from (2) hospital in Baghdad from 20/3/2014 to 1/8/2014 in which urine sample were collected using a sterile container. The classical methods for diagnosis pathogenic bacteria in urine is based on urine culture on different microbiological media including Blood agar, nutrient agar and MacConkey agar then incubated at 37°C for 24 hrs.

For further diagnosis of gram negative bacteria in urine sample, selected isolated colonies were further streaked on EMB agar plates, also non – lactose fermenting colonies were streaked on King A, King B medium for isolation of pseudomonas aeruginosa. For isolation of gram positive staph spp in urine sample, selected colonies from blood agar were sub cultured on Mannitol salt agar and incubated at 37°C for 24 hrs.

**Identification of bacteria**

The diagnostic procedures consisted of direct microscopy observation, Gram staining, Biochemical tests, Catalase and coagulase tests.

**Identification of Gram negative bacteria by API 20E system**

Identification of the isolates was carried out by sub-culturing suspected colonies from MacConkey agar plates on API 20E microtubes system. This system is designed for the performance of 20 standard biochemical tests.

**Identification of Staph aureus**

Selective isolated colony from mannitol salt agar were further identified by Catalase test and Coagulase test.

**Antimicrobial susceptibility**

Test All aspects of this procedure were standardized according to NCCLS to ensure consistent and accurate results.

**Preperation of bacterial samples**

Inoculum from test bacteria (E.coli, pesudomonas, staph aureus ) were cultured in brain heart broth and incubated at 37°C for 24hrs, then a broth culture was diluted to match a 10^7 McFarland turbidity standard, which is roughly equivalent to 150 million cells/ ml.

**Incubation Procedure**

Media used in this test was Mueller-Hinton agar. Using an aseptic technique, place a sterile swab into the broth culture of a specific organism then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube, streak on the Mueller-Hinton agar plate to form a bacterial lawn. Allow the plate to dry for approximately (5) minutes, then used a flame-sterilized forceps and gently press each disc containing specific antibiotics to the agar to ensure that the disc is attached to the agar. Plates should be incubated overnight at an incubation temperature of 37°C before reading the results.

**Preparation of aqueous extract of Thyme vulgaris leaves**

Aqueous extract of *Thyme vulgaris* leaves was prepared by mixing 15.0gm of dry plant leaves with 100 ml of sterile distilled water in a 250 ml sterile conical flask with occasional shaking. The extract was filtered through a gauze for coarse residue then filtered through Whatman filter paper No.1 and finally filtered with milipore filter 0.45 mm then through 0.22mm. The filtrates were then concentrated by using rotavapour and stored in universal bottles at 4°C in refrigerator prior to use.

Antibacterial Susceptibility test for aqueous extract of *Thyme vulgaris* leaves

Antibacterial Susceptibility testing for a aqueous extract of thyme leaves was done by using well diffusion method. To check antibacterial activity of thyme leaves water extracts, sterile Muller Hinton agar plates were used then with Pasteur pippete (5) well were done that used to put different
concentration of thyme leaves water extract in which the center well was used to put the negative control (normal saline). 0.2 ml of bacterial suspension was inoculated with micropipette and spread uniformly with a sterile cotton swab over agar surface then allowed to dry for 5 minutes. 0.1 ml of different concentrations of Thyme vulgaris leaves aqueous extracts (20, 40, 60, 80) mg/ml were loaded in wells, while 0.1 ml of normal saline were used as negative control in center well. Plates were observed after 24hrs of incubation at 37°C for appearance of zones of inhibition around the wells. Antibacterial activity was evaluated by measuring diameter of zones of inhibition in (millimeters) of bacterial growth.

Cell Lines

Cell lines used in this study were kindly provided by the Iraqi Center for Cancer and Medical Genetics Research (ICCMGR).


This murine mammary adenocarcinoma cell line was derived from first in vivo passage for spontaneous mammary adenocarcinoma of female BALB/c mice. Passage 50-55 was used throughout this study, cells were maintained using RPMI-1640 with 10% FCS.

Viable Cell Count

Viable cell counting were accomplished using trypan blue stain. Cell suspension was prepared at high concentration by trypsinization and resuspension in known volume. The cover slip fixed in its place on a clean haemocytometer. One part of cell suspension 0.2 ml of cells were add to one part of trypan blue 0.2 ml and eight parts 1.6 ml of PBS and mixed together. Transferred 20 µl of the sample to the edge of the cover –slip along running in to counting chamber. After counting by using light microscope under x40 objective was accomplished. Starting with one chamber of haemocytometer, cells were counted in 1 mm center square, then four 1 mm center square were counted and separate count of viable and non-viable cells were done. Cell concentration (cell /ml), total cell count and cell viability (%) were calculated.

Total cell count = C (cell /ml) × the original volume of fluid from which the cell sample was removed

Cytotoxicity assay

Cell cultures in microtitration plate (96 wells) were exposed to Thyme vulgaris water extract at different concentration during the log phase of growth and the effect were determined after recovery time. Cells were collected after adding 2-3 ml of Trypsin –versin not more than few minutes. After trypsinization, cells were counted in order to get final concentration 1×10^6 cell /well. Afterward, 200 µl of cell suspension was seeded in each well within sterilized microtitration plates, the plate was covered with microtitration lid and sealed with adhesive parafilm then incubated for 24 hours at 37°C. The plates were checked if the cell were formed confluent monolayer and there is no contamination, then the medium was removed and 200 µl of serial dilutions of thyme water extract at concentration (25, 50, 75, 100 and 125) mg/ml were added. Three replicates were used for each concentration. The middle two columns were used as a control (cells treated with serum free medium only). The plates were incubated at 37°C for the selected exposure time 24, 48 and 72 hours. After exposure, the medium was decanted off and cells in the well were gently washed by adding and removing 0.1 ml of warmed sterile phosphate buffer saline for two times. At the end, 200 µl of crystal violet were added to each well, the plates were incubated at 37°C for 20 min. At the end of incubation, excess dye was removed by washing the well three times with phosphate buffer saline and let it to dry. The absorbance was determined by using an ELISA reader at wave length 492 nm.

\[
\text{IR} = \frac{A - B}{A} \times 100
\]

IR: inhibitory rate, A: read of control, B: read of treated cell.

3. Results and discussion

Laboratory diagnosis

Isolation and Identification of Enteropathogenic Escherichia coli, Pseudomonas aeruginosa and staph aureus

Among the total of (41) urine samples that were collected, only (19) isolate (46.3%) were gave typical morphological characteristics and biochemical test that related to Escherichia coli while (10) isolates (24.3%) were gave typical morphological characteristics and biochemical test that related to Pseudomonas aeruginosa and (5) isolates (12.3%) related to staph aureus, while the rest (7) isolates (17%) related to different genera as shown in.
Table 1: Number of isolates and their percentage

<table>
<thead>
<tr>
<th>Hospitals</th>
<th>No of samples</th>
<th>No of Pseudomonas Aeruginosa isolates</th>
<th>No of staph aureus isolates</th>
<th>No of E.coli Isolates</th>
<th>No of other strain</th>
<th>Total No of selected isolates</th>
<th>Percentage of selected isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khadymsia</td>
<td>25</td>
<td>6</td>
<td>3</td>
<td>12</td>
<td>4</td>
<td>41</td>
<td>51.2</td>
</tr>
<tr>
<td>Al-yarmook</td>
<td>16</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>31.7</td>
</tr>
<tr>
<td>Total No</td>
<td>41</td>
<td>10</td>
<td>5</td>
<td>19</td>
<td>7</td>
<td></td>
<td>82.9</td>
</tr>
</tbody>
</table>

Diagnosis of Escherichia coli is depends upon isolation and laboratory identification of the bacterium. The samples were directly streaked on MacConkey and EMB agar and incubated at 37°C for 24 hrs. On MacConkey agar, deep red colonies are produced. Growth on EMB agar produces colonies with a greenish metallic sheen.

For isolation of Pseudomonas aeruginosa, samples were directly streaked on MacConkey agar and incubated at 37°C for 24 hours, its form colorless colonies, 1-3 mm in diameter when cultured on the surface of MacConkey agar. Frequently isolates obtained from urinary tract infections, has a mucoid appearance which is attributed to the production of alginate slime (Pavam et al., 2010). P. aeruginosa is often preliminarily identified by grape-like or tortilla-like odor of aminoacetophenone in vitro (Warren et al., 2000). For confirmative diagnosis of Pseudomonas aeruginosa, colonies were streaked further on selective medium (King A, King B) agar plates and incubated at 37°C for 24 hours. (King A) medium is very useful for isolating Pseudomonas spp from clinical specimens. Pseudomonas isolate form a blue or blue-green pyocyanin pigment on it. The pigment diffuses into the surrounding growth. Also Pseudomonas aeruginosa produced pyoverdine pigments on King B media that fluoresce under ultraviolet light.

For identification of staph aureus, colonies were cultured on blood agar. It's appears beta hemolysis on it. Then its subcultured on Mannitol-Salt agar that differentiates S. aureus from other catalase positive gram postive cocci likes S. saprophyticus. S. aureus grows on Mannitol-Salt agar medium containing 7.5% NaCl which inhibits the growth of many other organisms also can ferment mannitol into acid that detected by the change in pH indicator from red to yellow (Hanselman et al., 2009).

Identification of gram negative bacteria by API 20E system

In API 20E system, Escherichia coli colonies were subjected to further identifications. It's give negative results in Arginine Dihydrolase (ADH), Citrate utilization (CIT), H2S production, urease production (URE), Tryptophane deaminase (TDA), Voges Proskauer (VP), Gelatin liquefaction (GEL). Isolates were give positive results in Indole production (IND), Blactamase test (ONPG), lysine decarboxylase (LDC), ornithine decarboxylase (ODC). Glucose, mannitol, Sorbitol, rhamnose, melibiose fermentation but give negative results insucrose, inositol, amygdaline fermentation. The result of API 20E system reveal that only 19 isolates from 28 isolates were identified as Escherichia coli.

Pseudomonas aeruginosa colonies were identified by API 20E system. It give positive results in Arginine Dihydrolase (ADH), Citrate utilization (CIT), Arabinose fermentation (ARA). It give variable results in Gelatin liquefaction (GEL). Strains were gave negative results in B-lactamase test (ONPG), Lysine Decarboxylase (LDC), Ornithine Decarboxylase (ODC), H2S production, urease production (URE), Indole production 14 (IND), Tryptophane Deaminase (TDA), Voges - proskauer (VP), Mannitol, inositol, Sorbitol, Rhamnose, sucrose, Melibiose, Amygdaline, Glucose Fermentation. The results mentioned above were in agreement with those mentioned by (Kenneth 2011).

Identification of staph aureus

Pure colonies of staph aureus that ferment mannitol in mannitol salt agar show positive results for catalase and Coagulase enzyme.

Antibiotic susceptibility test

Results of Antibiotic susceptibility test for pathogenic bacteria isolated from UTI infections reveals that Escherichia coli isolates were 100% sensitive to trimethoprim/sulphamethozaxole, ciprofloxacine and nalidixic acid. Also 66.6% of them were sensitive to gentamycine, amoxicillin and tetracycline as shown in table (2).
Results also reveals that 100% of isolates were resistant to bacitracin, cefixime, erythromycin and ampicillin, 66.6% of them were resistant to chloramphenicol and nitrofurantoin as shown in figure (1.1).

Results of Antibiotic susceptibility test for pseudomonas aeruginosa isolates reveals that 100% of them were sensitive to Trimethoprim/sulphamethoxazole, ciprofloxacin, also 33.4% of them were sensitive to gentamycine, nalidixic acid, amoxicillin and chloramphenicol. Results also reveals that 100% of isolates were resistant to cefixime, erythromycin and ampicillin, and 66.6% of them were resistant to bacitracin and nitrofurantoin as shown in table (2).

Results of Antibiotic susceptibility test for staph aureus isolates reveals that 100% of them were sensitive to Trimethoprim/sulphamethoxazole, Gentamycine, chloramphenicol and nalidixic acid, 66.6% of them were sensitive to ciprofloxacin, amoxicillin and cefixime. Results also reveals that 100% of isolates were resistant to ampicillin, 66.6% of them were resistant to bacitracin, erythromycin and nitrofurantoin and tetracycline as shown in figure (1.2).

Table 2: Antimicrobial susceptibility test for E.coli, pseudomonas aeruginosa and Staph aureus isolates.

<table>
<thead>
<tr>
<th>NO. of bacteria</th>
<th>SXT (25 mg/ml)</th>
<th>CIP (5 mg/ml)</th>
<th>CN (10 mg/ml)</th>
<th>E (15 mg/ml)</th>
<th>AM (25 mg/ml)</th>
<th>F (100/100 mg/ml)</th>
<th>AX (25 mg/ml)</th>
<th>T (30 mg/ml)</th>
<th>NA (50 mg/ml)</th>
<th>B (10 mg/ml)</th>
<th>CFM (5 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E2</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E3</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>PS1</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>PS2</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>PS3</td>
<td>I</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>SA1</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>I</td>
<td>R</td>
<td>I</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>SA2</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>I</td>
<td>R</td>
<td>I</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>SA3</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
</tbody>
</table>

NO. number; PS. Pseudomonas; SA. Staph aureus; E. Escherichia coli, S. sensitive, R. resistant, I. intermediate.

In another survey, a total of (17) Escherichia coli isolates were collected from urine specimens of patients with urinary tract infection. Antibiotics sensitivity test indicated that amikacin followed by chloramphenicol and ciprofloxacin are the most effective antibiotics (Rosa et al., 2001)

Isolates from urine samples obtained during 1999 were identified and their susceptibility to antimicrobial agents were studied. The susceptibility studies showed that E.coli resistant to
E. coli bacteria often carry multiple drug resistance plasmid and under stress, readily to transfer those plasmids to other species. Indeed E.coli have a frequent member of biofilm where many species of bacteria in close proximity to each other. This mixing of species allows E.coli that are piliated to accept and transfer plasmids from and to other bacteria. Thus, E. coli and the other enterobacteria are important reservoirs of transferable antibiotic resistance (Hanselman et al., 2009).

The low susceptibility to antibiotics is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes (mexAB, mexXY) and the low permeability of the bacterial cellular envelopes.

Moreover, Pseudomonas resistance may obtained from antibiotic resistance plasmids, both R-factors and RTFs (Ravichandra et al., 2012). In addition to this intrinsic resistance, P. aeruginosa easily develops acquired resistance either by mutation in chromosomally-encoded genes or by the horizontal gene transfer of antibiotic resistance determinants (Rahual et al., 2009). Only few antibiotics were effective against Pseudomonas aeruginosa, isolated from UTI infections including fluoroquinolones, gentamicin and imipenem, and even these antibiotics are not effective against all strains (Nadia et al., 2004).

Detection of antibacterial activity of thyme vulgaris leaves water extract against different pathogenic bacteria isolated from UTI infection.

For detection antibacterial activity of thyme leaves water extract against different pathogenic bacteria isolated from UTI infections. Different concentration of Thyme vulgaris leaves water extract were used. Results reveals that thyme leaves water extract at lower concentrations(20,40) mg/ml have strong antibacterial activity against pathogenic bacteria (Staph aureus, Pseudomonas aeruginosa) in which the diameter of zone of inhibition range from (19-25)mm for the Staph aureus and from (15-24)mm for Pseudomonas aeruginosa as shown in figure (2.1) also increased the concentrations of thyme leaves water extract lead to increased the diameter of zone of inhibition for the higher concentration (60,80)mg/ml to range from (28-36)mm for Staph aureus as shown in figure (2.2) and (25-32)mm for Pseudomonas aeruginosa as shown in table (3).

While Escherichia coli were resistant to the thyme leaves water extract at lower concentration (20,40) mg/ml, but it has moderate zone of inhibition at higher concentrations(60,80)mg/ml to reach (15-20)mm.
Table 3: Antimicrobial activity of *Thyme vulgaris* leaves water extract on growth of *E. coli*, *Pseudomonas aeruginosa*, and *Staph aureus* isolates.

<table>
<thead>
<tr>
<th>Bacteria Spp</th>
<th>Concentration of thyme water extract (mg/ml)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 Mg/ml</td>
<td>40 Mg/ml</td>
</tr>
<tr>
<td>SA1</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>SA2</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>SA3</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>PS1</td>
<td>16</td>
<td>24</td>
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<td>PS2</td>
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<td>20</td>
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<tr>
<td>PS3</td>
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<td>21</td>
</tr>
<tr>
<td>E1</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>E2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>E3</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>

SA: *Staph aureus*; PS: *Pseudomonas aeruginosa*; E1: *Escherichia coli*

In a study in Oman, thyme vulgaris shows high antibacterial activity against all tested microorganism *staph aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* at different concentrations in which inhibition zone was between (12-20) mm as compared to the standard drug Amoxicillin (Aisha et al., 2013). The anti-bacterial activity of Thyme studied at different concentrations and using two types of pathogenic bacteria *Escherichia Coli* and *staph aureus*. Thyme leaves water extract has shown higher activity at concentration (50) mg/ml in which the diameter of zone of inhibition was (21) mm for *Escherichia Coli* and (15) mm for *staph aureus*, followed by the rest of the varying concentrations and rates (Fayad et al., 2013). Thyme constituent such as carvacrol and flavonoids exhibited a good antimicrobial properties against gram-negative bacteria such as *Salmonella typhimurium*. The main constituents of thyme include thymol, tannin, saponin and triterpenic acids have good antibacterial properties against Gram negative bacteria *Escherichia coli* (Al-Muhna, 2010).

Among the essential oils thymol, linalool, carvacrol, and cinnamon were found to be the most successful against various *Staphylococcus* species, including the dreaded MRSA. It is effective against other forms of bacteria like *Salmonella*, *Enterococcus*, *Escherichia*, and *Pseudomonas* species. (Mercola, 2010). The antimicrobial activity of active compounds like Thymol, a phenolic constituent of thyme oil, showed the highest activity against the *S. aureus* (20.1 mm), against the *B. subtilis* (20.0 mm) (Tamara et al., 2006).

Study of cytotoxic activities of *Thyme vulgaris* leaves water extract on cell line.

Study the inhibitory rate of *Thyme vulgaris* leaves water extract on AMN3 cell line passage 50-51 reveal that *Thyme vulgaris* water extract have a concentration dependent effect on the viability of AMN3 cells. Cell viability decrease significantly with increase the concentration of *Thyme vulgaris* water extract in which (IR) ranged from (15.44%) for the lower concentration (25) mg/ml to reach (39.75%) for the higher concentration (125) mg/ml after (24) hrs.

According to the time factor extended treatment for (48) hrs revealed that there was gradual increase in growth inhibition for lower concentration (50, 75) mg/ml to reach (30.1% and 38.18%) as seen in table (4). At concentration (100) mg/ml, thyme water extract caused significant growth inhibition (47.99%), also increase the concentration of thyme water extract to (125) mg/ml result in significant reduction in cell viability to reach (65.69%). According to the time factor, cell viability decrease significantly with increasing the concentration after (72) hrs of treatment as in (figure 4). The highest significant inhibition in cell viability (60.23% and 76.41%) respectively were obtained by the concentration (100, 125) mg/ml after 72 hrs. When concentration of toxin were fixed and compared IR at (24) (48) hrs and (72) hrs revealed that p value are significant among all (p<0.05) as shown in table (4).
Table 4: Inhibition growth rate of AMN3 cell line after exposure to *Thyme vulgaris* leaves water extract at different concentration for three exposure time (24, 48 and 72) hrs

<table>
<thead>
<tr>
<th>Conc. Of Thyme leaves water extract mg/ml</th>
<th>Mean (IR)</th>
<th>SE</th>
<th>Mean (IR)</th>
<th>SE</th>
<th>Mean (IR)</th>
<th>SE</th>
<th>P</th>
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<tr>
<td>25</td>
<td>15.44</td>
<td>2.00</td>
<td>28.44</td>
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<td>36.32</td>
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<tr>
<td>50</td>
<td>25.71</td>
<td>2.00</td>
<td>30.1</td>
<td>1.99</td>
<td>40.78</td>
<td>1.03</td>
<td>0.001</td>
</tr>
<tr>
<td>75</td>
<td>28.20</td>
<td>1.32</td>
<td>38.18</td>
<td>1.32</td>
<td>46.20</td>
<td>1.22</td>
<td>0.001</td>
</tr>
<tr>
<td>100</td>
<td>30.21</td>
<td>1.06</td>
<td>47.99</td>
<td>1.41</td>
<td>60.23</td>
<td>1.85</td>
<td>0.001</td>
</tr>
<tr>
<td>125</td>
<td>39.75</td>
<td>2.00</td>
<td>65.69</td>
<td>0.21</td>
<td>76.41</td>
<td>1.00</td>
<td>0.003</td>
</tr>
</tbody>
</table>

P1: differences among all, IR: Inhibitory rate, SE: Standard Error

**Figure (3):** Effect of *Thyme vulgaris* leaves water extract at different concentration of viability of AMN3 cell line at (24, 48, 72) hrs

In a study used to assess antileukemic effect of *thyme vulgaris* on monoblastic leukemic THP-1 cells lines demonstrated that these plants may display an anticancer effect by different mechanisms, including suppressing the initiation or reversing the promotion stage in multistep (Doa, 2013). The anticancer effect of *thyme vulgaris* extract (TVE) in CRC cells was inhibits proliferation in a concentration and time dependent manner. *T. vulgaris* could have an anticancer effect and that some of its bioactive compounds may prove to be effective treatment modalities for human CRC (Sepidehand Sadegh, 2016). Cytotoxic effects of thyme water extract. Against cancer cell line (HeLa, Hep) cell line and normal cell line (Ref) reveals a dose-dependent decrease in survival of the tumor and normal cell lines. However, thymol exhibited stronger cytotoxicity at concentration (30.5) mg/ml towards human cell lines. The inhibition effect of thymol on (HeLa, Hep) cell in significant manner higher than Ref cell line may be due to high cytotoxicity of thymol toward cancer cell line (Reema, 2011). Thyme essential oil which contains carvacrol and thymol are the major components that have an important in vitro cytotoxic activity against tumor cells. The molecular mechanism of the observed cytotoxicity may owing to their lipophilic nature, plant volatile compounds appear to accumulate in the cell membrane and increase its permeability, resulting in leakage of enzymes and metabolites (Tanaka, 2007). Cytotoxicity of thyme essential oil was investigated on head and neck squamous cell carcinoma (HNSCC) cell line. The IC$_{50}$ of thyme essential oil extract was 369 µg/ml. Genes involved in the cell cycle, cell death and cancer were involved in the cytotoxic activity of thyme essential oil at the transcriptional level. The three most significantly regulated pathways by thyme essential oil were interferon signaling, N-glycan biosynthesis and extracellular signal-regulated kinase 5 (ERK5) signaling, thus Thyme essential oil inhibits human HNSCC cell growth based on pharmacogenic approaches (Sertel et al., 2011). The anti-tumor effect of the Moroccan endemic thyme (*Thymus broussonetti*) essential oil (EOT) was investigated in vitro using the human ovarian adenocarcinoma IGR-OV1 parental cell line. These cell lines elicited various degrees of sensitivity to the cytotoxic effect of EOT. The cytotoxic activity begins at 3 hrs of incubation and increases in a
References


Doa’a F., 2013. Effect of Origanumsyrriacum, Hibiscus sabdariffa, Thyme vulgaris and Pelargonium graveolensExtracts on Human Leukemia THP-1 Cells in Vitro .MSC.Biological Sciences/ Medical Technology.Palestine. 28


Kenneth T., 2011. Pseudomonas aeruginosa.Rev. Todar online textbook of bacteriology diagnosis. 29


National committee for clinical laboratory standard,(2001). Performance standard for antimicrobial susceptibility testing.NCCLS.


