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Evaluating The Effect of Thyme Extract on Inhibiting Gene Expression of Virulence Factors in Acinetobacter Baumannii and Studying Its Effect on Biofilm Formation

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Abstract

Because of the resistance of this bacteria to many antibiotics, it has become necessary to find alternatives to antibiotics or materials that work synergistically with antibiotics in order to control and inhibit the growth of this bacteria, which poses a great danger to patients with burns and wounds. Therefore, it has been proposed that this plant extract be used to prove its effectiveness. Grind the dried thyme leaves to obtain a fine powder. Place 10 grams of thyme powder in a beaker containing 100 ml of distilled water (10% w/v concentration). Heat the mixture in a water bath at 60-70°C for 30-60 minutes with continuous stirring. After heating is complete, let the mixture cool to room temperature. Filter the extract using filter paper or a fine strainer to obtain the pure extract. To prepare several concentrations of the aqueous extract, dilute the basic extract (10%) to the desired concentrations, such as 2.5%, 5%, and 7.5%. Use the following equation to determine the volume of the aqueous extract and the volume of distilled water required for each concentration: C1×V1=C2×V2C1 \times V1 = C2 \times V2C1. Effect of different concentrations of thyme extract (2.5, 5%, 7.5, and 10), where a significant effect of this plant was observed in inhibiting the biofilms of these bacteria, and the concentration of 7.5 and 10 gave the best results in inhibiting the biofilm of these bacteria. The mechanism of inhibition can be explained by the fact that thyme contains active compounds such as thymol and carvacrol, which have antibacterial properties.

Keywords: Thyme Extract, Gene Expression, Acinetobacter Baumannii

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Introduction

Thymus Vulgaris is a common wild plant with a distinctive smell that is used as a type of flower or spice. [1,2] It belongs to the Labiatae family of plants. [3] It is colloquially called Zaatar or Thyme, as mentioned in Arabic medicine. [4,5] It is considered one of the most powerful antiseptics and natural antibacterial agents. Thyme is regarded as a perennial plant with many branches and is covered with stems. [6,7] Its height ranges from 30 to 80 cm. Its leaves are opposite, almost oval, covered with white fuzz. [8] The flowers appear from the tops of the plant in the form of a profuse group. [9] They are small, labial flowers of a purple or red color that appear in the spring and summer

months. [10] This plant usually grows in rocky and mountainous places, on roadsides, and around grain fields [11]. Thymol is considered the most active ingredient found in thyme. Anti-fungal and antibacterial properties characterize it, as it can prevent fungal and viral infections and fight different types of bacteria, thus working to raise and strengthen the immune system. [12] Thyme is also rich in vitamin C, which works to stimulate the production of blood cells. [13] White blood is the first line of defense in the body's immune system. [14] Thyme is considered a rich source of potassium and various minerals such as iron, calcium, and manganese [15]. These minerals play an important role in bone health and encourage bone growth, in addition to strengthening bones and reducing the incidence of various bone diseases. Thyme baths are useful in relieving joint pain, strengthening the body, and removing fatigue.

Methodology

Preparation of aqueous extract of thyme

Grind the dried thyme leaves to obtain a fine powder. Place 10 grams of thyme powder in a beaker containing 100 ml of distilled water (10% w/v concentration). Heat the mixture in a water bath at 60-70°C for 30-60 minutes with continuous stirring. After heating is complete, let the mixture cool to room temperature. Filter the extract using filter paper or a fine strainer to obtain the pure extract. To prepare several concentrations of the aqueous extract, dilute the basic extract (10%) to the desired concentrations, such as 2.5%, 5%, and 7.5%. • Use the following equation to determine the volume of the aqueous extract and the volume of distilled water required for each concentration: C1×V1=C2×V2C1 \times V1 = C2 \times V2C1. [12].

Preparation of bacterial samples

Use a fresh culture of Acinetobacter baumannii to obtain a sufficient number of bacteria. Cultivate the bacteria in a nutrient medium (such as nutrient broth) to obtain a logarithmic growth phase.

Treatment of samples with aqueous extract:

Divide the bacteria into different experimental groups, each treated with a specific concentration of aqueous extract (such as 2.5%, 5%, 7.5%, and 10%), plus a control group without extract. Incubate the samples at 37°C for a specific time (such as 24 hours).

Evaluation of the effect of the extract on the biofilm

Use microtiter plate assay to assess biofilm formation. Add crystal violet to distinguish biofilms formed on the walls of the wells, then measure the absorbance at a wavelength of 570 nm. Compare the biofilm formation between the groups treated with the different extracts and the control group. [5]

Gene expression analysis

Extract RNA from the samples after treatment

Use the reverse transcription technique to determine the effect of the extract on gene expression of virulence factors associated with the gene, such as genes responsible for enzyme production and adhesion. Perform quantitative polymerase chain reaction (qPCR) to determine the change in gene expression levels compared to the control group. If the aqueous extract of thyme is effective in inhibiting the biofilm, you will notice a decrease in absorbance when measuring the biofilm. For gene expression, the results are expected to show a reduction in the expression of genes associated with virulence factors. The target genes are ompA (associated with adhesion) and bap (associated with biofilm formation).

Results

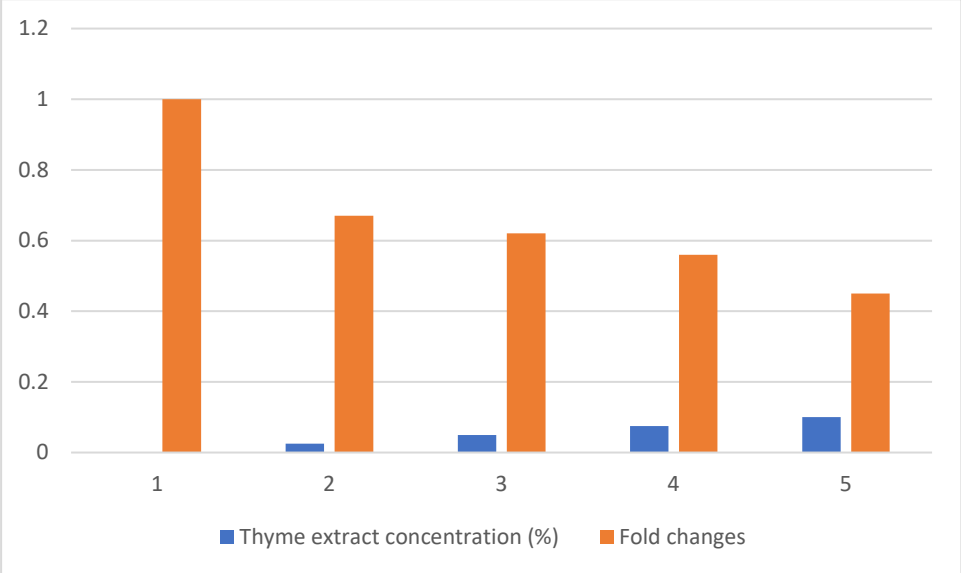

Figure 1 Effect of thyme extract on inhibiting biofilm formation

Figure 1 shows the effect of different concentrations of thyme extract (2.5, 5%, 7.5, and 10), where a significant impact of this plant was observed in inhibiting the biofilms of these bacteria, and the concentration of 7.5 and 10 gave the best results in inhibiting the biofilm of these bacteria. The mechanism of inhibition can be explained by the fact that thyme contains active compounds such as thymol and carvacrol, which have antibacterial properties. These compounds can affect the biofilm by penetrating it and interfering with its formation process. Thyme components may affect the cellular signals that regulate the response of bacteria to stress, leading to a reduction in their ability to form biofilms. Thyme can increase the permeability of the cell membrane to bacteria, leading to the leakage of vital substances and disruption of cellular activity. It can work synergistically with other antibiotics, enhancing its effectiveness in inhibiting biofilms.

Table 1 Effect of thyme extract on inhibiting bap associated with biofilm formation of Acinetobacter baumannii

Thyme extract concentration (%)	Fold changes
Control	1
2.5%	0.67
5%	0.62
7.5%	0.56
10%	0.45

Table 1 shows the effect of thyme extract on the inhibition of a gene associated with biofilm formation, where we notice a clear change in the effect of this extract on the gene, as gene expression decreased at a concentration of 2.5% to 0.67 compared to the control, and the decrease in gene expression increased at concentrations of 5%, 7.5% to 0.62, 0.56. Respectively, the concentration was given from 10% to 0.45, and it was the best concentration compared to the rest of the concentrations and compared to the control. Thyme contains active compounds, such as thymol and carvacrol, which have antimicrobial properties. These compounds can affect the permeability of the cell membrane of bacteria, causing disturbances in the structure and function of the cell membrane. This effect can lead to reduced stability of molecules associated with biofilm formation, thus reducing the gene expression of genes responsible for this formation. Inhibition of a gene associated with biofilm formation when using thyme extract could be the result of multiple effects, including

disruption of the cell membrane, inhibition of cell communication systems, increased oxidative stress, inhibition of enzymes, and influence on regulatory factors of gene expression.

Table 2 Effect of thyme extract on inhibiting ompA associated with adhesion of Acinetobacter baumannii

Thyme extract concentration (%)	Fold changes
Control	1
2.5%	0.55
5%	0.34
7.5%	0.23
10%	0.22

Table 2 shows the effect of thyme extract on inhibiting the gene associated with bacterial adhesion ompA, where a significant decrease in gene expression was observed as the concentration of the extract increased. The 10% concentration was the best concentration in inhibiting the gene compared to the rest of the concentrations, as the gene expression decreased to 0.22 compared with control 1. The decrease in gene expression of the adhesion-related ompA gene in Acinetobacter baumannii bacteria when using concentrations of thyme extract can be explained by several reasons, including cell communication systems playing a role in regulating the expression of adhesion-related genes. Thyme compounds may interfere with these chemical signals, reducing the activity of the systems and thus reducing gene expression of the ompA gene, which contributes to adhesion to host cells or surfaces.

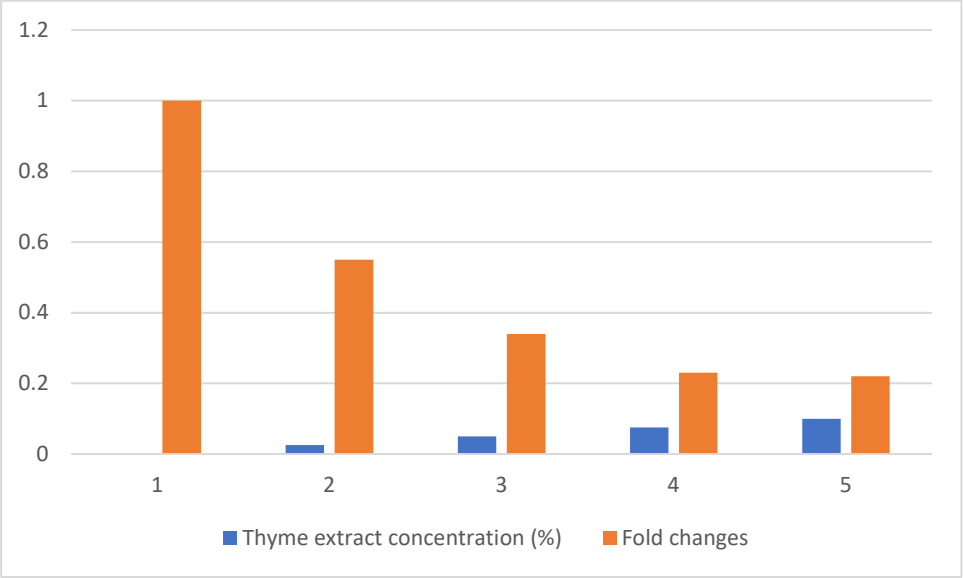

Figure 2 Effect of thyme extract on inhibiting ompA associated with adhesion of Acinetobacter baumannii

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