Jurnal Biologi Volume: 2, Nomor 3, 2025, *Page*: 1-10

Comparative Evaluation of the Antibacterial Activity of Aqueous and Ethanolic Extracts of (Thymus vulgaris) Against Acinetobacter baumannii

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DOI: https://doi.org/10.47134/biology.v2i3.4531
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Received: 11-03-2025
Accepted: 18-04-2025
Published: 30-05-2025

Abstract: Acinetobacter baumannii is a Gram-negative, pathogenic bacterium associated with multidrug-resistant hospital infections, making it a prime target for research into alternative treatments the work aimed to study the antibacterial properties of alcoholic and aqueous extracts of thyme. One hundread blood samples were collected from patients admitted to the Intensive Care Unit (ICU) at Sharqat General Hospital for the period from 1/9/2024 to 30/3/2025. Patient information was recorded, including patient name, age, and type of infection related to the patient's condition. Bacterial isolates were diagnosed based on phenotypic characteristics of bacteria, colony growth, and basic biochemical tests. The antibacterial activity of the alcoholic and aqueous extracts was evaluated by well diffusion method at concentrations of (5, 10, 25, 50, and 100) µg/ml. The results of testing the effectiveness of the alcoholic and aqueous extracts of thyme against Acinetobacter baumannii bacteria showed that the alcoholic extract recorded higher activity at all concentrations compared to the aqueous extract. At the lowest concentration (5 µg/mL), the diameter of the inhibition zone for the alcoholic extract was 4.5 ± 0.5 mm, while it was 3.2 ± 0.4 mm for the aqueous extract. When the concentration was increased to 10 µg/mL, the activity increased to 7.2 ± 0.7 mm for the alcoholic extract versus 6.3 ± 0.6 mm for the aqueous extract. At a concentration of 25 µg/mL, the inhibition zone reached 10.2 ± 0.6 mm for the alcoholic extract and 9.8 ± 0.7 mm for the aqueous extract, which are slight differences but continue to favor the alcoholic extract. At a concentration of 50 µg/mL, the values were 15.2 ± 0.7 mm for the alcoholic extract and 13.5 ± 0.8 mm for the aqueous extract. Finally, at the highest tested concentration of 100 µg/mL, inhibition reached its maximum levels, recording 19.3 ± 0.8 mm for the alcoholic extract and 17.2 ± 0.9 mm for the aqueous extract The alcoholic extract showed higher inhibitory activity than the aqueous extract against A. baumannii at all tested concentrations.

Keywords: A.Baumannii , Tymus Vulgaris,Alcoholic , Aqueous, Well Diffusion Methods

Introduction

The discovery of penicillin by Alexander Fleming in 1928 marked a pivotal turning point in the history of modern medicine, revolutionizing the fight against bacterial diseases, previously a leading cause of death worldwide. With the introduction of penicillin as the first effective antibiotic into clinical use, mortality rates from bacterial infections declined significantly, paving the way for the development of new generations of antibiotics. This

achievement improved the quality of healthcare and reduced the burden of many serious infections. This was clearly reflected in the increase in global life expectancy, which nearly doubled over the following decades, thanks to better control of infectious diseases and lower rates of complications.

Antibiotic resistance (AMR) is one of the major health challenges facing humanity today. Resistance arises primarily as a result of the excessive and inappropriate use of antibiotics in human medicine and agriculture, coupled with weak control and prescription systems in many countries. In 2017, the World Health Organisation published a list of twelve bacteria classified as problematic due to their resistance to numerous widely available antibiotics. The encompassed bacteria include

Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacteriaceae , Enterococcus faecium, Staphylococcus aureus, Helicobacter pylori, Campylobacter spp., Salmonella spp., Neisseria gonorrhoeae, Streptococcus pneumoniae, Haemophilus influenzae, and Shigella spp. (fluoroquinolone-resistant).

Due to this growing challenge, it has become imperative to seek safe and effective therapeutic alternatives. Relying on natural products, especially those extracted from medicinal plants, is one promising solution in this field.

Medicinal plants have long been a rich source of biologically active substances with antimicrobial activities, anti cancer, anti-oxidant. Among these plants, Thyme (Thymus vulgaris) is a popular aromatic medicinal plant in traditional medicine. It is characterized by its wide spectrum of biological properties, particularly antibacterial properties. Thyme extract contains biologically active compounds such as thymol and carvacrol, which are phenolic compounds that have been proven effective in inhibiting the growth of many pathogenic bacteria, both Gram-positive and Gram-negative. These compounds disrupt the integrity of the bacterial cell membrane, leading to the leakage of vital cellular components and cell death. Acinetobacter baumannii (A. baumannii) is a Gram-negative bacterium belonging to the Bacillus subfamily. It is an opportunistic extracellular pathogen of humans, arising from hospital-acquired infections (HAIs), also known as nosocomial infections. Also known as "Iraqi" due to its occurrence in US military treatment facilities in Iraq, A. baumannii has rapidly become one of the most troublesome pathogens in healthcare settings worldwide and currently tops the list of priority pathogens for the development of new antibiotics. From this perspective, the current research aims to evaluate the antibacterial efficacy of both aqueous and alcoholic extracts of cardamom, and to compare their effects on Gram-positive and Gram-negative bacterial strains.

Methodology

Plant collection

Fresh leaves of the local medicinal herb Tymus vulgaris were obtained in September 2024 from local nurseries in Sharqat city. The collected plants were washed with running tap water, air-dried, and then ground into a fine powder using an electric mixer (Bosch GmbH, Germany). The resulting powder was then stored in a polyethylene bag for further analysis.

Ethanolic Extract preparation

The ethanolic extract was prepared by immersing 25 g of finely ground dried leaves in 100 ml of 80% (v/v) ethanol solution in a tightly sealed glass vial. The vial was placed at room temperature (25 ± 2°C) for 15 days, with intermittent manual shaking (twice daily) to ensure improved extraction efficiency. After the soaking period, the mixture was filtered using Whatman No. 4 filter paper to separate the filtrate from the plant sediment. The filtrate was then evaporated under reduced pressure using a rotary evaporator at a temperature not exceeding 40°C to obtain a concentrated extract. The ethanolic extract was stored in dark, tightly sealed bottles and refrigerated at 4°C until further use.

Aqueous Extract preparation

To prepare the aqueous extract, 25 grams of finely ground leaves were weighed and added to 100 ml of boiling distilled water (100°C). The mixture was then left in a water bath at 80°C for 1 hour with continuous stirring. The mixture was then allowed to cool to room temperature and filtered using Whatman No. 4 filter paper. The resulting extract was partially dried using a rotary evaporator under reduced pressure and then concentrated to the desired volume. The concentrated aqueous extract was then stored in sealed, opaque bottles at 4°C until use.

Isolation and identification of bacterial isolates

A total of 100 clinical specimens were collected from patients in Sharqat General Hospital intensive care unit (ICU) between September 2024 and March 2025. After obtaining a single colony of isolated bacteria, the isolates were identified depending on phenotypic colony characteristics, including size, shape, mannitol fermentation, and Novobiocin disc diffusion.

Diagnosis of Bacteria (Morphological Diagnosis)

Colonies were initially identified based on the phenotypic characteristics of isolated bacterial colonies cultivated on MacConkey and blood agar media, encompassing the form, colour, texture, odour, and size of the colonies. The diagnosis was substantiated with biochemical assays, including the indole test, methyl red test, urease test, oxidase test, and catalase test.

Hemolysin Production

This test was used to detect the ability of bacteria to product hemolysis enzyme. this test were done by streaking of pure isolates on blood agar, then incubated at 37ºC for 24 hrs. the result showed either non-hemolytic with vague creamy colonies on blood agar or a hemolysis around the colony should be detected for the positive result.

Antimicrobial Activity

A standard well diffusion approach involves examining the inhibitory effect of Aqueous and alcoholic extract solutions on the growth of A. baumannii bacteria as a Gram-negative bacterium, according to method adopted by Allalah et al.,2023 (10) with some modifications: first, the bacterial isolates were cultured in nutrient broth for 18 to 24 hours

at 37°C. Then, 0.1 ml of bacterial suspension was transferred to nutrient agar and left for 24 hours at 37°C. Finally, a single colony was added to a test tube containing 5 ml of regular saline to make the bacterial suspension. plates were incubated for 10 minutes after putting a part of the bacterial suspension onto the Mueller-Hinton agar, and this was done in close proximity to a concentration of 1.5 x 108 bacterial cells/ml according to McFarland standards. Using a sterile cork borer, further 5 mm wells were bored. Using a micropipette, around 1 ml of different concentration (5, 10, 25, 50 and 100) µg/mL of Aqueous and alcoholic extract solution was added to each well, and the plates were left to incubate at 37°C for one day. Following the inhibitory zone width measurements. As a control , sterile distilled water putted in place of the enzyme solution. Each isolate was tested three times in the experiment.

Ethics approval

This research was performed in compliance with the ethical standards established in the Declaration of Helsinki. Before acquiring the sample, the patient's agreement was secured through both written and verbal communication, after the review and approval of the study protocol and subject information by the local ethics committee (3299 in 15/10/2024).

Statistical Analysis

The study data were analyzed utilizing SPSS version 22.0, with each experiment conducted thrice to assure precision. All data were expressed as Mean ± Standard Deviation.

Result and Discussion

Diagnosis of *A. baumannii* by the morphological characteristics

The traditional methods for bacterial diagnosis rely on the phenotypic identification of the causal organism by bacteriological procedures, including culturing on selective media, Gram staining for microscopic characteristics, and colony morphology assessment. All above mentioned characteristics were studied on MacConkey agar and selective medium Chrom agar Acinetobacter.

The microscopic morphology when examined under the with gram stain. The results of grams staining and microscope test showed that all of 25 clinical isolates of *A. baumannii* isolates were gram-negative, cocco bacilli shape, occurring in singly, and occasionally arranged in diplococcic. In general, their shape of *A. baumannii* isolates different forms according to growth phase from bacilli to cocco-bacilli. diplococcus and some had short chains (13). Figure (1) illustrate the developing colonies have different properties according to the variant agar they are using. When isolates cultured on MacConkey agar to get pure isolates, the colonies appeared small , pink colour, pale and non-lactose fermenter (14). Colonies when cultured overnight on chrom agar media at 37°C for 24h, appear light purple with a halo around the colonies(15). While on Blood agar colonies were showed non-pigmented creamy, convex, mucoid with smooth surface in diameter 0.5-2 mm, and the colonies non -productive hemolysine (γ hemolysis)

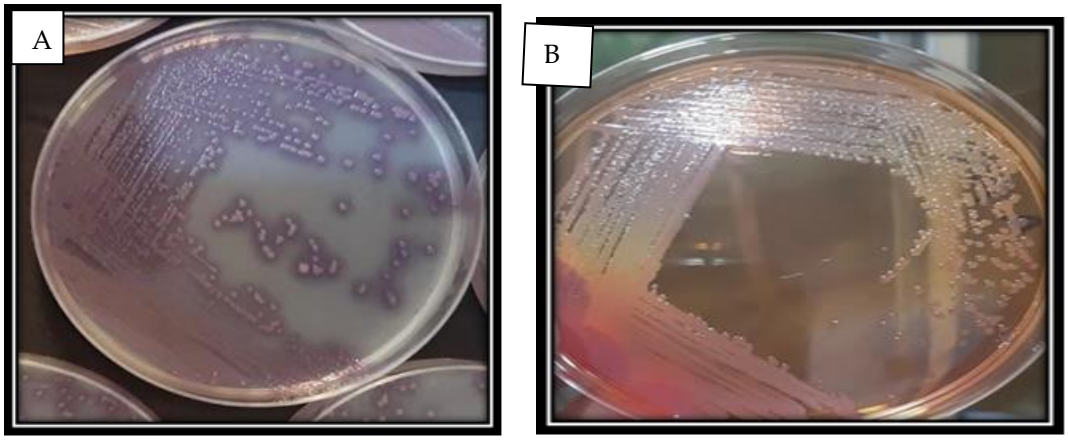

Figure 1: *A . baumannii* colonies (A) on Chrom media and (B) on MacConkey

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Identification by Biochemical tests

The results of a series of biochemical tests were used for detection *A. baumannii* in **table (1)** reveal that all isolates has the ability to grow at 44ºC and produce heavy growth after 24 hrs of incubation., The ability to grow at 44°C is an important characters that distinguishes *A. baumannii* from other species, belonging to the genus of *Acinetobacter* (13). All isolates showed a positive results for the Catalase test because the bacteria can produce the catalase enzyme, which mediated the breakdown of hydrogen peroxide into oxygen and water elaboration of oxygen bubbles occurrence. All isolates give negative reaction for Oxidase test, Lactose fermentation, Indole production , Methyl red. Voges- Proskauer. The findings of the test that were positive were found in the Simmons citrate, although the results of the urease test were variable, the results were agreed with results of Macfaddin (2000)(16) and Kadhom and Ali (2022)(17).

Table 1. Biochemical test for Identification of *A baumannii*

Biochemical test	Result
Oxidase production	-ve
Catalase production	+ve
Lactose fermentation	-ve
Growth at 44ºC	+ve
Indole production	-ve
Methyl red	-ve
Ureas production	Variable
Citrate utilization	+ve
Voges- Proskauer	-ve

Positive = (+ve) , Negative = (-ve)

After isolation and identification of *A. baumanii* by standard and molecular diagnostic methods. The growth results indicated that not all clinical specimens yielded growth following culturing and incubation; only 25 (12%) isolates of *A. baumannii* exhibited growth on the media from a total of 100 specimens, as illustrated in the figure (2)

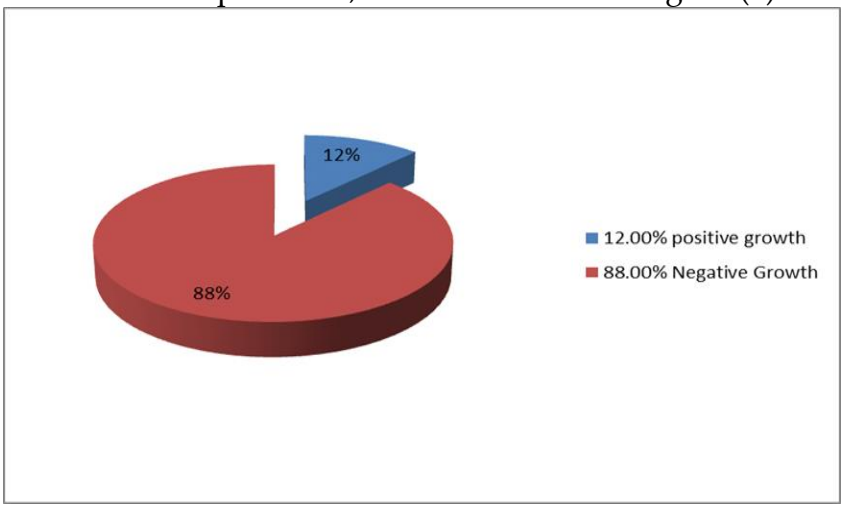

Figure 2. The percentage of positive and negative growth *A .baumannii*

Distribution of *A. baumannii* according to the diseases

The specimens were collected randomly from patients with various diseases in intensive care unit (ICU) during the course of the study. The source of clinical isolates that identified from patients were blood and distributed according to the type of infection between septicemia with burns 4(16 %) infections, surgical wounds 5 (20%), Diabetes foot (gas gangrene) 6(24%), Brain Bleeding 3 (12%), Respiratory tract infection 4(%16). Table (2) show the distribution similarities and differences in sites and distribution of infection of bacteria show difference from country to another due to patients condition number of patients examined, health practices, personal hygiene environment of condition and laboratory procedures.

Table 2. The percentages of *A. baumannii* among different diseases

Disease	Isolates number	%
Burns	4	16
surgical wounds	5	20
Renal Failure	3	12
Diabetes foot	6	24
Brain Bleeding	3	12
Respiratory Failure	4	16
Total	25	100

Haemolysin production

It was used to detect the product of hemolysis enzyme. In the study all 25 isolates were cultured on blood agar by streaking method after incubation at 37C for 24 hours .. The colonies of *A.baumannii* isolates on blood agar appeared white to cream-colored, smooth and circular with entire edges. Most species were non-hemolysis (γ hemolysis). Colonies

became more mucoidal up on further incubations (16). This appears on the figure (2). This result agreement with many of previous studies such as Abdulkareem et al.,(2024)(19) and Lee et al .

Figure 2. Hemolysis test of *A .baumannii* isolates on blood agar

Antimicrobial Activity

The findings presented in Table 3 shows the antibacterial effect of alcoholic and aqueous thyme extracts against *A. baumannii*, by measuring the diameter of the zone of inhibition in millimeters (mm) at different extract concentrations (5, 10, 25, 50, 100 µg/mL).

Table 3. Zone of inhibition (mm) for alcoholic and aqueous extracts against *A .baumannii*

Zone of inhibition (mm)		**Zone of inhibition (mm)**	
Dose of alcoholic extract (µg/mL)	*A .baumannii* **(Mean±SD)**	**Dose of aqauous extract (µg/mL)**	*A .baumannii* **(Mean±SD)**
5	4.5 ± 0.5	**5**	3.2 ± 0.4
10	7.2 ± 0.7	**10**	6.3 ± 0.6
25	10.2 ± 0.6	**25**	9.8 ± 0.7
50	15.22 ± 0.7	**50**	13.5 ± 0.8
100	19.3 ± 0.8	**100**	17.2 ± 0.9

The results show that inhibition increases with increasing concentration. The results of testing the effectiveness of alcoholic and aqueous thyme extracts against *A. baumannii* showed that both extracts possess concentration-dependent antibacterial activity. A gradual increase in the diameter of the zone of inhibition was observed with increasing concentrations tested (5, 10, 25, 50, and 100 µg/mL). The diameter of the zone of inhibition at the lowest concentration was 4.5 ± 0.5 mm for the alcoholic extract, compared to 3.2 ± 0.4 mm for the aqueous extract. At the highest concentration, it gradually increased to 19.3 ± 0.8 mm and 17.2 ± 0.9 mm, respectively, indicating that thyme contains compounds that are effective against the studied bacteria. It is worth noting that the alcoholic extract demonstrated a clear superiority over the aqueous extract at all concentrations, which is attributed to the Thyme (*Thymus vulgaris*) extract has demonstrated significant effectiveness in inhibiting the growth of pathogenic bacteria(21). This is attributed to its content of active phenolic compounds such as thymol and carvacrol, which are the primary components

responsible for its antibacterial activity. These compounds work through several complementary mechanisms(4). They begin by disrupting the integrity of the cytoplasmic membrane by binding to its constituent lipids, leading to increased membrane permeability and consequent leakage of proteins, ATP, and ionic components essential for cell survival. This leads to disruption of osmotic balance and cell death(7).

In addition, thyme extract directly affects the bacterial cell wall, disrupting the structural bonds in the peptidoglycan layer, weakening the cellular structure and creating holes that impair the cell's ability to withstand osmotic pressure. This effect is particularly critical for Gram-positive and Gram-negative bacteria(22). Recent studies have also shown that thyme compounds have the ability to inhibit essential bacterial enzymes, including those that synthesize nucleic acids and proteins, disrupting transcription and translation processes and halting growth and reproduction.The results of the current study are consistent with a study conducted Bankova *et al*., on the antibacterial activity of thyme extract (*Thymus vulgaris L*.), which showed very high activity against pathogenic strains such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Pasteurella multiforme,* and *Enterococcus faecalis*(23).

Conclusion

The results show that both extracts, alcoholic and aqueous, exhibited antibacterial activity against *A. baumannii* with a dose-dependent response, as the diameter of the inhibition zone gradually increased with increasing extract concentration.

References

Abdulkareem MA. Activity Of Ciprofloxacin and Resveratrol On Expression Of AdeI J Genes In Clinical Acinetobacter baumannii Isolates: Activity Of Ciprofloxacin and Resveratrol On Expression Of AdeI J Genes In Clinical Acinetobacter baumannii Isolates. Academic Science Journal. 2024;2(3):139–160.

Alaallah NJ, Abd Alkareem E, Ghaidan A, Imran NA. Eco-friendly approach for silver nanoparticles synthesis from lemon extract and their anti-oxidant, anti-bacterial, and anti-cancer activities. Journal of the Turkish Chemical Society Section A: Chemistry. 2023;10(1):205–216.

Alshiekheid MA, Dwiningsih Y, Sabour AA, Alkahtani J. Phytochemical Composition and Antibacterial Activity of Zingiber cassumunar Roxb. against Agricultural and Foodborne Pathogens. 2022.

AL-Sudani EA, Alash SA. Prevalence of Urinary Tract Infections in Adult and Child Patients. Indian Journal of Public Health. 2019;10(11):1861.

Asif M, Alvi IA, Rehman SU. Insight into Acinetobacter baumannii: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infection and Drug Resistance. 2018:1249–1260.

Bankova R, Popova TP. Antimicrobial activity in vitro of aqueous extracts of oregano (Origanum vulgare L.) and thyme (Thymus vulgaris L.). International Journal of Current Microbiology and Applied Sciences. 2017;6:1–12.

Chapartegui-González I, Lázaro-Díez M, Bravo Z, Navas J, Icardo JM, Ramos-Vivas J. Acinetobacter baumannii maintains its virulence after long-time starvation. PLoS ONE. 2018;13(8):e0201961.

Dahdouh E, Gómez-Gil R, Pacho S, Mingorance J, Daoud Z, Suárez M. Clonality, virulence determinants, and profiles of resistance of clinical Acinetobacter baumannii isolates obtained from a Spanish hospital. PLoS ONE. 2017;12(4):e0176824.

Ghaima KK, Saadedin S, Jassim KA. Isolation, molecular identification and antimicrobial susceptibility of Acinetobacter baumannii isolated from Baghdad hospitals. Burns. 2015;27:31.

Havenga B, Reyneke B, Ndlovu T, Khan W. Genotypic and phenotypic comparison of clinical and environmental Acinetobacter baumannii strains. Microbial Pathogenesis. 2022;172:105749.

Hutchings MI, Truman AW, Wilkinson B. Antibiotics: past, present and future. Current Opinion in Microbiology. 2019;51:72–80.

Kadhom HA, Ali MR. Epidemiological Molecular Analysis of Acinetobacter baumannii isolates using a multilocus sequencing typing and Global lineage. Revis Bionatura. 2022;7(1):29.

Lawandi A, Kadri SS. Can financial rewards for stewardship in primary care curb antibiotic resistance? The Lancet Infectious Diseases. 2021;21(12):1618–1620.

Lee JH, Kim J, Kim G-Y. Synergistic effects of a probiotic culture extract and antimicrobial combinations against multidrug-resistant Acinetobacter baumannii. Medicina. 2023;59(5):947.

MacFaddin J. Biochemical tests for identification of medical bacteria. Williams and Wilkins. Philadelphia, PA. 2000;113(7).

Mrabti NN, Mrabti HN, Doudach L, Khalil Z, Kachmar MR, Mekkaoui M, et al. Mineral contents, antimicrobial profile, acute and chronic toxicity of the aqueous extract of Moroccan Thymus vulgaris in rodents. International Journal of Secondary Metabolite. 2022;9(4):397–414.

Newman D. Old and modern antibiotic structures with potential for today's infections. ADMET and DMPK. 2022;10(2):131–146.

Nguyen M, Joshi S. Carbapenem resistance in Acinetobacter baumannii, and their importance in hospital-acquired infections: a scientific review. Journal of Applied Microbiology. 2021;131(6):2715–2738.

PEYMAnI A, FARAJnIA S, Nahaei MR, Sohrabi N, Abbasi L, Ansarin K, et al. Prevalence of class 1 integron among multidrug-resistant Acinetobacter baumannii in Tabriz, northwest of Iran. Polish Journal of Microbiology. 2012;61(1):57–60.

Soltani S, Shakeri A, Iranshahi M, Boozari M. A review of the phytochemistry and antimicrobial properties of Origanum vulgare L. and subspecies. Iranian Journal of Pharmaceutical Research: IJPR. 2021;20(2):268.

Strobel G. The emergence of endophytic microbes and their biological promise. Journal of Fungi. 2018;4(2):57.

Wirtu SF, Ramaswamy K, Maitra R, Chopra S, Mishra AK, Jule LT. Isolation, characterization and antimicrobial activity study of Thymus vulgaris. Scientific Reports. 2024;14(1):21573.

Yassin MT, Mostafa AA-F, Al-Askar AA, Sayed SR. In vitro antimicrobial activity of Thymus vulgaris extracts against some nosocomial and food poisoning bacterial strains. Process Biochemistry. 2022;115:152–159.