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Anti-bacterial activity of thyme aqueous extracts against *Pseudomonas aeruginosa* isolated from burned patients of Al-Hussein Teaching Hospital of Iraq

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Abstract

This study was conducted to examine the inhibitory activity of the aqueous and alcoholic extract of the thyme belonging to the Lamiaceae family against some pathogenic *Pseudomonas aeruginosa* (*P. aeruginosa*) isolated from burned patients of Al-Hussein Teaching Hospital, Iraq. The results indicated that the thyme aqueous extract in its alcoholic state is more effective in inhibiting bacterial growth than it is in its aqueous state. However, there were differences among the studied cases regarding the sensitivity of pathogenic *P. aeruginosa* towards this extract. The current investigation demonstrated a significant difference ($P < 0.05$) between males and females with 28 (70%) males infected, compared to 12 (30%) females. The differences may be attributed to social and anatomical differences between males and females, including smoking, lack of medical treatment, and prolonged hospitalization in male patients. Regarding the age factor, the results showed a significant difference among the groups in terms of infection with *P. aeruginosa* ($P < 0.05$). Patients within the age ranges of 16-30 and 31-45 years were more susceptible to *P. aeruginosa* as their infection rates reached 49.1% and 27.9%, respectively. In conclusion, the thyme extract indication can be used to control diseases associated with *P. aeruginosa*.

Keywords: Herbal supplement, *Pseudomonas aeruginosa*, Risk factor, thyme aqueous extracts

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1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative bacillus, which is widely distributed in nature. It is one of the most common non-fermenting bacteria in clinics, the flora of which is isolated on the surface of human skin and can contaminate medical equipment and even disinfectants, resulting in iatrogenic sensitization [1]. It is an important opportunistic pathogen of hospital-acquired infection and has the characteristics of easy colonization, easy mutation, and multi-drug resistance. The lower respiratory tract is the most common site of nosocomial infection, mainly including bronchiectasis, chronic obstructive pulmonary disease, and pneumonia [2] caused by multidrug-resistant. *Pseudomonas aeruginosa* has a high mortality rate and is difficult to be treated [3]. *Pseudomonas aeruginosa* is one of the most common clinical pathogens of MDR and PDR [4] and its drug resistance mechanism involves many aspects. Through producing inactivated enzymes, *P. aeruginosa* can produce β-lactamase, aminoglycoside modification enzymes, chloramphenicol B Acyltransferase, and some other enzymes, among which β-lactamase and aminoglycoside modification enzymes have important clinical value [5]. One of the causes of bacterial resistance to antibiotics is the presence of different lytic enzymes, including AmpC enzymes, extended-spectrum β-lactamases, metalloenzymes, and other enzymes, such as KPC enzymes [6]. Humans have known the use of plants in the treatment of diseases since ancient times, as evidenced by their historical and archaeological writings and literature. Some ancient peoples have excelled in this field, especially the ancient Egyptians since 3000 BC, as well as the Mesopotamian people who ubiquitously used herbal treatment [7], especially during the reign of King Ashurbanipal [8]. where the inscriptions on the clay figure could indicate humans' wide interest in medicinal plants, but the use of these herbs declined somewhat at the end of the nineteenth century and the beginning of the twentieth century as a result of the revolution in the use of chemicals and antibiotics [9, 10]. However, the harmful side effects of many chemical treatments, as well as the emergence of resistant bacterial strains and their economic cost have attracted researchers' attention to medicinal plants

at the national and international levels. Many studies have also confirmed the inability to dispense the benefits of medicinal plants and their effectiveness against many human diseases [11].

Thyme extract is documented as a bactericide herbal agent in numerous studies performed in clinical environments, such as dental clinics [12] and hospitals [13].

The diagnosis and treatment of *P. aeruginosa* in burn wound infections has become a critical problem worldwide. Therefore, the current study aimed to investigate the antibacterial activity of the dry aqueous and alcoholic extracts of the thyme extract against a variety of *P. aeruginosa* pathogens that cause infections in burn patients.

2. Materials and methods

The present cross-sectional study was conducted on hospitalized children and adults of both genders at Al Hussein Teaching Hospital, Karbala, Iraq, from January 2021 to July 2021. The Nutrient agar, Nutrient broth, MacConkey agar, Brain hearth infusion agar, water Pepton, Triple sugar iron agar, simmon citrate agar, gelatin medium were purchased from the limited M LAB company (England). All isolates were cultured on 10% blood agar, to show the type of blood hemolysis. All isolates with colonial morphology and specific odor which led to hemolysis, were assesed by oxidase test [14].

The thyme extract plant belonging to the Lamiaceae family [15] was collected from the Karbala market, and its classification was confirmed in the National herbarium of Iraq.

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Lamiales

2.1. Preparation of dry aqueous and alcoholic extracts of thyme:

The aqueous water extract was prepared in a fresh state according to the method elaborated by Kamel [16]. To begin, 40 g of the cut thyme was added to 160 cm^3 of distilled water and crushed using a blender (in cool conditions). The resulting mixture was stirred by an electric motor for 60 minutes and left at 4°C for 24 hours. In the next step, the repeated filtration was carried out by cutting transfer the resulting filtrate to a powder by means of a Buecher funnel using filter papers of type 1. No Whatmam and turning the filtrate into a powder using a Lyophilizer (Edwardi Company, England). Finally, the powder was stored in the refrigerator until use in tightly closed glass bottles. To prepare the alcoholic extract of thyme, the method of Mallahi et al [17] was used. At first, 40 g of thyme was added to 160 cm^3 of ethanol alcohol (95%) and crushed in a Blender device under refrigerated conditions followed by refrigerating the mixture for 24 hours. Afterward, the mixture was filtered by gauze, and then by a Buechner funnel. In the next step, the filtrate was put in the rotary evaporator vacuum Rotary (Electrothermal Company (English). Finally, the product was dried by lyophilization and stored in the refrigerator until use in airtight glass bottles.

To obtain a concentration of 200 mg/ml, 1 g of the plant extract was dissolved in 5 ml of distilled water. It was sterilized by filtering, when the alcoholic extract weighed 1 g, it was then dissolved for 10 minutes in Dimethyl Sulfoxide (DMSO). Afterward, 5 ml of the obtained substance was added to 0.1 ml of each extract in a glass vial containing 10 discs from sterile Bauer filter papers (disc diameter 6.5 mm). The final substances were left to saturate at laboratory temperature [18]. The Bauer method [19] was followed to study the inhibitory effect of these extracts on *P. aeruginosa*. In this regard, 4-5 colonies of bacterial type were transferred to bottles containing the nutrient broth medium and incubated at 37°C for 18 hours. Then, it was diluted with a physiological salt solution to match tube No. (0.5). The control tubes were considered 10^8 cells/ml, then 0.1 ml was transferred to a medium and then incubated at 37°C and spread with a glass rod in the shape of L on Muller Hinton agar for 30 minutes for impregnation, the sterile forceps were used to fix the tablets impregnated with the plant extract on the surface of the inoculated media, then the dishes were incubated at 37°C for 24 hours [20]. The results were recorded by observing and measuring the diameter of the inhibition.

2.2. Statistical study

To analyze the data, mean +/-SD was used for continuous variables. the frequency and percentages were calculated for categorical variables, and the Chi-square test was employed for categorical variables.

3. Results and Discussions

Table (1) shows the effect of some risk factors associated with the infection of *P. aeruginosa*. The results of the current study showed a significant difference between male and female participants ($P < 0.05$), as the number of infected males reached 28 (70%), compared to 12 (30%) of infected females. The results of the current study matched those of Suman et al. [21]. However, Nihad et al [22] reported a nonsignificant effect of sex on the distribution of *P. aeruginosa* bacteria except in urinary tract infection, as it was found that 26 (78.78%) of the isolates were from infected females, while only 7 (21.21%) were from infected males. The differences may be attributed to social and anatomical differences between males and females. For instance, smoking, lack of hospital care, and prolonged hospitalization were common attributions among male patients [23].

Table 1: Number and percentage of *P. aerogenoza* associated with risk factor

Risk Factor	Total	No of *P. aeruginosa* (%)

	No.	
Sex		
Male	57	(%49.1) 28
Female	43	12 (.27.9%)
Statistical analysis	-	X^2 Cal.=4.59
		P< 0.05
Age Years		
(1-15)	13	(%23) 3
(16-30)	24	(%54.1) 13
(31-45)	39	(%48.71) 19
(46-60)	24	(%20.8) 5
Statistical analysis	-	X^2 Cal.=8.46
		P< 0.05
Immunocompromised Patient	4	(%100) 4
Prolonged antibiotics treatment	10	(%80) 8

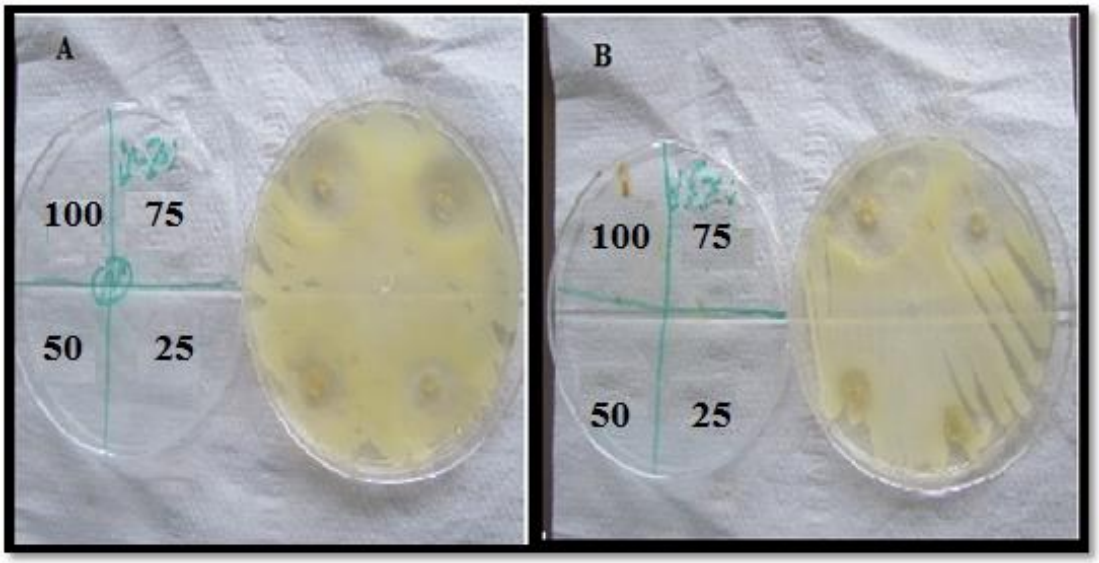

Figure 1: Inhibitory activity of aqueous (A) and alcohol (B) extract of thyme against *Pseudomonas aeruginosa* isolated from burn patients

Table 2 : Average diameters of the inhibition zone for thyme against *Pseudomonas aeruginosa* isolated from burn patients for both watery and alcoholic extracts

Average diameters of the inhibition zone for thyme extract	Concentration (mm)			
	%25	**%50**	**%75**	**%100**
	Mean ±SD	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**
Watery	**1.2±8.5**	**3.1±15.3**	**1.6 ±20.4**	**2.7 ±24.6**
Alcoholic	**2.3 ±10.1**	**2.4± 17.2**	**2.5 ±22.2**	**1.9 ±26.4**

Regarding the age factor, the results showed a significant difference ($P < 0.05$) among participants of different age groups regarding the infection with *P. aeruginosa*. Patients within the age ranges of 16-30 and 31-45 years were at a higher risk of infection as the percentages reached (49.1%) and (27.9%), respectively. The obtained result of the study differed from those by Herfindal and Gourley [23] indicating that children and elderly patients were more susceptible to infection, compared to other age groups because some disorders, such as immunodeficiency, malnutrition, and poor health conditions may increase the chance of *pseudomonas* infection [24].

The results of the current study indicated that 100% of the immunocompromised patients were infected by the disease. Kanj and Sexton [25] also indicated that cancer patients with resulting neutropenia for chemotherapy or hematological malignancies were more likely to develop *Pseudomonas* infection. This bacterium is one of the major opportunistic human pathogens that causes serious and sometimes fatal infections in immunocompromised hosts.

Prolonged use of antimicrobials as a treatment for the patient appears to be another risk factor for infection with *P. aeruginosa*, especially multidrug-resistant *P. aeruginosa* (MDRPA), which was confirmed by other researchers [26]. According to these results, as most isolates were detected in 80% of patients who had previously received courses of antibiotic treatment, *P. aeruginosa* rapidly evolves against antibiotics, either through physical adaptation and/or tolerance or genetically represented by auto-resistance [27, 28]. On the other hand, Jurado and colleagues [29] reported that in hospitalized patients exposed to various antimicrobial agents, the intrinsic and acquired resistance enables the organism to survive, thus the chance of infection increases as well as the spread of the organism around the environment especially the increase of the hospital environment. Improper use of antibiotics may induce *Pseudomonas* antibiotic resistance [30].

Efficacy evaluation of aqueous and alcoholic thyme extract

The minimum inhibitory concentration of aqueous and alcoholic thyme extract was investigated. The selection of these extracts was based on the increase in the resistance shown by *P. aeruginosa* isolates to broad-spectrum beta-lactam antibiotics in general, as the anti-biotics were and still represent the optimal therapeutic option for treatment against negative bacilli infections. Gram staining especially for *P. aeruginosa* shows an increase in bacterial resistance as a result of the excessive, repeated, and unstudied use of these antibiotics locally and internationally [31]. As can be seen in Table 2, all *P. aeruginosa* isolates (n = 40) diagnosed by the Kirby-Bawer disc diffusion method [32] were tested.

The aqueous and alcoholic extracts of the thyme plant showed its inhibitory activity against *P. aeruginosa* bacteria, and the increase of this activity correlated with the increase in the concentration. The effectiveness is in the best condition at 75% and 100% concentrations, as the diameters of inhibition were 20-22 mm and 24-26 mm, respectively, for the aqueous and alcoholic extracts of thyme, respectively (Fig). It was clear that the alcoholic extract of the thyme plant was superior in activity to the aqueous extract, and these results were in line with the findings of a study performed by Nzeako et al. [33] where the thyme extract was used for *P. aeruginosa.*

Essential oils (such as thyme, oregano, mint, cinnamon, cumin, alluvium, cloves, and eucalyptus) have a significant effect on bacteria as they have powerful antimicrobial properties. Due to these properties, the essential oils are used in food inductry and meat products [34]. If used in meat products, these aromatic oily liquids obtained from a variety of plant materials can reduce the incidence of foodborne diseases and delay the oxidation of fats as well. According to Ozogul et al [35], it is possible to use some plant extracts to determine the antimicrobial activity of many essential oils, including thyme and oregano essential oils, which can have significant antibiotic effects on germs that provoke hospital-acquired infections. The broad activity of essential oils and their synergistic actions have made them a valuable treatment against multidrug-resistant bacterial strains [36].

Thyme is a plant that offers a variety of benefits due to the presence of many chemicals and ingredients. An essential oil with a concentration of 0.5 percent contains up to 15 percent thymol. Thymol is an odorless phenolic chemical that is responsible for the characteristic smell of thyme.

4. Conclusions

In conclusion, the indication of the possibility of using the extract of the thyme of this plant in controlling diseases associated with *Pseudomonas aeruginosa* is sensitive to this extract. Thyme extract can be used to control the diseases associated with *Pseudomonas aeruginosa.*

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