Determining the inhibitory effect of *Nigella sativa* extract and comparing it with some antibiotics on gram-positive and gram-negative bacteria

Fatima Mustafa Al-najar
Department of Biology, College of Science, Tikrit University, Tikrit, Iraq
Correspondence Author: Fatima Mustafa Al-najar
Received 21 Feb 2023; Accepted 6 April 2023; Published 17 April 2023

Abstract
The most typical bacterial infection is a urinary tract infection, and if it is not treated, problems may result. In order to examine, identify, and test the microorganisms responsible for urinary tract infections for susceptibility to antibiotics and *Nigella sativa* plant extract, 100 urine samples were collected as part of the study. According to the findings of the bacterial culture, all isolates grew positively and displayed two types of growth: growth that was positive for gram stain and growth that was negative for gram stain. The results showed that IPM was the most effective antibiotic, as all the isolates were 100% sensitive to it, and the least effective was the CRO antibiotic, as most of the isolates were 100% resistant to it. The results also showed the effectiveness of *Nigella sativa* extract against the isolates under study and at all concentrations used.

Keywords: *Nigella sativa*, extract, antibiotics, gram-positive and gram-negative bacteria

Introduction

It should be noted that women are more susceptible to urinary tract infections than men for a variety of reasons, including the shortness of the urethra, the close proximity of the urine opening to the anal and genital areas, and pregnancy, which causes physiological changes that make the urinary tract more susceptible to infection. While antibiotics have helped and still help treat urinary tract infections, their efficacy has started to decline due to bacteria's growing capacity to resist them in a variety of ways, rendering the treatment ineffective. This resistance manifests itself most noticeably when the use of antibiotics is increased randomly (Bone *et al.* 2019) [8]. The body's immune defenses play a key role in protecting the human body from diseases if they give indications and warnings of the occurrence of infections, and the most important of these indicators is the secretion of cytokines, which are similar to hormones in terms of function to transmit chemical signals between the body's synthetic cells and immune cells 6-IL-6 (Interleukin) is One of the types of cytokines, which is produced as an immune response and increases in concentration minutes after the injury (Diepold *et al*., 2008). Urinary tract infections are detected by measuring the concentrations of cytokines in the sera of people with UTIs, which are a vital indicator of the level of UTIs and severity of infection, including IL-6 and 8-Li *et al*., 2017 [16] (Interleukin).

Study objectives

1. Determine the source of the bacteria isolates under study.
2. Investigating the sensitivity of the bacteria isolates used under study to some antibiotics and *Nigella sativa* plant extracts, and making a comparison between the effectiveness of antibiotics and plant extracts against This type of bacteria.

Materials and methods

Culture media preparation

The culture media was created in accordance with the processing company's specifications. According to the instructions on the packaging, it was autoclaved for 15 minutes at 121°C and 15 pounds/inch of pressure, and then, after adding the media, it was incubated at (37) C for 24 hours to make sure it wasn't contaminated before being stored at (4) C until use.

Sample collection

The current study included the collection of (100) samples (50) samples of Gram-positive *Staphylococcus aureus* and (50) of Gram-negative Escherichia coli. The urine samples were collected according to the method (Cheesbrought, 2012) [11]. Sterile plastic containers were used, noting that the sample was taken from the middle of the Midstream urine and washing the area around the urethra with soap and water and drying it with paper towels. Emphasis on closing the container directly and not touching the skin to prevent contamination with the natural flora that resides on the surface Skin.

Macroscopic and microscopic examination of the urine

A part of the urine sample was placed in a test tube and examined with urine strip, then it was deposited in the centrifuge at a speed of 3000 rpm for five minutes, the filter was discarded and a drop of the sediment was transferred to a clean glass slide, then a slide cover was placed over it and it was examined with a light microscope at 10X magnification. and 40.

Urinary culture

Urine samples containing purulent cells with an average of more than 10 cells were inoculated dropwise by means of a

sterilized Loop Full carrier on blood agar medium and MacConkey agar medium, in order to isolate the bacteria that induce inflammation, the dishes were incubated for 24 hours at a temperature of 37 °C under air conditions. Colonies on the medium were counted and the growth was examined. The isolates were purified after the initial culture by sub-culturing the proper substrate for their growth to produce a pure culture. As for the urine samples of healthy pregnant women, they were cultured after conducting a microscopic examination to ensure that there was no UTI on the medial blood acres and MacConkey.

Antibiotic sensitivity test

Thirteen types of antibiotics were tested for sensitivity, as follows:

1. Mueller Hinton Agar was prepared according to paragraph and poured into dishes Petri and leave until it solidifies.
2. The bacterial inoculum was prepared by transferring (4-5) single and pure colonies of the bacteria into the Nutrient Agar medium for the purpose of development and revitalization.
3. The cultures were incubated at (37) C for (24) hours, then the bacterial suspension was diluted with sterile physiological saline to obtain a homogeneous suspension (1.5×10) cells/mL by comparing its turbidity with the turbidity of the MacFarland tubes No. (0.5).
4. Spreading the bacterial suspension on the surface of a dish containing Acar Mueller-Hinton medium, using a sterile cotton swab dipped in the bacterial suspension, and the excess inoculum was removed by pressing on the sides of the tube, then the cotton swab was passed on the dish in three directions at an angle of (60) degrees between one stroke and another, and the dishes were left at a temperature room temperature for (5) minutes.
5. The antibiotic tablets were fixed using sterile forceps to the plate, at the rate of (5) tablets for each plate, and an appropriate distance was left between one tablet and another to avoid overlapping between the inhibition areas, then the plates were incubated at a temperature of (37) C for a period of (18-24) hours.
6. The results were recorded by measuring the diameters of the inhibition zones in millimeters around each disc and compared with the standard rates of the zones of inhibition for antibiotics.

Table 1

No.	Name of antibiotic	Concetration	Inhibition zone		
			S	I	R
1	Ceftriaxone	30	≤ 28	23-27	≥ 22
2	Imipenem	10	≤ 16	14-15	≥ 13
3	Ciprofloxacin	5	≤ 21	16-20	≥ 15
4	Azthromycin	15	≤ 26	22-25	≥ 21

Plant collection and preparation

The Salah al-Din Governorate's local markets provided the plants, which were then transported to the lab, thoroughly cleaned, put in clean containers at laboratory temperature, and continuously stirred to prevent rotting. They were then thoroughly pulverized in the lab mill and stored in sterile containers. And kept under dry circumstances until the plant extracts, including an alcoholic extract, started operating by dissolving 40 gm of the powder from each plant in 160 ml of 95-percent ethyl alcohol.

Then it was left for 24 hours for the purpose of soaking, then it was filtered through several layers of sterile gauze, then the solution was concentrated using a rotary evaporator at a temperature of 40 until a thick liquid was obtained, then the solution was filtered with Milipore filters with fine holes and a diameter 0.22 mm micrometer, then dried in a thermal oven at a temperature not exceeding 40 to obtain on the extract in its solid form. Each sample was placed in glass tubes sealed and labeled and kept in Refrigerate until use.

Sterilization of alcoholic extracts

The alcoholic extract was sterilized by combining one gram of the plant's alcoholic extract with 2.5 milliliters of DMSO, then pasteurizing the mixture at 62 degrees Celsius for ten minutes to achieve the standard concentration of the alcoholic extract that was used to create the subsequent dilutions used in the study.

Testing the inhibitory effectiveness of plant extracts against bacteria (inhibition zone diameter)

The agar diffusion method was used by digging (Wells) to test the sensitivity of the bacteria to plant extracts at a standard concentration (400 mg / hole) of the nutrient medium and as in (Egorove 1985), and the method included making 4 holes (one hole for each concentration). After spreading (0.1) ml of the bacterial hindrance on the medium, equal holes were made in the Mueller Hinto Agar solid medium with a diameter of (6) ml to contain plant solutions at an amount of (0.2) ml for each hole. The dishes were then placed in the refrigerator for an hour to spread the plant solutions, and the occlusion was then incubated at a temperature (37) C) and for a duration of (24) hours. The results were then read by measuring.

Results and discussion

Isolation and diagnosis

The isolates were diagnosed by the traditional method adopted in the hospitals under study based on the culture characteristics of the colonies, the microscopic characteristics of the bacterial cells, and the biochemical tests.

Resistance of bacterial isolates to antibiotics

The sensitivity of the isolates under study to (5) antibiotics was tested using antibiotic tablets, as the majority of them showed sensitivity to most of the antibiotics used in this study, as shown in Table (2) and Figure (1) of the results, and when conducting a sensitivity test For antibiotics of 100 isolates of bacteria under study, it was found that some of the antibiotics used in the research had significant differences between resistant, sensitive, and medium-sensitive isolates, while it was found that other antibiotics were ineffective Significant differences.

Table 2: The effect of antibiotics on the bacteria under study

Concentration	IPM	CIP	CRO	DO	AZM
Staph					
Mean	45	34.4	12.2	22.8	34.1
Std. Deviation	0.8165	1.174	1.751	1.549	1.37
Std. Error of Mean	0.2582	0.3712	0.5538	0.4899	0.4333
E. coli					
Concentration	IPM	CIP	CRO	DO	AZM
Mean	37.8	39	0	10.1	31.4
Std. Deviation	1.619	0.8165	0	0.9944	1.174
Std. Error of Mean	0.5121	0.2582	0	0.3145	0.3712

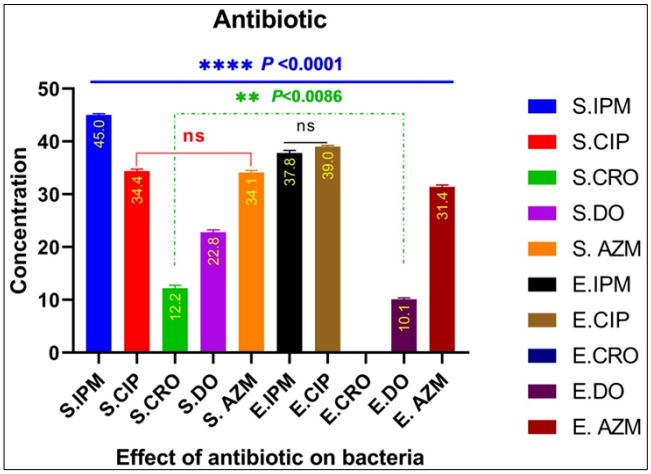


Fig 1

Figure (1) The effect of antibiotics on the bacteria under study

And through the results of the sensitivity of isolated bacteria, the most antibiotics that *Staphylococcus aureus* resisted is penicillin, which is commonly used in Qatar and widely used in hospitals to treat diseases caused by this bacterium, even if it is an easy-to-treat disease that does not require the use of antibiotics, although The fact that antibiotics are widely used without a prescription sometimes leads to the emergence of resistant isolates to them, which has become known and confirmed by the scientific community, and that the production of *Staphylococcus aureus* bacteria for beta-lactamase enzymes that inhibit the action of penicillin is behind their high resistance to this antibiotic, and in general, *aureus* isolates. isolated from the community are more sensitive to antigens.

Antibiotics that do not belong to the group of non-beta-lactamase compared to those isolated from the hospital environment. The results showed that there was a discrepancy in the resistance of the isolates under study towards the antibiotics used, as these results were identical to the results of Al-Nasseri (2002) [6], Al-Juma (2012) [4], and Muhammad (2013) [19]. As for the resistance of MRSA bacteria to antibiotics from the cephalosporin group, these were results it was an approach to what Muhammad (2013) [19] reached.

The study also showed that the isolates under study were sensitive to the antibiotic Azithromycin, and these results were far from what Muhammad (2013) [19] reached (46%) and (54%), respectively, and Hamada (2008) [13] (39%) As for the antibiotic Ciprofloxacin it, all the isolates were sensitive to it, as these results were close to what was reported by Al-Nasiri (2002) [6], as the percentage of isolates resistant to this antibiotic was, and close to what Muhammad (2013) [19] reached, as the percentage of The resistant isolates were (0%) and also close to the study of Yamada (1997) *et al* [29]. This antibiotic is a broad-spectrum antibiotic against Gram-positive and Gram-negative bacteria as for the anti-imipenim, the percentage of resistance to it was (60%), and these results were close to what came with it (Al-Nasiri 2002) [6] and (2013). Abdullah and Muhammad (2013) [1] as the rates of resistance to it were 0%. It is very different from the findings of Friday (2012) (22%) and the study of Zaidan (2007) [30].

The reason for the different rates of sensitivity and resistance to the antibiotics used in this test is that these Antibiotics such as penicillin's and cephalosporins are widely used, inexpensive, and more readily available the most modern and expensive antibiotics, such as Imipenem, are therefore expected to resist these MRSA bacteria.

Antibiotics are commonly used, especially in developing countries, as they are frequently used by the public even without antibiotics Consult a doctor and that the spread of resistance to these antibiotics leads to the high cost of treatment and the survival period longer hospitalization and treatment failure, which leads to life-threatening illnesses and more mortality.

The resistance of bacteria to antibiotics is often due to the accidental transmission of genes that encode resistance from external sources, or as a result of chromosomal mutations, or as a result of frequent exposure of a person to different antibiotics (Chambers and Deleo, 2009) for a long time. What complicates the problem is the high ability of this bacteria to develop its resistance against many antibiotics such as beta-lactam antibiotics (Grundman *et al* 2008). The reason for the resistance of bacteria to penicillin antibiotics may be attributed to their ability to produce the enzyme penicillinase that destroys the beta-lactam ring - which interferes with the synthesis of peptides and glycans of the cell wall (Prescott *et al*. 2005) [21]. The reason for the resistance may be changes in the process of making the antibody-sensitive cell wall, leading to an increase in its thickness or a lack of cross-links, causing a change in the target position to this antagonist (Chambers, 2001) [10].

Inhibitory effectiveness of medicinal plants Medicinal plants currently occupy a large position in industrial production and are the main source of medicinal plant drugs or the source of materials that enter into the preparation of medicine in the form of extracts or active substances that are used as raw materials for the production of some chemical compounds that are the nucleus of chemical manufacturing. For some general pharmacological substances and causes problems related to the use of antibiotics, such as penicillin resistance, interest in medicinal plants has increased as antimicrobial agents, and for this reason some plants that are believed to have antimicrobial activity were used in this study, which were chosen based on their use in folk medicine to treat some diseases. This study included the effect of extracts of each plant (*Nigella sativa*) on gram positive and gram negative, where the inhibition zone was examined and the result was considered positive if the zone of inhibition was (10 mm) or more, as shown in Tables (3) and Figure (2).

Table 3: Effect of different concentrations of *Nigella sativa* extract on the bacteria under study

Staph				
Concentration	400	200	100	50
Mean	30.9	25.1	20.5	15
Std. Deviation	1.792	0.9944	1.269	0.8165
Std. Error of Mean	0.5667	0.3145	0.4014	0.2582
E. coli				
Concentration	400	200	100	50
Mean	21.3	15.1	10.5	7.6
Std. Deviation	2.003	0.8756	1.08	0.9661
Std. Error of Mean	0.6333	0.2769	0.3416	0.3055

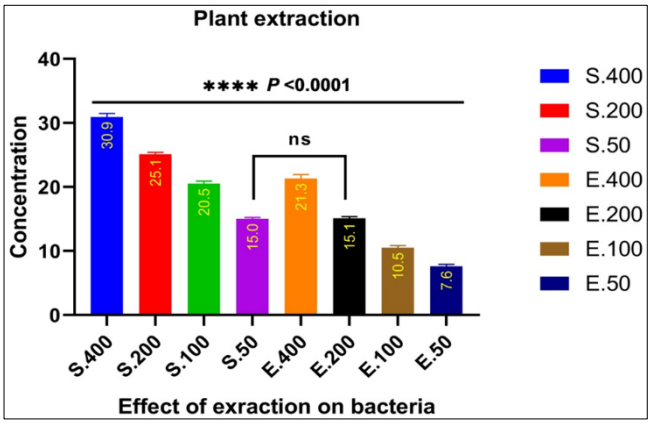
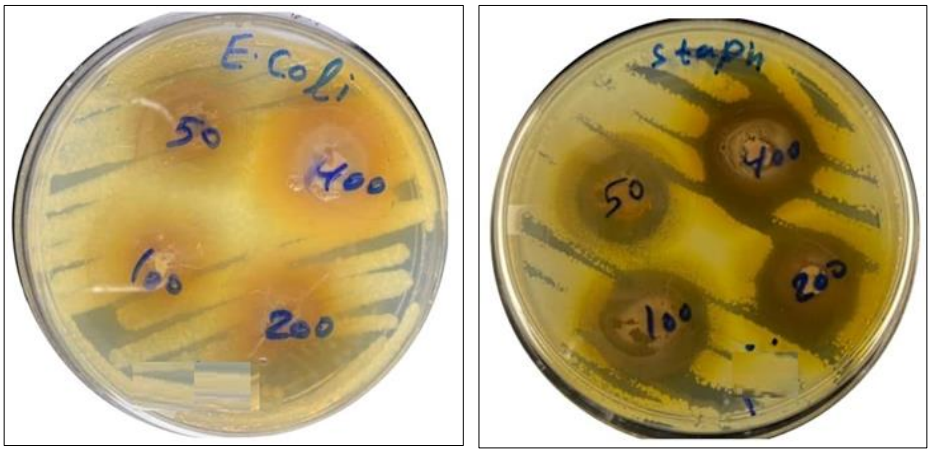


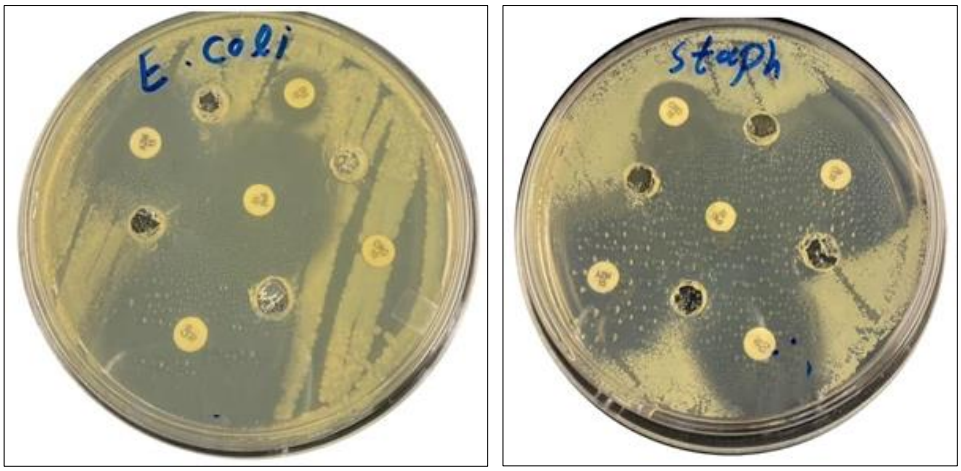
Fig 2: The effect of different concentrations of the extract on the bacteria under study

The results of the current study showed that there was a significant inhibitory effect of Nigella sativa extracts on the growth of bacteria, where the average diameter of inhibition of the alcoholic extracts of Nigella sativa on bacteria was similar to the results of a study conducted by (Vahabi et al 2011) [28] (2014 AL-Wazni et al) [7] and Ahmed et al. (2010) [2], who showed an inhibition zone with a diameter of (20mm), (23mm), and (20mm), respectively, as well as agreement with the results of (Mohammad & Bane, 2005) [18], who showed an inhibition zone with a diameter of (23 mm) for both the alcoholic and aqueous extracts, as well as The results of (Braga et al. 2005) [9] and (Irina et al. 2005) [14] that the zones of inhibition were (18mm), (20.1mm) and (20.1mm), respectively, as well as in a study of the German extract of Nigella sativa conducted by (Majeed et al., 2002) [17], and it was evident from This study shows that the Nigella sativa extract has a wide effect on the microbiota, and it is noted that the S. aureus bacteria that cause diseases important to humans, therefore, Nigella sativa are used in the possibility of producing a therapeutic substance (antimicrobial agents) and thus reducing the incidence of resistance that appears among bacteria as a result of use The wide range of commonly used antibiotics.

We conclude that there is a clear inhibitory effect of *Nigella sativa* on the growth of S. *aureus*, so we recommend conducting more studies on *Nigella sativa* and extracting and isolating the active substances from them to increase their effect on Microorganisms.



a) Alcoholic extract of *Nigella sativa* on bacteria *E. coli* b) Alcoholic extract of *Nigella sativa* on bacteria Staph



a) Effect of antibiotics on bacteria *E. coli* b) Effect of antibiotics on bacteria Staph

Fig 3: Susceptibility of plant extracts and antibiotics to bacteria under study

Conclusions

- There is a high sensitivity ratio by isolates for antibiotics used.
- Alcoholic plant extracts for *Nigella sativa* pants may be good alternatives to produce alternative medicines for UTI treatment.

References

1. Abdullah BH. Prevalence and antimicrobial susceptibility pattern of methicillin-resistance Staphylococcus aureus (MRSA) in AL-Yarmouk teaching hospital – Baghdad. AJPS J., 2013, 13(1).
2. Ahmed, Osama Ibrahim, Khurshid, Berri Ahmed, Nawab Al-Din, Faten Muhammad. Effect of tannin extract of some plants on the activity of some pathogenic bacteria. College of Agriculture - University of Kirkuk, 2010.
3. Al-Halim, Saba Muayad Suleiman Muhammad. The inhibitory effect of a number of medicinal plants and some of their active components on some types of salmonella isolated from patients with diarrhea, master's thesis, Faculty, 2001.
4. Al-Juma, Israa Taha Qassem. Prevalence of Staphylococcus aureus strains resistant to the antibiotic vancomycin and the effect of the CO2 laser in this resistance. Master Thesis, College of Science - University of Mosul, 2012.
5. Al-Mebairik NF, El-Kersh TA, Al-Sheikh YA, Marie MAM. A review of virulence factors, pathogenesis, and antibiotic resistance in Staphylococcus aureus. Clin. Microbiol. Rev. 2016;27:50-56.
6. Al-Nasiri, Osama Nijres. Molecular bacteriological and genetic study of Staph bacteria. aureus and studying its resistance to beta-lactam antibiotics and modern quinolones, PhD thesis, University College of Sciences Al-Mustansiriya, 2002.
7. Al-wazni WS, Al-dwri S, Darweesh MF. The antibacterial· & antibiofilm activity of Punica granatum peel aqueous extract against some oral pathogen, Magazin of Al-Kufa University for Biology, 2014, 6(2).
8. Bonev BB, Brown NM. (Eds.). Bacterial resistance to antibiotics: from molecules to man. John Wiley & Sons, 2019.
9. Braga LC, Shupp JW, Cumming C, Jett M, Takahashi JA, Carmo LS. Pomegranate extract inhibits Staphylococcus· aureus growth and subsequent enterotoxin production.J Ethnopharmacol. 2005;96:335-339.
10. Chambers HF. The changing epidemiology of Staphylococcus aureus?· Emerg Infect Dis. 2001;7(2):178-182.
11. Cheesbrough M. District Laboratory Practice in Tropical Countries.· Second edition update (part 2), Cambridge university press: India, 2012.
12. Fabricant DS, Farnsworth NR. The value of plants used in tradition medicine for drug discovery. Enviromental Health prespective supplements. 2001;109:69-75.
13. Hamada, Yasser Hamad. Bacteriological and genetic study of Staphylococcus aureus bacteria isolated from wound infections. Master's thesis, College of Education, Tikrit University, 2008.
14. Irina S, Vinograd or E, Flahant S, Grigoris. Extraction· and purification of fibronectin Staphylococcus aureus, Jour. Infect Immun. 2005;73:3007-3017.
15. Jawetz E, Brooks GS, Butel JS, Morse SA. Jawetz Melnick· and Adeibergs. Medical Microbiology. 23 thed. McGrow Hill, Singafor, 2004.
16. Li M, Kouzmina E, McCusker M, Rodin D, Boutros PC, Paige CJ, *et al*. Cytokines and depression in cancer patients and caregivers. Neuropsychiatric Disease and Treatment. 2017;13:2903-2911.
17. Majid, Guitar Rashid, Al-Shatti, Sabah Malik Habib. The effect of the permeability of some plant extracts on the growth of some microorganisms. College of Agriculture - Basra University, 2002.
18. Mohammad Hassan J, Bane MY. Evaluation of anti-bacterial activity of punica granatum peels extracts, on growth of gram-positive bacteria isolated from clinical samples, Director General of General Commission for Industrial Research & Development, 2005.
19. Muhammad Osama Ismail. Prevalence of methicillin-resistant Staphylococcus aureus bacteria among the health staff of some hospitals in Salah al-Din Governorate, diploma thesis - College of Science - University of Tikrit. – Iraq, 2013.
20. Navarro V, Villarreal ML, Rojas G, Lozoya X. Antimicrobial evaluation of some plant used in Mexican, J. Ethnopharmacology. 1996;53:143-147.
21. Prescott LM, Harley J, Klein DA. Microbiology. 6thed, McGraw-Hill, USA, 2005.
22. Rashid Zuhair Najeeb. Medicinal herbs in the civilization of Mesopotamia, Dar Al-Kutub for Printing and Publishing, University of Mosul, Iraq, 2008, 8-10.
23. Soltani S, Emamie AD, Dastranj M, Farahani A, Davoodabadi A, Mohajeri P. Role of Toxins of Uropathogenic Escherichia coli in Development of Urinary Tract Infection. JPRI. 2018;21(1):1-11.
24. Souman L. The rapeutic uses of herbs and medicinal plants in traditional. Arab medicine J. The Arab Board of medical specialization. 2001;3(2):53-56.
25. Stari Francesik, Jerassik Vaclav. Medicinal herbs Translated by: Saad Al-Din Shrooq Muhammad Kazem, first edition, House of General Cultural Affairs, Ministry of Culture and Information, Baghdad, 1986.
26. Swamy MV, Faraz MAA, Mendem S, Shubham P, Vinyas M. Urinary Tract Infections: A Comprehensive Review. Int. J. Curr. Microbiol. App. Sci. 2020;9(7):773-786.
27. Terlizzi ME, Gribaudo G, Maffei ME. Uro Pathogenic Escherichia· coli (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-Antibiotic Antimicrobial Strategies. Front Microbiol. 2017; 8(1566):1-23.
28. Vahabi S, Najafi E, Alizadeh S. Invitro antimicrobial effects of some· Herbed essences against oral pathogens. J of Med Plants Res. 2011;5(19):4870-4878.
29. Yamada H, Chiba A, Matsumuva K, Inazu T, Shimizu T,

Kusunoki S, *et al*. Structures of Sialylated OLinked Oligosaccharides of Bovine Peripheral Nerve - Dystroglycan J. Biol. α Chem. 1997;272:2156-2162.

30. Zaidan, Israa Ali. Bacteriological and genetic study of Staphylococcus aureus resistant to the antibiotic vancomycin, Master Thesis, College of Science, University of Baghdad, 2007.