STUDY ANTIBACTERIAL ACTIVITY OF *LAURUS NOBILIS* LEAVES WATER EXTRACT ON SOME ISOLATES OF PATHOGENIC BACTERIA

[1] B. H. Saleh [2]H.N. Yahya [3]R N. Ibrahim
Assist. Prof. Assist. Lecturer Lecturer
[1]Dept. Mole. and Medi. Biotech.- Coll. of Biotech., AL-Nahrain University.
[2]Dept. Plant Biotech.- Coll. of Biotech., AL-Nahrain University.
[3]Dept. Mole. and Medi. Biotech.- Coll. of Biotech., AL-Nahrain University, Baghdad, Iraq
*Corresponding author's Email: HaiderN.Yahya@gmail.com

ABSTRACT:

This study was aimed to isolation of pathogenic bacteria from different clinical cases like burns, wounds &UTI infections, then study the antimicrobial activity of *Laurus nobilis* leaves water extract on it. From a total of (80) samples were taken from these cases, the most isolated bacteria were related to *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staph aureus & Escherichia coli*. Antibiotic sensitivity test was done for isolated bacteria against (9) antibiotic and most of them revealed sensitivity to gentamycin, ciprofloxacin& trimethoprim/ sulphamethoxazole. Different concentration of *Laurus nobilis* leaves water extract (25, 50, 100, 200) mg/ml were tested for detection its antibacterial activity against isolated bacteria .Results revealed that concentration (50, 100, 200) mg/ml revealed high antibacterial activity against *Staph aureus* & *Klebsiella pneumoniae*, also showed intermediate level against *Escherichia coli*, while higher concentration only (100, 200) mg/ml of extract revealed antibacterial activity against *Pseudomonas aeruginosa*

Keywords: ***Laurus nobilis*, antmicrobial activity, pathogenic bacteria, burns, wounds, UTI**

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دراسة التاثير البكتيري المضاد للمستخلص المائي لاوراق الغار *Laurus nobilis* على بعض العزلات البكتريه المرضية

بشرى هندي صالح حيدر ناطق يحيى ريم نعيم ابراهيم
استاذ مساعد مدرس مساعد مدرس
كلية التقنيات الاحيائية– جامعة النهرين–بغداد

المستخلص:

هدفت هذه الدراسة الى عزل البكتريا المرضية من حالات سريرية مختلفة مثل التهابات الحروق والجروح والتهابات المجاري البولية ومن ثم دراسة التاثير البكتيري للمستخلص المائي لاوراق نبات الغار عليها حيث من مجموع 80 عينة سريرية , كانت معظم البكتريا المرضية المعزولة تعود لبكتريا الزوائف الزنجارية,الكبسيلة الرئوية ,المكورات العنقودية الذهبية والاشرشيا القولونية. تم اجراء اختبار الحساسية للبكتريا المعزولة ضد (9) انواع من المضادات الحيوية وقد اظهرت اغلب العزلات حساسيتها تجاة الجنتاميسين، السبروفلوكساسين والترايميثبريم/سلفاميثاكزازول. تم تحديد الفعالية الضد بكتيرية للمستخلص المائي لاوراق الغار بتراكيز مختلفة (200,100,50,25) ملغم /مل ضد البكتريا المعزولة حيث اظهرت التراكيز (200,100,50) ملغم / مل تاثير عالي ضد بكتريا المكورات العنقودية الذهبية والكبسيلة الرئوية كما اظهرت تاثير متوسط ضد بكتريا الاشرشيا القولونية, في حين اظهرت التراكيز العالية للمستخلص (200, 100) ملغم /مل تاثير ضد بكتريا الزوائف الزنجارية.

الكلمات المفتاحية: اوراق الغار, الفعالية الضد ميكروبية, البكتريا المرضية, حروق, جروح, التهاب المجاري البولية

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INTRODUCTION

Laurus nobilis is a flowering plant that related to Lauraceae family. This family is consisting of about 2850 *spp* & 45 genera in the world (28). It's an aromatic evergreen shrub or tree that regarded as high-content spice found in Europe, South America, Asia, and endemic in Morocco, Spain &Turkey (13). *Laurus nobilis* leaves are used as preservative in the industry of food (27). Chemical analysis of the plant revealed the presence of many compound such as flavonoids, volatile and non-volatile oils, tannins, alcohols, many minerals, alkaloids& different vitamins (23). Also it's containing many secondary metabolites as active constituents like hydroxyl group (3). *Laurus nobilis* leaves aqueous extracts have been used in herbal medicine for treatment of several dermatological & neurological disorders. It's also have antimicrobial activity against different pathogenic bacteria *in vitro* (4, 5, 21). Infection of urinary Tract (UTI) is defined as the invasion and spread of pathogenic microorganisms (M.O) in the tissues of urinary tract (19). Many factors may leads to UTI infections such as predisposition genetically, sexually intercourse, abnormalities in UTI structure, diabetes, low immunity, pregnancy, formation of stone, and use of catheterization (7). The bacteria are able to adhere, resistant to host defenses, then colonization and infection of UTI by two important routes: the hematogenous and ascending pathways (15). Burn wound injuries are common nosocomial infections in the world. Burn wound area are predispose for colonization with M.O of exogenous and endogenous origin during thermal injury (11). Many factors like burn nature, patient immunity, age, depth & extent of injury, also number & type of M.O. at the burn site, enzyme and toxin production that dissemination systemically, all of these factors enhance burn wound infections (20). Burn wounds become colonized and infected with gram negative bacteria like *P. aeruginosa* that regarded as most predominant bacteria isolated from burn wound site, followed by other gram negative bacteria like *klebsiella pneumoniae, E. coli, Proteus spp, Acinetobacter baumannii,* also gram positive bacteria *like S. aureus* (22).

MATERIALS AND METHODS

Diagnosis of bacteria: During this study (80) samples were taken from UTI & burn wounds infections from 2 hospitals in Baghdad during the period from 30/8/2020 to 15/1/2021 for isolation of different pathogenic bacteria. Swaps from clinical samples were cultured directly on nutrient agar, Blood gar and MacConkey agar, incubated for 24 hrs. at 37°C. Selected isolated colonies were sub-cultured on different selective media such as EMB, King A, King B, and mannitol salt agar, and then incubated at 37°C for 24 hrs. for diagnosis of *E.Coli, K. pneumoniae, P. aeruginosa* & *S. aureus* respectively. The identification of pathogenic bacteria start by staining of bacteria by gram stain, and finally identified by vitek 2 systems.

Antimicrobial susceptibility Test

This test was done according to Kirby-Bauer method (14). Nine antibiotics disks were used in this test, Trimethoprim / sulphamethoxazole (SXT), ciprofloxacin (CIP), Gentamycine (CN), Chloramphenicol (C), Erythromycin (E), Amoxicillin (AX), Nalidixic acid (NA), Bacitracin (B), and Cefixime (CFM). A few colonies from each tested bacteria (*Escherichia coli, klebsiella pneumonae, Pseudomonas aeruginosa* & *staph aureus*) were inoculated in 4ml of brain heart infusion broth, put overnight in incubator at 37°C, centrifuged at 5000 rpm for 5 min, then diluted to match $1\text{-}2x10^8$ turbidity in compared with Mcfarland turbidity tube (0.5). A sterilized Mueller-Hinton agar were distributed in a plates, after solidification, 0.1 ml of overnight bacterial broth from each tested M.O was put in a plate and streaked in many direction by a sterile swab, then leave it to dryness for a few minutes, the disc for each antibiotics was fixed by a sterile forceps on the agar surface and incubated at 37°C for 24 hrs. before reading the results that was compared with a NCCLS standardized ranges (25).

Preparation of *Laurus nobilis* leaves aqueous extract: *Laurus nobilis* leaves were obtained from local market of Baghdad-Iraq during June (2020), which has been previously identified by National Herbarium of Iraq. The plant's leaves were grinded; 50 gram of plant powder was prepared by maceration with 250 ml of sterile distilled water. boiled for 30 min

over a hot plate, and then put in shaker incubator at 37 °C for 48 hrs. The plant was firstly filtered by multiple layers of gauze, then by Whatmans filter paper No.1. The filtrates were concentrated finally by oven at 45 °C. Stock solution was made with D.W (1:1), then different concentrations were prepared (25, 50, 100, and 200) mg/ml that filtered by Millipore filters 0.45 & 0.22µm before use.

Estimation of antimicrobial activity for *Laurus nobilis* leaves water extract

Well diffusion method (29) was used for detection antibacterial activity of aqueous extract of *Laurus nobilis* leaves. A sterilized Muller Hinton agar medium has been used, 4 well were made to put different concentration of *Laurus nobilis* leaves water extract, then a sterile swap was dipped in the broth for each tested M.O. and spread uniformly on agar surface, left it to dryness for a few min. A 100µl of various concentrations of water extracts of *Laurus nobilis* leaves (25, 50, 100, and 200 mg/ml) were put in the wells, incubated overnight at 37°C, then examined for estimation the diameter of zone of the inhibition area around each well.

RESULTS AND DISCUSSION

Isolation & Identification of *Pseudomonas aeruginosa, K. pneumonae, S. aureus* & *E.Coli:* From a total of (80) bacterial specimens that were taken, 27 isolate (33.75%) belong to *Pseudomonas aeruginosa*, (17) isolates (21.25%) were related to *Klebsiella pneumoniae*, (13) isolates (16.25%) were related to *Staph aureus*, (7) isolates (8.75%) were identified as *Escherichia coli*, while the remaining (22%) of them were related to various bacterial *spp*. Such as *Serratia marcescens*, *providencia*, *Proteus mirabilis*, *Enterobacter cloacae*. These results is depend on diagnosis of bacteria based on their morphology on selective media, gram stain and finally diagnosis by vitek 2 system as shown in (Table 1). All gram negative bacteria like *K. pneumoniae, E.Coli* & *P. aeruginosa* appears in gram stain as rods shaped, pink arranged either as singular or pairs, non-forming spore. *S. aureus* appeared as blue, gram (+) cocci. *Pseudomonas aeruginosa* was identified on MacConcky agar, its form a fate yellow colonies, sometimes mucoid due to production of biofilm. This result has been agreed with (14, 26). On blood agar it's able to produce different types of haemolysis. Also on (king A, king B) agar plates, bacteria produce blue green Pyocyanin and Fluorescence Pyoverdines pigment on the later media under UV light.

Table 1. The number of bacterial isolates and their ratio of isolation

Hospitals	No of samples	No of *P. aeruginosa* isolates(%)	No of *S. aureus* Isolates(%)	No of *E.coli* Isolates(%)	No of *K. pneumonae* Isolates(%)	No of other strain(%)	Total ratio of isolation(%)
Al-ammamein kademin	30	10(12.5)	5(6.25)	3(3.75)	5(6.25)	7(8.75)	37.5%
Baghdad teaching hospital	50	17(21.25)	8(10)	4(5)	12(15)	9(11.25)	62.5%
Total No	80	27(33.75)	13(16.25)	7(8.75)	17(21.25)	16(20)	100%

These results are compatible with (29). *Klebsiella pneumoniae* isolates produce pink, slime colonies on MacConcky agar due to presence of capsule. *Escherichia coli* colonies appear as pink colonies on MacConcky agar, Also its forms green metallic sheen on EMB agar (14). *Staph aureus* colonies cultured on blood agar produce beta hemolysis onit & on Mannitol-Salt agar its appears as yellow colonies due to mannitol fermentation ,its used to differentiates *S. aureus* from other coagulase negative *Staph* like *Staphylococcus saprophyticus & Staphylococcus epidermidis*. (18, 1). A study in Intensive care burn unit in India to determine bacterial pathogens responsible for infections of burn wounds, then detected their sensitivity to antibiotics recorded that from a total of 272 wound swabs were taken, 62.8% of them were gave a positive results for the presence of bacteria. The predominant bacteria isolated from these cases were *P. aeruginosa, K. pneumoniae, Acinetobacter baumannii* - and *Escherichia coli* (17). Another study revealed that from a total of 300 specimens that were taken, (90%) of isolated bacteria were from infections of burn & skin injures, 124 (52%) of them was identified as gram (-) rods, the rest 112 (48%) appeared as gram (+) bacteria (1). The ratio of bacteria isolated from burn wound infection are76% gram (-) rods, 20.18% gram (+) bacteria &3.82% of them belong to fungus (12). A study by (22) recorded that 90 samples were taken from people with a second degree burns in two

hospitals in Baghdad .The most frequent M.O. isolated was *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, also *Acinetobacter. baumannii* were isolated at high ratio. UTI infections are more common in children less than 10 years old. Most of them are caused by gram (-) bacteria related to Enterobacteriacae family like *E. coli* that isolated from more than 80% of cases, followed by *K. pneumoniae*, *p. aeruginosa*, *Proteus spp*. & Enterobacter *spp.* Also gram (+) Bacteria like *S. spp* was isolated (20).

Antibiotic sensitivity test

Antibiotic sensitivity test for pathogenic bacterial *spp*. isolated from infections of Urinary tract & burn wounds revealed that all *S. aureus* bacteria showed a sensitivity to nalidixic acid ,Trimethoprim / sulphamethoxazole, ciprofloxacin, & Gentamycine, also 50% of them were sensitive to erythromycin, amoxicillin & chloramphenicol, while 100% of them have a resistance to bacitracin & cefixime as seen in (Figure 1).

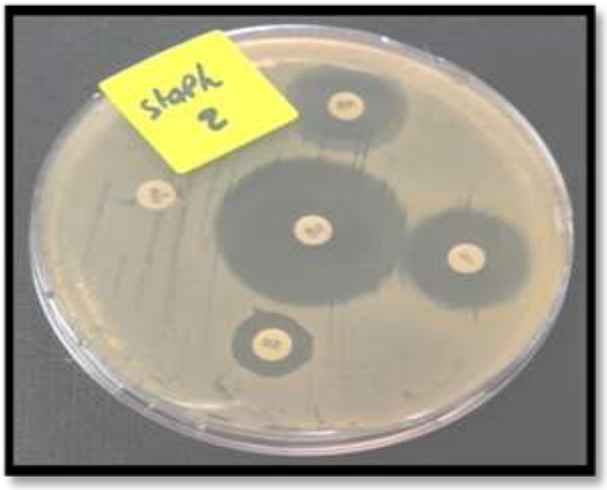

Figure 1. *Staph aureus* antibiotic sensitivity test

All isolates of *K. pneumoniae* showed sensitivity to cefixime, Gentamycine & chloramphenicol, also fifty of isolates have sensitivity to Trimethoprim / sulpha-methoxazole, ciprofloxacin & nalidixic acid, while all of them revealed a resistance to erythromycin, amoxicillin & bacitracin. Antibiotic sensitivity results for *P. aeruginosa* isolates revealed that 100% of them have sensitivity to ciprofloxacin & gentamycin, 50 % of isolates revealed sensitivity to nalidixic acid, chloramphenicol & erythromycin, otherwise100% of them have a resistance to cefixime, bacitracin and Trimethoprim /sulphamethoxazole. *Escherichia coli* isolates showed a high sensitivity rate 100% to gentamycine. Trimethoprim/sulphamethoxa-zole, ciprofloxacin, chloramphenicol and erythromycin, also 50% of them were sensitive toamoxicillin & nalidixic acid, while 100% of *E. coli* isolates were resistant to bacitracin & cefixime as shown in Table 2.

Table 2. Antibiotic sensitivity test for isolates of *K. pneumonia, E.coli, P. aeruginosa, & S. aureus*

Number Of Bacteria	SXT 25 mg/ml	CIP 5 mg/ml	CN 10 mg/ml	C 30 mg/ml	E 15 mg/ml	AX 25 mg/ml	NA 30 mg/ml	B 10 mg/ml	CFM 5 mg/ml
K1	R	R	S	S	R	R	R	R	S
K2	S	S	S	S	R	R	S	R	S
PS1	R	S	S	S	S	R	S	R	R
PS2	R	S	S	R	R	R	R	R	R
ST1	S	S	S	R	R	S	S	R	R
ST2	S	S	S	S	I	I	S	R	R
E1	S	S	I	S	I	R	S	R	R
E2	S	S	S	S	I	S	S	R	R

Trimethoprim / sulphamethoxazole (SXT), Ciprofloxacin (CIP), Gentamycine (CN), Chloramphenicol (C), Erythromycin (E), Amoxicillin (AX), Nalidixic acid (NA), Bacitracin (B), and Cefixime (CFM), Resistant (R), Sensitive (S), and Intermediate (I).

A study by (2) revealed that Imipenem was the most effective antibiotic against gram (-) bacilli & gram (+) cocci isolated from burn wound infections at ratio (90.8%).Also the isolated bacteria have a resistance to Ampicillin, Cefotaxime, Cefepime, Amoxicillin-clavulanic acid, & Cefpodoxime. Another study showed that from a total of 100 clinical samples that were taken from UTI infections for isolation of pathogenic bacteria & examined their sensitivity against many antibiotics like ampicillin, gentamicin, tobramycin, streptomycin, ceftriaxone & imipenem. From a total of 42 bacterial isolates, twenty of them were multidrug-resistant (MDR), eight revealed extensive drug-resistant (XDR) & two isolates recorded as pan drug-resistant (PDR) (9). A study by (12) recorded that many antibiotics were active against *P. aeruginosa* isolated from infections of urinary tract & burn wound include Gentamicin, fluoroquinolones & imipenem, but it's not effective on all isolates.(16) reported that a high rate of resistance was

detected among isolates of *E.Coli* to many antibiotics such as Beta-lactamase, tetracycline, aminogltcosides, chlorom-phenicol that cause major health problems.

Detection of antimicrobial activity for leaves aqueous extract *of Laurus nobilis* against pathogenic bacteria isolated from infections of urinary tract& Burn wounds:

Laurus nobilis leaves water extract at different concentration (25, 50, 100, and 200) mg/ml were used for determine its antibacterial activity against isolated bacteria .The results revealed that *Laurus nobilis* leaves water extract at concentration (50, 100, and 200) mg/ml have a high antimicrobial activity against *S. aureus & K. pneumoniae* isolated from infections of urinary tract & Burn wounds as shown in (Figure 2).

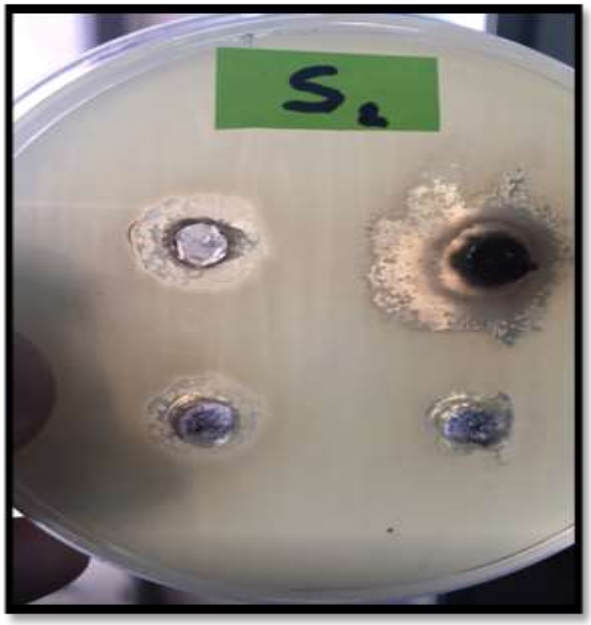

Figure 2. Antimicrobial susceptibility test for leaves aqueous extract of *Laurus. nobilis* against *staph aureus* isolate

Also the plant leaves water extract have intermediate level of antibacterial activity against *E.Coli*, While its revealed antibacterial activity against *P. aeruginosa* at higher concentrations only (100, 200) mg/ml, but the bacteria was resistant to antibacterial activity for leaves aqueous extract of *Laurus nobilis* at low concentrations (25, 50) mg/ml as shown in (Table 3).

Table 3. Antimicrobial activity for *Laurus nobilis* leaves water extract against *E.coli, P. aeruginosa, S. aureus* & *K .pneumona*e isolates

Concentration of *Laurus nobilis* extract (mg/ml)				
Zone of inhibition (mm)				
Bacteria *Spp*	25 Mg/ml	50 Mg/ml	100 Mg/ml	200 Mg/ml
ST1	16	20	24	27
ST2	15	19	23	25
K1	14	20	22	23
K2	13	19	21	24
PS1	R	R	14	17
PS2	R	R	15	18
E1	11	13	17	19
E2	12	13	16	18

***Staph aureus* (ST), *Klebsiella pneumona*e (K), *Pseudomonas. aeruginosa* (PS), and *Escherichia Coli* (E).**

A study by (4) revealed that *in vitro* antibacterial activity for *Laurus nobilis* leaves water extracts that was prepared by two methods of extraction, maceration and decoction were tested against *S. aureus* and *E. coli* isolates. Results showed that aqueous extract of both extraction methods have a higher antibacterial activity against *S. aureus* isolates at concentration 50% and 75% in comparison to *E. coli* that showed intermediate sensitivity at these concentrations. The inhibitory action of the plant leaves water extract was increased with increased concentrations. Another study revealed that aqueous extract of *Laurus nobilis* leaves showed an inhibitory activity against *S. aureus* at different concentration (25, 35, and 50) mg/ml because its content a strong biological components & secondary metabolites (10). Antibacterial activity of *Laurus nobilis* leaves water extract was detected against gram-positive bacteria like *Listeria monocytogenes*, *S. aureus* & gram-negative bacteria such as *E. coli, P. aeruginosa, K. pneumoniae* & *Enterobacter cloacae.* Results revealed that the average of inhibition zone of leaves water extract against M.O. was ranged from (18-25) mm for *P. aeruginosa, K. pneumoniae (*14-20) mm, *S aureus* (12-15) mm, *Enterobacter cloacae* (10-13) mm (28). Results in this study was agreed with that reported by (8) because various agents, such as the season, variance in the plant components &the Essential oils variations within a plant *spp.* may lead to differences in the antibacterial activity. Laurel

leaf EO are regarded as a natural supplement & have antibacterial activities against different gram (-) & gram (+) M.O. like *Staph aureus* & *Bacillus subtilis*, *Salmonella spp., P. aeruginosa & E.coli* (27). It was noted that terpenes was the main components responsible for antimicrobial activity of laurel leaves essential oils that cause disruption in the cellular membranes and increase permeability of it ,also may cause membrane-embedded proteins alteration and finally lead to membrane transport system disruption (6).

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