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Antibacterial Activity of *Lantana camara* Flower Extracts Against Growth of Pathogenic Bacteria Isolated from Wounds and Burns Infections

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Abstract

Clinical samples 200 were collected from patients hospitalized in Baquba Teaching Hospital / Diyala during September 2021 to the end of January 2022. The clinical samples were distributed into 145 (72%) of wounds and 55 (27.5%) of burn samples. The samples were sub cultured on Blood agar Macckonky agar and Mannitol salt agar, isolated were examined microscopically and diagnosed by using biochemical tests which showed, *Staphylococcus aureus* 30 (17.6%) and *Staphylococcus epidermidis* 13 (7.64%) and *Acinetobacter baumannii* 56 (32.9%) *Pseudomonas aeruginosa*, 32(18.8%), *Klebsiella* spp., 20 (11.7%) *Proteus* spp. 8 (4.7%), *Escherichia coli* 11(6.47) respectively. Susceptibility test examined against 13 antibiotic the isolates were multiple drug resistance MDR. APiE20 and Vitek- 2 system were used to confirm the identification the flower extract of *Lantana camara* was used and evaluated the antibacterial efficacy against five bacterial isolated from wounds and burns infection. The aqueous extract showed moderately antibacterial activity at the concentrations (12.5, 25, 50, 100, 200) mg/ml against tested bacteria with highest inhibition zones (15) mm at the concentration 200 mg/ml against *Klebsiella* spp. followed by *Pseudomonas aeruoginosa Staphylococcus aureus Acinetobacter baummannii* and *Proteus* spp (13,12.5,12.5) mm respectively. Concentration 12.5 mg/ml did not show any inhibitory activity the alcoholic extract showed higher

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antibacterial activity than the aqueous extract with the widest inhibition zones (18) mm against *Klebsiella* spp. followed by *Proteus* spp., *staphylococcus*, *Pseudomonas* and *Acinetobacter* with inhibition zones (16, 15, 15, 14) mm respectively.

Keywords: Antibacterial, *Lantana camara*, Cutaneous infections, Susceptibility test.

الفاعلية الضد بكتيرية لمستخلصات زهرة المينا الشجيرية ضد نمو البكتريا المرضية المعزولة من اخماج الجروح والحروق

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الخلاصة

تم جمع 200 عينة سريرية من المرضى الراقدين في مستشفى بعقوبة التعليمي / ديالى خلال الفترة من أيلول 2021 إلى نهاية كانون الثاني 2022. تم توزيع العينات السريرية على 145 (72٪) من الجروح و 55 (27.5٪) من عينات الحروق. زرعت العينات على وسط اكار الدم والماكونكي ووسط ملح المانيتول و تم تشخيص العزلات مجهريا و باستخدام الاختبارات البيوكيميائية التي أظهرت أن *Staphylococcus aureus* 30 (17.6٪) و 13 (7.64٪) *Staphylococcus epidermidis* و *Acinetobacter baumannii* 56 (32.9٪) *Pseudomonas aeruginosa* 32 , (18.8٪) ، *Klebsiella* spp 20 (11.7٪) ، *Proteus* spp. 8 (4.7٪) *E.coli* , 11(6.47%) على التوالي. تم اختبار الحساسية لجميع العزلات تجاه 13 مضاد حيوي وكانت العزلات ذات مقاومة متعددة للمضادات الحيوية ، وتم تأكيد التشخيص بواسطة نظامي APiE20 و Vitek- 2. تم اختيار زهرة المينا لتحضير المستخلصات النباتية ، وتم تقييم فعالية المستخلصات المضادة للبكتيريا على خمس سلالات بكتيرية معزولة من اخماج الحروق والجروح ،اظهر المستخلص المائي فعالية متوسطة في تثبيط البكتريا بتركيز (12.5 ، 25 ، 50 ، 100 ، 200) ملغ / مل حيث بلغ اعلى قطر تثبيط (15) ملم بتركيز 200 مجم / مل ضد عزلات *Klebsiella* spp. تليها (13) *Pseudomonas aeruoginosa* ملم و *Staphylococcus aureus* و *Acinetob*acter *baummannii* كانت (12.5, ,13,12.5) ملم بتركيز 12.5 ملجم / مل *Proteus* spp. لم يظهر أي نشاط مثبط. أظهر المستخلص الكحولي فعالية أكبر كمضاد للبكتيريا من المستخلص المائي. لوحظ أن أوسع مناطق التثبيط كانت 18 ملم *Klebsiella* spp. تليها *S. aureus* *P.aeruoginosa* ., *Proteus* spp و *A. baummannii* مناطق تثبيط (16، 15، 15، 14) ملم على التوالي.

الكلمات المفتاحية: الضد بكتيري، المينا الشجيرية، الاخماج الجلدية، اختبار الحساسية.

Introduction

The World Health Organization (WHO) reports estimated 265,000 of mortality occurred every year as a result of burn injuries and wound infections that occurred when a large areas of skin are disrupted ,microbial nutrient availability and vascular supply destruction as well as systemic immunosuppression [1] . Skin damage caused by many reasons such as trauma including cuts, abrasions chemical burns, fire burns, cold, heat, radiation, surgery, or as a consequence of diabetes ulcers[2].Burn injuries are a global public health problem and still remain the leading cause of disability and unintentional death [3]. Resistance to antibiotics is rising to high levels in all parts of the world accompanied by new resistance mechanisms that are spreading globally and can threat the ability to treat common infections. Recently, antibiotics can be bought for human or animal use without a prescription or standard treatment guidelines ,the over prescription of drugs by health workers and veterinarians and the overuse of antibiotics without physicians monitoring in health care facilities lead to increase of the resistance toward common antibiotics[4]. Multi-Drug Resistance(MDR) is a term refered to the acquired non-susceptibility to at least one agent in three or more antibiotic groups another term used to express the ability of pathogens to resist antibiotics was Extensive Drug Resistance (XDR) that defined as the bacterial resistance to at least one antimicrobial agent in all but two or fewer antimicrobial categories as the bacterial isolates remain susceptible to only one or two categories and the term PDR (Pan Drug Resistance) explain that the bacterial isolates are non-susceptibility to all agents in all antimicrobial categories [38].As a result to the antibiotic resistance issue there was an urgent need for alternative drug therapies to overcome this challenge by using the natural alternatives such as medicinal plants and their extracts that has become the safest alternatives. *Lantana camara* from Family: Verbenaceae is one of the most important medicinal plant which have various traditional uses[5]. Parts of plant extracts were traditionally used for healing of wounds, cuts, skin itches, and eczema. Plants containing many phytoconstituents such as alkaloids, glycosides, saponins, steroids, terpenoids, carbohydrates, flavonoids, and coumarins. It has various pharmacological activities such as an antioxidant ,antimicrobial, antibacterial,

antifungal, antiulcerogenic, anthelmintic, anti-hyperglycemic, anti-inflammatory, analgesic, anticancer, antitubercular and insecticidal activity [5].

Material and method

Collection of clinical samples

Two hundred surface swabs have been collected from patients cared in Baquba Teaching Hospital / Diyala during September 2021 to the end of January 2022. The swabs was taken from wound and burn infections site of patients from both genders of different ages admitted in surgical, burn wards and surgical operation rooms .

Isolation and identification of the clinical samples

Morphological examination based on the morphological characteristic, Isolates were grown on MacConkey agar and Blood agar(incubated at 37°C for 24h) after incubation colony color, shape, edges, specific material production, blood hemolysis and texture were studied . Microscopic examination is done by taking one bacterial colony and transported to the microscopic slide, fixed by quick pass over a burner flame then stained using Gram stain technique. Cell arrangement and shape were observed , Identification by biochemical tests was made these tests included Oxidase test, Catalase, .IMiVC test and Triple Sugers Iron TSI, confirmation of the clinical isolates were done by API 20E Diagnostic Systems that depends on 20 tests and according to the manufacturer's instructions (Biomerieux), Confirmation of bacterial isolates identification by VITEK-2 system.

Susceptibility of the bacterial isolates to antibiotics

All the bacterial isolates were tested for antimicrobial susceptibility according to the CLSI (2021) criteria using agar diffusion method. Few bacterial colonies (2-4) from overnight culture were transferred to 2 ml of normal saline to prepare the bacterial suspension which was adjusted to McFarland turbidity (1.5×10^8) CFU/ml. Then inoculated in Muller-Hinton agar and incubated for 24 h at 37°C. The diameter of the inhibition zone of each antibiotic disc was

measured and the results were interpreted by referring to CLSI (2021) recommendation. Table 1 showed different antimicrobial discs.

Table 1: Antibiotics potencyused in the study

Antibiotics	Conc. µg/ml	Antibiotics	Conc. µg/ml
Pencillin	10	Levofloxacin	5
Pipracillin	100	Meropeneme	5
Ampicillin	10	Impeneme	10
Cefotaxime	30	Vancomycin	10
Ceftriaxone	30	Trimethoprim/sulfamethoxazole	1.25/23.75
Cefoxitin	30	Amikacin	30
Ceftazidime	30	Gentamycin	10
Azithromycin	15	Tobramycin	10
Aztereonam	30	Ciprofloxacin	5

Collection of plant sample

Lantana camara flowers were collected from domestic and public gardens in Diyala governorate/ Iraq during October 2021. The plant was identified by Professor Dr. Khazal. D. Wadi in department of Biology - College of Sciences - University of Diyala. The flowers were washed in distilled water (to remove dust, dirt, and other contaminants), dried in shad , the dried flowers were ground by an electric grinder to get a fine powder which then were kept in sterile glass airtight container till further analysis [7] .

Preparation of *Lantana camara* flower hot aqueous extracts

Preparing hot aqueous extracts was done by following the method of Thorat *et al* [7] through mixing 50 gm of plant powder per plant sample with 500 ml of boiled distilled water after cooling to 60°C in a 1000ml volumetric flask. Then placed on a magnatic sterile hot plate For 2 h, the mixtur filtered using multiple layers of medical gauze, then filtrated using Whatman No.1 filter paper , then filtrate distributed into sterile plane tubes and was centifugated on a device Centrifuge at 3000 rpm for 10 minutes and evaporated at 40 ° C until the solvent evaptrated completely .the extract was dried by spread in sterile glass petri dish with a large surface area in incubator at 37 ºC to get a dry powder of the aqueous extract, It was placed in

sterile sealed glass tubes, and after marking Stored in the refrigerator at a temperature of 4°C until use this procedur was repeated to get enough amount of the flower extracts .

Preparation of *Lantana camara* flower alcoholic extract

Alcoholic extract was performed by the maceration method according to Sadat *et al* [8] 50 grams of the dried flower powders were soaked in 500 ml of pure ethanol and left in the dark for 3 days. The resulting solution was filtered through filter paper. The extract was concentrated using a rotary apparatus at 45°C and dried in incubator at 37°C to obtain the dry powder of the extract. Then it was placed in an airtight tube coated with aluminum foil and kept in the refrigerator at a temperature of 4°C Until use.

Biological effectiveness of *L. camara* flower extracts on bacterial growth

The antibacterial activity of the plant extracts have been tested with five different concentrations(12.5, 25, 50, 100, 200) mg/ml against five kinds of pathogenic bacteria isolated from pateints in Baquba Teaching Hospital / Diyala. The bacterial isolates included *Staphylococcus auerus,Acinetobacter baumannii* , *Pseudomonas aeroginosa* , *Klebsiella oxytoca* and *Proteus vulgaris* . The bacterial suspension was prepared by taking a number of bacterial colonies by loop to tubes containing heart and brain infusion broth the tubes were incubated for 18 hours at a temperature of 37 °C for the purpose of activating, then the bacterial suspension was compared with McFarland standard solution, which depends on the degree of turbidity of the bacterial suspension is close to 1.5 x 10^8 (CFU/ml).

Determination the antibacterial activity of the flower extracts

2 gm of flower extrat was dissolved in 10 ml of distilled water using vortex to obtain a 100% stock solution at a concentration of 200 mg/ml .Four different concentrations were prepared from the stock (12.5, 25, 50,100) mg/ml. Antibacterial activity of the flower extracts was determined by agar well diffusion method according to Dadi *et al* [9] . Each tested bacterium was cultured on Mueller- Hinton agar after comparison with McFarland tube (1.5x10^8) CFU/ml by streaking method and the five wells 5 mm were made in the plate by sterilized cork borer.

100 µl of five concentration added to wells using micropipette,the sixth well was taken as control add 100 µl of distilled water.Three repeats were made for each bacterium and then incubated at 37 °C , 24 h.

Statistical Analysis

The descriptive and ordinal variables were described in the form of number and percentage using excel 2010. All values were expressed as Mean ± SE values and the same are presented in tables. These analyses were done by using SPSS (Statistical Package for Social Sciences) program version 16.0 for windows.

Results and Discussions

The overall number of samples collected was 200 clinical samples and about 170 (85%) showed positive bacterial growth where as 30(15%)of the subcultured swabs did not show any growth, the positive growth of the clinical isolates divided into 43(25%) gram positive bacteria represented by *S. aureus* 30 (17.6%), *S.epidermidis* 13(7.64%). And 127(74.7%) gram negative bacteria that include *A.baumannii* 56 (32.9%) , *P.aeruginosa* 32(18.8%) , *Klebsiella* spp. 20 (11.7%), *Proteus* spp. 8(4.7%) and *Escherichia coli* 11(6.47%). The results showed that the number *P. aeroginosa* 15 (27.7%) collected from burns infections were the highest percentages of bacterial isolates followed by *A. baumannii* , *S. aureus* 13 (24%) , 10(18.5%) , respectively. The results also showed that the rate of isolation of the other bacterial isolates from burns was for *Klebsiella* spp .8 (40%) , *S.epidermidis* , *Proteus* spp. 6 (11.1%) , 2 (3.7%) . The results presented that the highest rate of bacterial isolates from wound infections was for *A. baumannii* 43(37%) followed by *S. aureus* and *P. aeruginosa* 20(17.2%),17 (14%) respectively. The rate of isolation of *E. coli*,*Klebsiella* spp., *Proteus* spp.and *S. epidermidis* from wounds were 11 (100 %),12(60%),4 (3.4%),7 (6%) respectively .The current results found that the numbers of gram negative bacteria isolated from wound and burns were higher than gram positive bacteria, *S. aureus* and *S. epidermidis* were the most frequent gram-positive bacteria isolated from wound and burn infections these results agreed with Nouri *et al.* [10] .The most commonly pathogenic bacteria isolated from wound and burns was *A. baumannii* followed by *S.aureus* and

P.aeruginosa these results were correspond with Gouran *et al* [11]. *A. baumannii* survived on dry surfaces under nutrient limiting conditions and colonized in medical devices and equipment that serve as reservoirs in prolonged hospital outbreaks. This pathogen has the ability to persist longer than *E. coli* on dry surfaces and remain viable for more than 4 months. *Acinetobacter* spp. are more frequently found on inanimate objects and hands of medical staff than *S. aureus* and *P. aeruginosa* [12].

Antibiotics susceptibility test

The susceptibility test of the 30 of *S.aureus* and the 12 isolates of *S . epidermidis* were tested to 10 antibiotics as shown in table (2) for *S . aureus*, The results showed high resistance to Penicillin, Cefoxitin, Co-trimaxzole, Levofloxacin, Meropeneme, Doxycycline, Ciprofloxacin, Gentamycin and Vancomycin (86.6%, 80%, 70%, 53.3%, 56.6 %, 46.6%, 46.6%, 40%, 40%, 30%). This pathogen developed many mechanisms of resistance to all *β-lactams*, tetracyclines, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole and vancomycin [13]. This bacteria can produce penicillinase, which can hydrolyze the penicillin *β-lactam* ring [14,15]. Resistance to tetracycline is mediated either through ribosomal protection proteins, which act to dislodge the tetracycline from its ribosomal binding site, or through the presence of efflux pumps. *S. aureus* is also resistant to trimethoprim-sulfamethoxazole results from the production of specific enzymes that contain amino acid substitutions that make them resistant to the antibiotic combination [13]. The resistance to Ciprofloxacin was 40%. which agreed with Raheema [16] . Fluoroquinolones resistant is either mediated through efflux pumps or through mutational amino acid substitutions in the fluoroquinolone binding site of topoisomerase IV and DNA gyrase. As *Staphylococci* are very sensitive to fluoroquinolones, mutations in both enzymes are required for resistance to develop [13]. The results also showed moderate resistant toward Imepenim 46.6%, that did not agree with Yosef [17] The resistance to Doxycycline 46.6%. and Gentamycin 43.3% matched with Raheema[16] . The resistance of *S.aureus* to aminoglycosides is caused by a decrease in membrane permeability Which results in a decrease in drug intake[18].and also mediated through enzymatic inactivation, specifically through enzymes that acetylate and phosphorylate aminoglycosides [19] . The lowest resistant

percentage was toward Vancomycin 30%, this antibiotics has long been contributed the drug of choice for the treatment of severe *S.aureus* infections [20] Vancomycin is mainly resisted by specific binding of the bacterial cell wall via peptidoglycan precursor small peptides, which are terminated with D-alanyl-D-alanine. this binding inhibits the elongation and cross-linking of bacterial cell wall peptidoglycans,which cause repressing to cell wall synthesis and leading to bacterial death [21]. *S.epidermidis* isolates were resistance to Penicillin,Cefoxitin ,Cotriamaxzole,Meropeme,Levofloxacin ,Doxycycline, Ciprofloxacin , Gentamycin(76.9% ,61.5%, 53.8% ,53.8%, 46.1% , 38.4% , 23%) All *S.epidermidis* isolates were 100% sensitive to Vancomycin. *S. epidrmidis* has been shown the same susceptibility patter n of *S.aureus* toward the tested antibiotic the results agreed with Chapi and Momtaz [22].

Table 2: Number and percentage of resistant and sensitive *S.aureus* and *S.epidermidis*

Antibiotic	*S. aureus* n=30 percentage %				*S. epidermidis* n= 13 percentage %			
	R	%	S	%	R	%	S	%
Penicillin	28	93.4	2	6.6	10	76.9	3	23.1
Cefoxitin	24	80	6	20	6	46.2	7	53.8
Gentamicin	12	40	18	60	5	38.4	8	61.6
Ciprofloxacin	12	40	18	60	6	46.2	7	53.8
Levofloxacin	16	53.4	14	46.6	7	53.8	6	46.2
Impeneme	14	46.6	16	53.4	4	30.7	9	69.3
Doxycyclin	13	43.3	17	56.7	8	61.5	5	38.5
Vancomycin	4	13.3	26	86.7	0	0	13	100
Trimethobrim sulfamethazole	17	56.6	13	43.4	5	38.4	8	61.6
Azithromycin	9	30	21	70	3	23	10	77

Susceptibility test of 56 *A. baumannii* to 13 antibiotics was tested . This pathogen was resistant to Piperacillin, Ceftazidim, Cefotaxime, Aztereonam ,Amikacin, Tobramycin, Ciprofloxacin, Levofloxacin , Impenem, Meropenem ,Trimethobrim sulfamethazole , Doxycylin, Pipracilin/ Tazobactam (53.2% ,73.2%,80.3%, 73.2% , 44.6% , 60.7%, 46.4%,46.4% , 69.6%, 58.9% , 50%, 44.6% and 41%,.) these results matched with what was found by Hamza [23] . The clinical isolates showed moderately resistance pattern toward Pipracillin 53.2%, Ciprofloxacin 46.6%, Amikacin 44.2% ,Pipracillin-Tazobactam 41%, which agreed with Twiari *et al* [24] Resistance to aminoglycosides in this microbe is due to the production of all types of aminoglycoside modifying enzymes[25]. Resistance to fluoroquinolones is mediated through mutations in the

genes of DNA gyrase and topoisomerase IV, reducing their affinity for fluoroquinolones, and through the production of qnr-type protection proteins, which inhibit the binding of fluoroquinolones to topoisomerase IV and DNA gyrase. In addition, *A. baumannii* can produce efflux pumps and decrease the expression of porins, reducing intracellular concentrations of fluoroquinolones [26].

All 30 *P. aeruginosa* were tested for susceptibility against 13 different antibiotics the results showed high resistance to Piperacillin, Cefotaxime, Ceftazidim Amikacin, Tobarmmycin, Ciprofloxacin, Levofloaxin, Meropenem, Impenime, Doxycyclin, Aztereonam, cotrimaxzol, Azithromycin (65.6%, 75%, 87.5%, 53.1%, 46.8%, 53.15%, 43.7%, 84.3%, 50%, 68%, 56.2%, 62.5%, 53%). The isolates had shown a varied levels of resistances to the tested antibiotics and the highest resistance were to penicillins, cephalosporins, aminoglycosides, monobactam, fluoroquinolones and carbapenems. The current findings partially correspond with another local study done by Al Saadi [27]. this pathogen can display resistance to many kinds of antibiotics groups including aminoglycosides, quinolones and β-lactams, Aminoglycoside resistance is due to multiple factors including the reduced cell membrane permeability, increased efflux, ribosomal changes and enzyme modification. Among these mechanisms, the enzymatic modification of amino and glycoside groups in the aminoglycoside molecular structure plays a predominant role in resistance to this class of antibiotics [28].

Table 3: Distribution numbers and percentages of resistant and sensitive *A. baumanii* and *P. aeroginosa*

Bacteria	*Acintobacter baummanni* n=56 percentage %				*Pseudomonas aeroginosa* n= 32 percentage %			
Antibiotics Symbol	R	%	S	%	R	%	S	%
Piperacillin	30	53.5	26	46.5	21	65.6	11	34.4
Cefotaxime	41	73.3	15	26.7	28	87.5	4	12.5
Ceftazidime	45	80.4	11	19.6	24	75	8	25
Amikacin	25	44.6	31	55.4	17	53.1	15	46.9
Tobramycin	34	60.7	22	39.3	15	46.8	17	53.2
Ciprofloxacin	26	46.4	30	53	17	53.2	15	46.8
Levofloxacin	26	46.4	30	53.6	14	43.7	18	56.3
Impeneme	39	69.6	17	30.4	16	50	16	50
Meropeneme	33	58.9	23	41.1	27	84.4	5	15.6
Doxycyclin	25	44.6	31	55.4	22	68.8	10	31.2
Aztereonam	-	-	-	-	17	53.2	15	46.8

Trimethobrim sulfamethazole	28	50	28	50	20	62.5	12	37.5
Azithromycin	41	73.2	25	44.8	-	-	-	-
Piperacillin-Tazobactam	23	41	33	58.9	18	56.2	10	31.8
R Resistance S sensitive (-) Not used for bacteria								

All of the 20 isolates of *Klebsiella* spp. were resistance to Piperacillin, Ampicillin, Cefotaxime Ceftriaxone,Ceftazidime, Aztereonam, Impeneme, Meropeneme, Tobramycin, Levofloxacin, Amikacin, Azithromycin, Ciprofloxacin, Co-trimaxzole, Amoxicillin-Clavulanic acid (30%, 60%, 95%, 90%, 95%, 90%, 65%, 40%, 56%, 40%, 40%, 30%, 40%, 35%, 90%). These results matched with Nirwati *et al*. [29]. *Escherichia coli* and *Klebsiella* spp. producing extended spectrum β-lactamases (ESBLs) represent a worldwide growing threat to public health these bacteria easily develop resistance to antibiotics through the production of enzymes such as Extended Spectrum β- Lactamase (ESBLs) and Carbapenemase which confer resistance to Penicillins, Cephalosporins (including third-generation Cephalosporins) and Aztreonam. [30]

The 11 isolates of *E.coli* showed a varied levels of resistances to Piperacillin, Aztereonam, Ampicillin, Levofloxacin, Doxycycline, Ceftazidime, Ceftriaxone, Tobramycin, Cotrimaxzole, Amikacin, Ciprofloxacin, Cefotaxim (63.6%, 81.8%, 72.7%, 45.4%) the isolates were to 100%sensitive Impeneme, Meropeneme and Amoxicillin-Clavulanic acid. These results agreed with Alharbi *et al*. [31]. Meropenems are members of carbapenems considered clinically important antibiotics that is used in the treatment of Multidrug-Resistant (MDR) bacterial infections. The clinical isolates of *E. coli* found to be susceptible to Amoxicillin/ Clavulanic acid which indicates that this pathogen can be inhibited by Clavulanic acid [32].

E. coli strains in this study were resistant to β- lactam antibiotics by producing wide spectrum β -lactamase (ESBL) that is known as plasmid-mediated β-lactamase. These enzymes are capable of hydrolyzing and inactivating penicillins, cephalosporins and monobactams [33]. The highest percentages of resistance were observed for Tobramycin (72.7%) followed by Amikacin (45.4%) these antibiotics can be resisted by several mechanisms that include enzymatic modification, inactivation of their effect mediated by aminoglycoside

acetyltransferases, nucleotidyl transferases, or phosphotransferases which is commonly observed with gram positive and negative bacteria, increased efflux, decreased permeability and modifications of the 30S ribosomal subunit that interferes with binding of the aminoglycosides by either mutations (nucleotide replacement) or post transcriptional modifications associated with aminoglycoside resistance [34].

The total number of the n= 8 *Proteus* spp. revealed resistance to Aztreonam Impenem, Meropenem, Tobramycin, Amikacin, Azithromycin, Ciprofloaxcin, Co-trimaxzole and Levofloxacin (62.5%, 25%, 75%, 25%, 50%, 95%, 37.5%, 50 %, 50% 25%) respectively.

All the tested isolates were (100%) sensitive to Amoxicillin\clavulanic acid, Piperacillin, Ampicillin, Cefotaxime, Ceftazidime, Ceftriaxone and Doxcyclin. The results agreed with [35]. *Proteus* spp. is naturally resistant to several antibiotics and shows reduced susceptibility to Imipenem. However higher levels of resistance to Imipenem commonly occur in this pathogen consecutively to the loss of porins, reduced expression of penicillin binding proteins (PBPs), or acquisition of several antibiotic resistance genes, including carbapenemase genes. Emergence and spread of MDR *P. mirabilis* isolates, including those producing cephalosporinases and carbapenemases, are increasingly reported which complicates the treatment of infections caused by this pathogen [36] Narrow-spectrum β-lactamases produced by this bacteria have been inhibited by Clavulanic acid or resistant to the action of Clavulanic acid have been reported in *Proteus* spp. These enzymes hydrolyze narrow-spectrum penicillins, slightly 1st generation cephalosporines but are not active on 2nd and 3rd generations and carbapenems [37].

Table 4: Number and percentage of resistant and sensitive clinical isolates of *Klebsiella* spp., *Proteus* spp. and *E.coli*

Bacteria	*Klebseilla* spp n = 20 % percentage				*Proteus* spp n=8 % percentage				*E.coli* N=11 % percentage			
Antibiotic	R	%	S	%	R	%	S	%	R	%	S	%
Piperacillin	12	60	8	40	0	0	8	100	7	63.6	4	36.4
Ampicillin	19	95	1	5	0	0	8	100	9	81.8	2	18.2
Cefotaxime	18	90	2	10	0	0	8	100	5	45.4	6	54.4
Ceftazidime	18	90	2	10	0	0	8	100	9	81.8	2	18.2
Cefrtiaxone	19	95	1	5	0	0	8	100	8	72.7	3	27.3

Aztereonam	13	65	7	35	5	62.5	3	37.5	7	63.6	4	36.4
Impeneme	8	40	12	60	2	25	6	75	4	36.4	7	63.6
Meropeneme	13	65	7	35	6	75	2	25	8	72.7	3	27.3
Tobramycin	11	55	9	45	2	25	6	75	8	72.7	3	27.3
Amikacin	8	40	12	60	4	50	4	50	0	0	11	100
Azithromycin	6	30	14	70	7	95	1	5	6	54.5	5	45.5
Doxycyclin	10	50	10	50	0	0	8	100	9	81.8	2	18.2
Ciprofloxacin	8	40	12	60	3	37.5	5	62.5	5	45.4	6	54.6
Levofloxacin	6	30	14	70	4	50	4	50	9	81.8	2	18.1
Trimethobrim sulfamethazole	7	35	13	65	4	50	4	50	8	72.7	3	27.3
Amxycillin-clavulanic acid	18	90	2	10	2	25	6	75	0	0	0	100
R Rsistance S Sensitive												

The isolates in the recent study possess different resistance mechanisms to various antimicrobial agents. These species are classified as multi-drug resistant (MDR), extensively-drug resistant (XDR), pan-drug resistant (PDR)[38]

The results of the current study showed that all the clinical isolates of *Staphylococcus* spp. were multiple drug resistant and the number of *S.aureus* and *S. epidermidis* resistant to one antibiotic from three or more antibiotic categories MDR were 21(70%), 12(95%) respectively. Numbers of isolates resistant to one antibiotic from all except two or fewer antibiotic categories XDR isolates were 9(25%), 1(5%) while the isolates are resistant to all antibiotic categories the isolates number and resist 10 antibiotics was 0(0%) respectively, table (5).

Table 5: Antibiotics resistant patterns of gram positive bacteria

BACTERIA	MDR NO.(%)	XDR NO.(%)	PDR NO. (%)
S.aureus	21(70%)	9 (30%)	0(0%)
S.epidermidis	12(95%)	1 (5%)	0(0%)

Gram negative bacteria *A. baumannii* and *P. aeroginosa*, *Klepseilla* spp, *Proteus* spp and *E.coli* were multiple resistance to antibiotics, the number of MDR were37 (66%), 16(53%), 16 (80%), 7 (87.5%) and 8 (72.7%) respectively, XDR 18 (32.1%), 14 (46.6%), 20%, 12.5%, 27.3% respectively The antimicrobial resistance pattern obtained from this study gave a serious

concern because the isolates were highly resistant to the commonly antimicrobial agents used in Iraq

Table 6: Number and percentages of antibiotic resistant pattern of gram-negative bacteria

Bacteria	MDR NO.(%)	XDR NO.(%)	PDR NO. (%)
A.baumannii	38(67.85%)	18(32.15%)	0(0%)
P. aeroginosa	16 (53.33%)	4 (32.15%)	0(0%)
*Klebsiella*spp.	16 (80%)	4(20 %)	0(0%)
E.coli	8 (72.7%)	3(27.3%)	0(0%)
Proteus spp .	7 (87.5%)	1 (12.5%)	0(0%)

Study of the biological effectiveness of *L. camara* flower extracts against pathogenic bacteria

The results of the antibacterial effectiveness of flower extracts against bacterial strains showed that the extracts displayed moderate to high inhibitory activities on all the clinical isolates and susceptibility to extracts was increased with increasing concentration of the extracts. The hot aqueous extract recorded the highest antimicrobial activity against the growth of *K. oxytoca* at a concentration of 200 mg/ml (15 mm) followed by *P. aeroginosa(*13mm), *S. aureus (*12mm). *A. baumannii(*12mm) *and Proteus spp.* (12mm) while the concentration 12.5 mg/ml did not show any inhibitory action toward all isolates as shown in table (7), The study also found that the bacteria under studying were more susceptible to alcoholic extract compared to the hot aqueous extract with a high inhibition zone recorded in *Klebseilla* spp. (18mm) followed by *Proteus* spp., *S. aureus* , *A.baumannii* and *P. aeruginosa* (16,15.5 ,14.5,15)mm respectively .This activity could be attributed to the presence of different phytochemicals and bioactive compounds in the extract such as high flavonoid, tannins, and phenol contents of the alcoholic extract,alkaloids, flavones, isoflavones ,saponins triterpenoids. and phenylethanoid. glycosides, were highly present in the alcoholic extracts than aqueous extracts [5].

The systematic screening of plant extracts for antimicrobial activity is a continuous effort to find new antibacterial compounds [39] Previous studies using extracts from *Lantana* species showed that they were able to inhibit the growth of gram positive and gram negative bacteria strains [40] *Lantana camara* was listed as one of the important medicinal plants of the world It

has been used to possess various biological activities such as anti-protozoal, anti-inflammatory, antibacterial and antioxidant activity The therapeutic potential of this plant is due to the presence of bioactive compounds [41].

Table 7: Antibacterial activity of *Lantana camara* flower extract against pathogenic bacteria (inhibition zones)

Bacteria	Conc. mg/ml	Bacteria Inhibition zone diameter (mm) Mean ± St. Error, the level of significant at P< 0.05				
		S.aureus	*A.baumannii*	*P.aeroginosa*	*Klebsiella* spp.	*Proteus* spp.
Aqueous Flower extract	12.5	0.00±0.00	0.00 ±0.00	0.00±0.00	0.00 ±0.00	0.00 ±0.00
	25	9.00 ±1.00	8.5 ±1.50	0.00 ±0.00	11.00±0.00	9.50± 0.50
	50	10.50±0.50	9.50 ± 1.50	11.00±1.00	12.5± 0.50	10.5 ± 0.50
	100	11.5± 0.50	11.5± 0.50	12.00±1.00	13.5±0.50	11.5±0.50
	200	12.50±0.50	12.50±0.50	13.00±1.00	15.00±0.00	12.50±0.50
Alcoholic flower extract	12.5	9.00 ±0.00	8.00 ±0.00	8.00±0.00	9.00±0.00	8.00±0.00
	25	11.50±0.50	9.00 ±4.50	9.00 ±0.00	12.50±0.50	11.00 ±1.00
	50	13.00±0.00	11.00± 1.00	12.5± 1.50	14.00±0.50	13.00±1.500
	100	14.5 ± 0.50	12.00 ± 1.00	13.0 ± 1.50	15.00±0.00	14.00± 2.00
	200	15.50±0.50	14.50 ±0.50	15.00±1.00	18.00±0.00	16.00±2.00

Conclusion

The present study supports the possible of *Lantana camara* as a medicinal plant had antibacterial effectivness against MDR bacteria . Ethnomedical and scientific reports about the medicinal properties represent it as a valuable plant and establishing it as a candidate for the future drug development.

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