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**Isolation and identification of common bacteria from
urinary tract exit and skin of rabbits, with their sensitivity
to ethanol extracts of some medical plants**

ABSTRACT

To isolate the common bacteria present in urinary tract orifices, and skin of rabbits, 18 adults rabbits of either sex, of 1-2 kg b.wt. local and albino breed were depended. In current study the isolated symbiotic bacteria from the skin and urinary tract exit of rabbits were identified based on their morphological, cultural, physiological and biochemical characters in addition to their sensitivity to ethanol extract of pomegranate peel, *Melia azedarach* stem, *Lantana cammra* fruit, *L. cammra* flowers and *L. cammra* leaves using well diffusion methods. The results showed that the most common isolates were Staphylococcus, Streptococcus, Pseudomonas, Klebsiella, Bacillus and *E. coli*. The ethanol - aqueous extracts of pomegranate peel and *L. cammra* leaves were of the more inhibitory effects against Staphylococcus , *E. coli* and Streptococcus .

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Introduction

There is a strong link between microbiology and dermatology. Fields together has long documented that the surface of the skin is colonized by many microorganism such as bacteria, fungi plus viruses; these contagious groups are closely related to humanoid health and disease (Grice and Segre, 2011), and the skin is the largest organ of the mammalian organism direct protection from external factors (Elias and Schmuth, 2009).

Plants are a potential source of antimicrobial intermediaries in diverse states about 60 toward 90 % of people in developing states usage plant-derived medicines; conventionally, raw plant extracts are used as herbaceous medicine to treated contagious infections (Alviano and Alviano, 2009). The Plants are rich in a variety of phytochemicals including alkaloids, terpenoids, flavonoids and tannins which created in the laboratory to have antimicrobial properties (Talib and Mahasneh, 2010). The mode of action of these herbaceous extracts and their effectiveness in utmost cases is

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still required to be precisely certified, these preparations assist central host reactions (Cruz *et al.*, 2007)

Microorganisms are generally considered to be of two groups; Residing microbes have its place to a relatively constant group of microorganisms that are regularly present in the skin and which recreate themselves afterward the disorder, The resident microbes are often seen as commensal; which means these microorganisms are not dangerous and many of them afford an advantage to the host. (Grice and Segre, 2011).

Skin micro flora mostly can be categorized into 2 collections ; transient and resident flora , Transient flora is introduced from the environment and only attach if the skin is disturbed ; Resident flora establish protected attachments to the skin are existent in stable numbers, also are able to withstand an acid atmosphere (Schroder, 2010).

Residential and transient microbes are not pathogenic in typical environments if suitable hygiene is conserved and if they are normal; However after perturbation, transient and or residential bacterial inhabitants can multiply ; colonize and cause disease. For sample, *Staph. epidermidis* is a skin commensal but can be an opportunistic pathogen in immune compromised hosts (Otto, 2009).

Resident G+ bacteria include *Corynebacterium sp.* besides *Micrococcus Streptococcus pyogenes* and *Staphylococcus aureus* are particularly pathogenic in the skin. G- organisms such as *Pasteurella multocida* , *Pseu. aeruginosa* , *Bartonella asp.*, *Caprocytophage canimorsaus*, *Klebsiella*, *Vibrio vuluificus* and *Rhinoscleromatis* are not normal resident skin micro flora but may reason skin infection (Katarina *et al.*, 2001).

Micro-organism may be useful to human microbiota, complex infective bacteria that populate positions in and on the human body such as skin, gut and oral cavity, distinct circumstances as in patients whose typical native defenses fail to function appropriately can lead to an imbalance of an individual class that are pathogens in the conventional sense such As *Escherichia coli* , *Enterobacter sp.* and *Pseudomonas aeruginosa* (Rogers *et al.*, 2013).

Skin is the chief organ in the mammalian organism and the situation direct defense from outward factors (Elias and Schmuth, 2009). Skin is not just an inert coverage of the body but it performs many major roles: thermoregulatory, defensive, excretory, sensory and metabolite, healthy skin affords security of the body from ecological factors: chemical (destructive agents, allergens , xenobiotics, surface active substances) physical (thermal injury, mechanical trauma and radiation), and biological (bacteria, viruses, fungus etc.) (Elias and Choi, 2005).

Masses of bacteria, viruses, fungi, archaea, and small arthropods Inhabit the surface of the skin, communally containing the skin micro biome (Heidi and Julia, 2012).

The microbial elements in this community are determined by many environmental and physiological factors such as the hormonal status ,local humidity, structural location, sebum and sweat creation and age of the host. This microbial communal contains bacteria, viruses, protozoa and fungi and its strength is based on a delicate Balance between the characteristics of the microbial population and the hosting of the human being with his defense mechanisms (Christensen and Bruggemann, 2014).

Bacterial skin flora is commensal, symbiotic or parasitic in relation to the host; Although it is known that alterations in the host's immune state have an important influence, the type of relationship established is often inherent to the bacteria. Continuous colonization is the result of bacteria's ability to adhere to the epithelium of the skin, growing in a relatively dry and acidic environment, and re-sticking quickly during the natural desquamation process (Feingold, 1986).

Aim of study was to isolate the common bacteria from the skin and urinary tract exit of rabbits and study its sensitivity for ethanol extracts of some medical plants .

Materials and Methods

The study was conducted, in Branch of Microbiology; College of Veterinary Medicine; University of Diyala; Iraq .from September 2018- April 2019.

Collection of samples

At the period from September 2018- April 2019, swabs from skin and urinary tract orifice were collected from local and albino rabbits. Urine samples were placed in a sterilized test tube then

transmitted to laboratory at less than two hour for examination ,While skin samples located in a sterile test tubes contained 5 mL of a sterilized brain heart infusion broth and placed in ice box for bacteriological examination .

Methods

Isolation and diagnosis of bacteria

Swabs cultured on nutrient broth for 24 hours then cultured on nutrient agar. Isolated colonies classified according to their shape, color and morphological characters . Then cultured on blood agar (reading according to hemolysis) if there is hemolytic zone (stained and examined microscopically) or cultured on MacConkey agar (Lactose fermenting and Lactose none fermenting) Lactose nonfermenting was examined by oxidase test for *pseudomonas* and *Proteus* Lactose fermenting bacteria was examined by Indol test : red ring (*E. coli*), no red ring *Klebseilla* , *Klebsiella* mucus strip.

Identification of isolates:-

The isolates were identified based on the morphological characters ,microscopical examination and biochemical tests

Microscopic examination:-

Single colony were picked up after the isolation of bacteria on MacConkey agar and blood agar, stained with Gram stain , then examined under microscope to recognize their shape and length according to (MacFaddin, 2004).

Biochemical tests:-

For the confirmation of any suspected isolate , a numerous biochemical tests had been done according to (Garrity *et al.*, 2005).

plants extract preparation

The fruits, flowers and leaves of the plants were gained from the native marketplaces of Baquba City or cool from trees in the gardens of the City. Wash it from dirt tap water formerly through distilled water, Air dryer devoid of contact to sunlight inside the laboratory for 3 weeks at 22 ± 2 °C, they are ground in an electronic crusher to obtain a fine powder and then extracted with ethanol according to standard extraction methods (Harborne, 1998) kept until used.

Preparation of Ethanol extract

Total of 50 grams of shade dried, powder from each of Pomegarante peel; *Melia azedarach* stem; *L. cammara* fruits, leaves and flowers). It is mixed with 250 ml of 70% ethanol by soxhlet machine for six hrs. At 60-70°C, formerly by a rotary evaporator at 40°C the solvent was removed under reduced pressure. then the final result of the extracted material was kept and then different concentrations (50, 100, and 200 mg/ ml) were prepared from the stock solution by dilution with dimethyl sulfoxide (DMSO) solution ; and used as the test extracts for antimicrobial activity assay(Karaman *et al.*,2003) McFarland solution was prepared according to (McFadden, 2000) ,The combination is shaken fine and placed a screw capped test tube and saved in dark place at 4 °C. The solution is mixed well before using it to compare it through bacterial turbidity as it gives a turbidity equivalent to (0.5×10^5) bacteria\ ml.

For the sensitive test to plant extract we used Muller Hinton agar.

Antimicrobial activity

The adapted agar diffusion technique has been well relied upon (Olurinola, 1996) to assess the antibacterial activity of the extracts, (10^5 CFU/ ml) was aseptically extent onto the surface of Muller Hinton agar and formerly leftward to dry for 30 min. A well (5 mm) in diameter was worked in media using sterile pipette ends. Each well was occupied with 100 µl of the crude extract (50, 100, 200 mg /ml), The dishes were leftward at room temperature for 30 min. to allow materials to diffuse into the media. The controls were prepared using the like solvent. incubated the plates at 37 °C for (18-24) hr. Inhibition zones in mm containing well diameter around well were measured. Antibacterial activity was expressed by way of the diameter of inhibition areas formed by the extracts against tested bacteria. Completely tests were completed in triplicate.

Results

Isolation of bacteria .

The results of current study showed that from 18 rabbits submitted to the study from which swabs were collected from skins, and external orifice of urinary tract of both male and females (Vagina in females and Prepuce in male). A total of *E. coli* 11, *Staphylococcus aureus* 9, Proteus 2, Bacillus 5, Pseudomonas 3, Streptococcus 2 and Klebsiella 1 were isolated either in pure or mixed forms (Table 1).

Table 1- Total number of isolates

Sp.	Pure	Mixed	Total
<i>E. coli</i>	5	6	11
<i>Staphylococcus</i>	6	3	9
<i>Proteus .</i>	1	1	2
<i>Bacillus .</i>	2	3	5
<i>Pseudomonas</i>	1	2	3
<i>Streptococcus</i>	0	2	2
<i>Klebsiella</i>	0	1	1

The results of current study showed the isolates from:

Skin:

Staphylococcus aureus . in pure colonies from 6 cases, and 2 cases mixed with either (*E. coli* and *Pseudomonas* .) *E. coli* 5 cases mixed with (*Proteus* ., *Pseudomonas* ., *Bacillus* and *Streptococcus* . *E. coli* mixed with *Staphylococcus aureus* and *Pseudomonas* .). *Pseudomonas* 2 cases mixed with either (*E. coli* and *Staphylococcus aureus* .). *Bacillus* pure in 2 cases and 2 cases mixed (*E. coli* and *Streptococcus* .). While *Streptococcus* . Isolated 2 cases mixed 1 case with (*E. coli* and *bacillus*). *Proteus* were isolated from 2 case mixed with *Staphylococcus aureus*. table(2).

Urinary:

Bacillus. mixed with *Klebsiella* 1. *E. coli* in pure form from 5 cases, or mixed with *Staphylococcus aureus* 1. *Pseudomonas* . in pure colonies 1, and *Proteus* in pure colonies from one case .table (2).

Table -2- Number of isolates (pure and mixed)

Sp.	Skin		Urinary	
	Pure	Mix	Pure	Mix
<i>Staphylococcus sp.</i>	6	2	-	1
<i>E. coli</i>	-	5	5	1
<i>Proteus sp.</i>	-	1	1	-
<i>Bacillus sp.</i>	2	2	-	1
<i>Streptococcus sp.</i>	-	2	-	-
<i>Pseudomonas sp.</i>	-	2	1	-
<i>Klebsiella sp.</i>	-	-	-	1

Sensitivity to ethanol Extracts: Sensitivity of isolates to ethanol extract of medicinal plants showed in table (3)

Staphylococcus aureus .

The results revealed that *Staphylococcus aureus* isolates were sensitive to ethanol: aqueous extract of Pomegranate peel and *Lantana cammara* leaves, with higher inhibitory zones in case of Pomegranate peel extract and lowest in *Melia azedarach* stem and *L. cammara* fruits and flowers extracts .

E. coli

E. coli were sensitivity to ethanol: aqueous extract of Pomegranate then to *L. cammra* leaves extract., with lowest sensitivity to *Melia azedarach* stem, *L. cammara* fruits and *L. cammara* flowers extract .

***Klebsiella* sp.:**

Klebsiella only mild inhibitory zones exhibited to Pomegranate and Melia with no effect of *L. cammara* extracts .

***Streptococcus* sp.**

The sensitivity of Strep. to: best was in cases of *L. cammara* leaves extract then Pomegranate peel extract.

***Pseudomonas* sp. :**

Pseudomonas best to Pomegranate peel extract .

***Bacillus* sp.:**

Bacillus showed sensitivity to *L. cammara* extract only .

Table -3- Sensitivity of isolates to ethanol extract of medicinal plants

Extract	Concentration mg/ml	<i>Staphylococcus</i>	<i>E.coli</i>	<i>Klebsiella</i>	<i>Streptococcus</i>	<i>Pseudomonas</i>	<i>Bacillus</i>
		Mean of inhibition zone(mm)					
<i>Pomegranate peel</i>	50	32.5	30	8.5	13.5	11.5	25
	100	37	29.5	11.5	19.5	28.5	-
	200	24	27	11.5	19	24.6	-
<i>Melia azedarach</i>	50	11	5	10	13.5	20.5	-
	100	13.5	9	10.5	11	-	-
	200	-	12	-	10	10	7
<i>Lantana camara fruit</i>	50	10.5	6.5	-	4	10	6
	100	-	10.5	-	4	5.5	8.5
	200	10	16.5	-	8	5.0	13
<i>Lantana camara Flower.</i>	50	-	12	-	13	5	22.5
	100	23	10	-	10	5	-
	200	10	8	-	11	5	-
Lantana camara leaves	50	17	15.5	-	17.5	7	13
	100	19.5	17.5	-	24.5	5	20.5
	200	36	16.5	-	36.5	5	22.5

Discussion

Isolated bacteria:

The results of current study showed a total of *E. coli* 11, *Staphylococcus aureus* 9, *Proteus* 2, *Bacillus* 5, *Pseudomonas* 3, *Streptococcus* 2 and *Klebsiella* 1 were isolated either in pure or mixed isolates.

The skin micro biome is much further different than was previously described established on culture based approaches. Serial documents reveal major individual changeability amongst samples cool from dissimilar patients. Significant contrast was also observed amongst the several skin regions within the animals, through a greater total of bacterial types saw on the hair skin (i.e. groin, pinna, periocular, dorsal nose, axilla, and interdigital lumbar) Whereas compared to skin with weak hair, mucosal surfaces or mucocutaneous junctions (such as lips ,conjunctiva and nose).

In studies using culture based approaches certain of the utmost commensal bacteria in the skin of healthy animals were displayed to include *Micrococcus* spp., Coagulase – negative *Staphylococci*- mainly *Staph. epidermidis* and *S. xylosus* – alpha – hemolytic *Streptococci* , *Clostridium* spp., *Acinetobacter* spp ,*Propionbacterium acnes* , besides several G- negative aerobs , *Proteus mirabilis*, *E. coli* ,*Bacillus* spp, *Corynebacterium* spp., and *Pseudomonas* spp. were considered to be transient microbes in the skin of animals (Milier, 2012).

Generally culture based approaches It is the standard for describing microbial diversity. However, particular bacteria need particular growth surroundings and are extremely hard to isolate. Additional bacterial types, e.g., *Staphylococcus aureus*, develop readily below normal culture

surroundings and then over crowded additional fastidious bacteria. The comprehensive cutaneous microbial surveys conducted decades ago were limited due to the ability to provide the appropriate growth conditions required for culturing and isolating highly sensitive microbes. Even with these limits, original studies used cultivation procedures to detect memberships of skin microbial communities, including *Staphylococcus. epidermidis* and other coagulase negative staphylococci. Additional microorganisms that are typically considered as skin colonizers contain those of the phylum *Actinobacteria* (the genera *Corynebacterium*, *Brevibacterium* and *Propionibacterium*) and the genus *Micrococcus*. The utmost fungal species are usually isolated are *Maslassezia spp.*, which are specially predominant in sebaceous regions .

Sensitivity to aqueous: ethanol (30:70) Extracts:

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism, these compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds(Zakaria *et al.*, 2007).

The organic extracts provided more powerful antimicrobial activity as compared to aqueous extracts. This observation clearly indicates that the existence of non-polar residues in the extracts which have higher both bactericidal and bacteriostatic abilities (**antara and** amla, 2012).

The well diffusion technique was further essential to determine the antibacterial properties of together alcoholic and aqueous extracts of cotoneaster sp., in contrast through agar disc technique, the alcoholic extract was further active and exhibited greater antibacterial influence alongside all bacteria sp.in comparison by way of aqueous extract as well both aqueous and alcoholic extract were concentration independent (Al-Khafaji *et al.*, 2014).

Staphylococcus . The results revealed that *Staphylococcus* isolates were sensitive to ethanol: aqueous extract of Pomegranate peel and *Lantana cammara* leaves, with higher inhibitory zones in case of Pomegranate peel extract and lowest in *Melia azedarach* stem and *L. cammara* fruits and flowers extracts.

E. coli.: *E. coli* were sensitivity to ethanol: aqueous extract of Pomegranate then to *L. cammara* leaves extract., with lowest sensitivity to *Melia azedarach* stem, *L. cammara* fruits and *L. cammara* flowers extract

Klebsiella sp.: *Klebsiella* only mild inhibitory zones exhibited to Pomegranate and *Melia* with no effect of *L. cammara* extracts.

Streptococcus sp. : The sensitivity of *Streptococcus* . best was in cases of *L. cammara* leaves extract then Pomegranate peel extract.

Pseudomonas sp.: *Pseudomonas* best to Pomegranate peel extract .

Bacillus sp.: *Bacillus* showed sensitivity to *L. cammara* extract only.

The patterns of antimicrobial activity diverged according to the solvent used for the extraction and plant extract. The organic raw extracts presented further inhibition than the aqueous extracts on the average for entirely plants extracts tested (Ashish *et al.*, 2011). It is probable that the aqueous crude extracts may have antimicrobial constituents insufficient before efficacy and which may explain why large amounts of the decoctions must be drunk by the patients (Holetz *et al.*, 2001).

commonly antimicrobial special effects can be attributed to the plant's phytochemicals used in our study In polyphenols and phenols have phenolic poisonousness to microorganisms which comprise Enzyme inhibition by oxidizing complexes, may be through response with sulfhydryl groups or through more nonspecific communications with the proteins (Mathabe *et al.*, 2006) .

Quinones, they afford a basis of a constant free radicals and are besides known to complex irreversibly with nucleophilic amino acids in protein (McCarrell *et al.*, 2008), frequently leading to inactivation of the protein and hurt of role, It may also make substrates unapproachable to microbes; Flavones, flavonoids and flavonols, Plants have been identified to create them in response to microbial infections (Al-Zoreky and Nakahara, 2003). And found in the laboratory they are

effective anti-microbial substances alongside a extensive range of microbes. Possibly due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, more lipophilic flavonoids may too disturb microbial membranes (Gould *et al.*, 2009).

Tannins one of their molecular activities is to combined proteins through so – named nonspecific forces such as hydrogen bonding and hydrophobic properties, as well as by covalent bond development (McCarrell *et al.*, 2008). Tannins own inhibitory actions and described to be toxic to filamentous fungi, yeast and bacteria (Orak *et al.*, 2011).

Terpenoids and necessary oils activities of terpenoids or terpenene have been recognized in contradiction of bacteria (Ullah *et al.*, 2012). polypeptides and Lectins, Peptides are inhibitory to microbes are commonly positively charged and contain disulfide bonds. The development of ion channels in the microbial membrane (Deepak *et al.*,2009) or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Ashish *et al.*, 2011) could be their mechanism of action.

The inhibitory result of pomegranate was permanently recognized to the antioxidant activity that depends mainly on the anthocyanin and phenolic contented of the fruit . Abdel Moneim (2012) pronounced the concentrated antibacterial activity was documented against *Klebsiella pneumoniae* and among fungi high activity against *Aspergillus* was documented.

The common of the *Lantana camara* action is due to bioactive complexes by, tannin, alkaloids, flavones, is flavones, flavonoids, triterpenoids ,isocatechins and saponins. Akiyama *et al.*, (2001) have described that the leaves extract of *Lantana camara* be active alongside numerous G+ and G- bacteria. The vital oil of *Lantana camara* showed prominent antibacterial activity alongside wholly the bacterial strains tested. G- *Kl. pneumoniae* and *Ps. aeruginosa* were not vulnerable to the essential oil at poorer concentration (Prashanth *et al.*, 2001).

In conclusions:

From results we can concluded that the *E. coli* and Staphylococcus are the most common bacteria among aerobic bacteria that present on the skin and natural orifices of the body of rabbits as saprophytic and pathogenic. And the ethanol: aqueous extracts of Pomegranate peel and *Lantana cammara* leaves are the most effective in sensitivity point of views. Further study of the all aerobic and anaerobic bacteria in rabbits as such workers are available rarely .

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عزل وتمييز البكتيريا الشائعة من المسالك البولية وجلد الأرناب ، مع حساسيتها لمستخلصات الإيثانول لبعض النباتات الطبية

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الخلاصة

لعزل البكتيريا الشائعة الموجودة في المسالك البولية ، وجلد الأرناب ، تم الاعتماد على 18 بالغاً من كلا الجنسين ، بوزن 1-2 كجم وزن محلي وسلالة ألبينو ، في الدراسة الحالية ، تم التعرف على البكتيريا التكافلية المعزولة من الجلد وخروج المسالك البولية للأرناب بناءً على خصائصها الشكلية والزرعية والفسولوجية البايوكيميائية. فضلاً عن إجراء اختبار الحساسية لمستخلصات الماء – الإيثانول للرب الرمان ، سيقان السبج ، ثمار المينا الشجرية ، وازهارها واوراقها بطريقة الانتشار في الحفر. بينت النتائج ان اغلب العزلات من المكورات العنقودية ، المكورات السبجية ، الاشريشيا القولونية، الكلبسيلا ، العصويات ، والزوائف . وان مستخلصي لب الرمان واوراق المينا كان لها تأثيرات فعالة ضد المكورات العنقودية , الاشريشيا القولونية ثم المكورات المسبجية.

كلمات المفتاح: الارانب، مستخلص الإيثانول ، النباتات الطبية