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Isolation Of Bacterial Pathogens From Ear, Nose And Mouth Of Domestic Rabbits With Antimicrobial Activity Of Pomegranate Peel, *Melia Azedarach* And, *Lantana Cammra*

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Abstract

Aims: isolation of bacterial pathogens from ear, nose and mouth of domestic rabbits with antimicrobial activity of pomegranate peel, Melia azedarach and, lantana cammra.

Methods: bacterial pathogens were isolated from ear, nose and mouth of (18)domestic rabbits by standard microbiological procedures .Antimicrobial activity of ethanolic extract for Pomegranate Peel, Melia Azedarach stem, Lantana Cammra flower and fruits were tested by agar diffusion method

Results:

Staphylococcus, Streptococcus, Pseudomonas, Klebsiella, Bacillus, and E. coli were the most prevalent isolates. The strongest inhibitory effects were the ethanol extracts of pomegranate peel and L. cammara leaves.

Conclusion: from ear, nose and mouth of domestic rabbits represent a major source for pathogenic bacteria of zoonotic importance. Pomegranate peel and L. cammara leaves can be used as alternative antimicrobial agents for eradication of the Staphylococcus, Streptococcus, Pseudomonas, Klebsiella, Bacillus, and E. coli

Key words: Rabbits , ethanol extracts, pomegranate peel ,L. cammara

Introduction

In many nations, plants constitute a potential source of antibacterial mediators.Between 60 and 90 percent of people in poor nations use medicines made from plants. Traditionally, infectious infections are treated with herbal medication made from unprocessed plant extracts (1).A variety of phytochemicals, such as tannins, terpenoids, alkaloids, and flavonoids, are abundant in plants and

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have been shown to exhibit antimicrobial effects in vitro (2). Although the majority of the time, scientific validation of the mechanism of action and efficacy of these herbal extracts is still needed, these preparations facilitate considerable host responses (3).

There are typically two groups of the microbiota. The microorganisms that are frequently established in the skin and that reestablish themselves after disturbance are known as "resident microbes," and they are a relatively stable group of microbes. According to (4), resident microorganisms are frequently regarded as commensal, which means they benefit the hosts and are not harmful.

The human microbiota, a complex community of infectious bacteria found in and on the human body at locations like the stomach, skin, and oral cavity, may benefit from the usage of microorganisms. Individual species that are pathogens in the traditional sense, such as Enterobacter sp., Escherichia coli, and Pseudomonas aeruginosa, among others, can become out of balance under certain conditions, such as in patients whose typical innate defenses are compromised (5).

Numerous environmental and physiological factors, including anatomic position, local humidity, the production of sweat and sebum, the host's hormonal state, and age, affect which microorganisms are present in this community. A careful balance between the characteristics of the microbial populations and the human host's defense mechanisms underlies the stability of this microbial community, which is made up of bacteria, viruses, fungi, and protozoa (6)

The purpose of the study was to isolate a common bacteria from rabbits' noses, mouths, and ears in order to test how sensitive it was to ethanol extracts of various medicinal herbs..

Materials and Methods

From September 2018 to April 2019, the research was carried out at the Department of Microbiology, College of Veterinary Medicine, University of Diyala, Iraq .

Collection of samples

Swabs were taken from local and albino rabbits' nose, mouth, and ears

Methods

Isolation and diagnosis of bacteria

Identification of bacterial isolates: -

Swabs were cultivated for 24 hours in nutrient broth and then transferred to nutrient agar. Colonies that are isolated and categorized based on their physical characteristics, including color, shape, and size. If a hemolytic zone exists, it is stained and studied under a microscope before being cultured on blood agar (reading according to hemolysis) or MacConkey agar (Lactose fermenting and Lactose none fermenting). Oxidase tests were used to check the lactose-nonfermenting bacteria for pseudomonas and Proteus. The Indol

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test was used to check the lactose-fermenting bacteria for red ring (E. coli), no red ring, and Klebsiella mucus strip**.**

Based on visual characteristics, microscopic analysis, and biochemical tests, the isolates were identified.

Microscopic examination: -

According to (7), single colonies were selected following the isolation of bacteria on MacConkey agar and blood agar, stained with Gram stain, and then studied under a microscope to identify their form and length

Biochemical tests:-

Numerous biochemical tests had been carried out in order to confirm any suspected isolate, according to (8)

plants extract preparation

The fruits, flowers, and leaves of the plants were obtained from Baqubah City's native markets or were kept cold by trees in the city's gardens. They are cleaned using distilled water and dirty tap water, then dried indoors in the dark for three weeks at 22°C before being ground into a fine powder and extracted with ethanol using conventional extraction techniques (9). They are then stored until needed

Prepration of Ethanol extract

Fifty grams of powder that has been shade-dried and is composed equally of L. cammara fruits, leaves, and flowers, Melia azedarach stem, and Pomegarante peel. It is blended for six hours while being sonicated with 250 cc of 70% ethanol. When the temperature reached 60–70°C, the solvent was earlier removed under reduced pressure using a rotary evaporator at 40°C. The stock solution was then formed into different concentrations (50, 100, and 200 mg/ml) by dilution with a dimethyl sulfoxide (DMSO) solution, and these concentrations were then used as the test extracts for an antibacterial activity experiment (10). According to (7), the McFarland solution was created.The mixture is well shaken, transferred to a test tube with a screw top, and stored at 4 degrees Celsius.

Antimicrobial activity

Agar Well Diffusion Method

(11) modified agar well diffusion method was used to assess the extracts' antibacterial efficacies. On the surface of Muller Hinton agar, a fresh colony forming unit (105 CFU/ ml) was aseptically disseminated before being allowed to dry for 30 minutes. Using pasteurized pipette ends, wells 5 mm in diameter were created in the media. 100 l of the crude extract (50, 100, or 200 mg/ml) were poured into each well. For 30 minutes, the plates were kept at room temperature to allow medial material diffusion. The same solvent was used to prepare the controls. For 18 to 24 hours, plates were incubated at 37 oC. The diameter of the well and the surrounding inhibition zones were measured in mm. The ability to combat bacteria

Results

Isolates spp.

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The most common bacterial isolates were illustrated in Table 1

Table 1- Bacterial pathogens isolated from nose, ear and mouth of domestic rabbits

Bacterial spp.	Pure	Mixed	Total	Percentage %
E. coli	13	8	21	61.9
S.aureus	6	10	16	37.5
Proteus sp.	3	3	6	50
Pseudomonas	3	2	5	60
Bacillus sp.	0	4	4	0
Klebsiella	3	1	4	75
Streptococcus	1	3	4	25

Table -2- Pure And Mixed Bacterial Pathogens Isolated From Nose, Ear And Mouth Of Domestic Rabbits

Bacterial spp.	Mouth		Nose		Ear		
	Pure	Mix	Pure	Mix	Pure	Mix	Total
E. coli	3	2	4	4	6	2	21
Proteus	1	1	-	1	2	1	6
Klebsiella	-	-	1	1	2	-	4
Streptococcus	1	2	-	1	-	-	4
Pseudomonas	-	-	3	1	-	1	5
Bacilli	-	1	-	2	-	1	4
Staphylococcus	3	2	3	4	-	4	16

Sensitivity To Ethanolic Extracts:

As sown in table (3) Staphylococcus isolates were susceptible to ethanol. Pomegranate peel extract had higher inhibitory zones than Lantana cammara leaf extract, and Melia azedarach stem and L. cammara fruits and flower extracts had the lowest inhibitory zones .On the other hand E. coli were sensitive to ethanol, followed by pomegranate aqueous extract and lilac cammara leaves extract, with Melia azedarach stem, lilac cammara fruits, and lilac cammara flowers extract having the lowest sensitivity .Pomegranate and Melia only caused weak Klebsiella inhibition, while L. cammara extracts had no effect . The best examples of Streptococcus . sensitivity were with L. cammara leaves extract, followed by Pomegranate peel extract. The finest pomegranate peel extract for Pseudomonas .Only L. cammara extract demonstrated sensitivity in bacilli

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Table -3- Sensitivity of Bacterial pathogens isolated from nose, ear and mouth of domestic rabbits to ethanol extract of medicinal plants

Extract	Concentrationmg/ml	***Staphylococcus***	*E. coli*	***Klebsiella***	***Streptococcus***	***Pseudomonas***
	Mean of inhibition zone(mm)					
Pomegranate	50	32.5	30	8.5	13.5	11.5
	100	37	29.5	11.5	19.5	28.5
	200	24	27	11.5	19	24.6
Melia	50	11	5	10	13.5	20.5
	100	13.5	9	10.5	11	-
	200	-	12	-	10	10
L. cammara fruits.	50	10.5	6.5	-	4	10
	100	-	10.5	-	4	5.5
	200	10	16.5	-	8	5.0
L. cammara Flow.	50	-	12	-	13	5
	100	23	10	-	10	5
	200	10	8	-	11	5
L. cammara leaves	50	17	15.5		17.5	7
	100	19.5	17.5		24.5	5
	200	36	16.5		36.5	5

Discussion

Much effort has been placed into finding compounds that can function as suitable antimicrobials agents to replace synthetic ones in the search for antimicrobials derived from natural sources. The development of less harmful and more potent medications to control the growth of microorganisms is facilitated by phytochemicals derived from plant products. These substances also have important therapeutic uses against bacteria, fungi, and viruses that are human pathogens. There have been numerous research done using plant extracts to screen antimicrobial activity as well as find new antimicrobial chemicals (12). In comparison to aqueous extracts, organic extracts showed stronger antibacterial activity. This finding unmistakably demonstrates the presence of non-polar residues in extracts with greater levels of bactericidal and bacteriostatic activity (13). The plant's phytochemicals used in our study are known to have antibacterial special effects. phenolic toxicity to microorganisms is a property of

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phenols and polyphenols, and this property includes enzyme inhibition by the oxidized compounds, perhaps by reactivity with sulfhydryl groups or through less specific interactions with the proteins (14); Quinones are known to irreversibly complex with nucleophilic amino acids in proteins (15), which frequently results in the protein becoming inactive and losing its function. They may also prevent the microorganism from accessing substrates; Plants are known to generate flavones, flavonoids, and flavonols in response to microbial infection (16). were discovered to be potent antibacterial agents in vitro against a variety of pathogens. More lipophilic flavonoids may also disrupt microbial membranes. Their activity is likely a result of their capacity to combine with extracellular and soluble proteins as well as bacterial cell walls (17).

Tannins connect with proteins by so-called nonspecific forces such hydrogen bonding and hydrophobic effects, as well as through the creation of covalent bonds (15). According to research by (18), tannins have Terpenoids and aromatic compounds Terpenene or terpenoids have been shown to have antibacterial effects (19). Microorganisms are inhibited by lectins and polypeptides, which are frequently positively charged and contain disulfide bonds. Their mode of action may involve the development of ion channels in the microbial membrane (20) or competitive suppression of microbial protein adhesion to host polysaccharide receptors (21).

Inhibitory properties and are poisonous to bacteria, yeast, and filamentous fungi. Pomegranate's inhibitory impact is always linked to its antioxidant activity, which mostly depends on the fruit's phenolic and anthocyanin content.According to Abdel Moneim (22).

Klebsiella pneumoniae had the highest antibacterial activity. The bioactive substances flavones, isoflavones, flavonoids, isocatechins, alkaloids, tannin, saponins, and triterpenoids account for the majority of lantana camara's action. According to Akiyama et al., Lantana camara leaf extract is effective against a variety of gram positive and gram-negative microorganisms. Against every studied bacterial strain, the Lantana camara essential oil shown strong antibacterial action. At lesser concentrations, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, both Gram negative bacteria, were resistant to the essential oil (23).

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