RESEARCH ARTICLE

Comparative Study Antibacterial Activity of some Medicinal Plants Extracts (Leaves and Peel) against some Multi-drug Resistant Bacteria from Clinical Isolates

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ABSTRACT

The study was conducted of tested the effect of three aqueous and alcoholic extract of medicinal plants from (*Ziziphus spina-christi*, *Punica granatum* L. and *Eucalyptus camaldulensis*) 100 mg/mL concentration and the sensitivity of to nine antibiotics (ciprofloxacin, azithromycin, augmentin, amoxicillin + clavulanic acid, ceftazidime, vancomycin, tobramycin (10 µg), tobramycin(5µg), ampicillin) against 20 antibiotic-resistant pathogenic bacteria isolate by using Mueller-Hinton agar (MHA) well diffusion method. Antibacterial activity of alcoholic (ethanol) extraction represented by inhibition zones diameters of three medicinal plants showed a strong activity comparison of aqueous extracts. Especially in the pomegranate extract as the proportion of sensitive bacteria 40 and 25% in *Eucalyptus camaldolehsis* leaves And less inhibitory in the alcoholic extract of *Ziziphus spina-christi* as estimated (5%). The ethanolic extracts of *Pomegranate* Peel have shown an interesting activity against *Yersinia enterocolitica***,** *Salmonella spp***,** and *Brucella abortus* with inhibition zones diameters of 30.0, 27.0 and 25.0 mm, respectively. Ciprofloxacin was the most effective antibiotic against almost all the studied pathogenic bacteria and less resistance to other types. It is worth mentioning the existence of results showing the efficacy of plant extracts in inhibiting bacteria resistant to all and many types of antibiotics used in this study, such *as Staphylococcus aureus HM*, *Shigella spp, Enterobacter spp, Salmonella spp T, Salmonella spp, Yersinia enterocolitica, Listeria monocot genus.*

Keywords: Antibacterial activity, Disk diffusion, *Eucalyptus camaldulensis*, *Punica granatum L.*, *Ziziphus spina-christi*, Zone of inhibition,

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INTRODUCTION

Plants, herbs, vegetables, and spices used in indigenous and folk medicines have been generally accepted currently as one of the essential sources of chemo-preventive drug discovery and development.[1] It has been observed the use of medicinal plants and herbs in the field of health prevention does not cause side effects in the event of the patients compliance with the conditions of treatment.[2] Many plants found in the world have been submitted to pharmacological tests like sidr (Ziziphus spina-christi) pomegranate (Punica granatum L.) and Eucalyptus camaldulensis. Ziziphus spina-christi, it is one of the most popular plants in the field of folk medicine. it belongs to the Rhamnaceae family. it is spread in areas where the temperature is moderate, the land is somewhat dry and the hemispheres of the hemisphere, whose climate is as warm as Iraq[3] Eucalyptus camaldulensis, it is an important medicinal plant, which belongs to family Myrtaceae, and it contains many active compounds like α_pinenene, 1, 8_cineol Isoledene, α_pinocareol_trans terpineol, cymene, terpinenegamma, 2_pentanone, 4_hydroxy _4_methel, Eucalyptol, Globulol, Limonene, Spathulenol, Pinacarvon, Guaiene.[4] Several studies have been conducted to identify the nature of the chemical compounds contained effectiveness of antifungal, antimicrobial and its effect in the treatment of many of the intractable diseases such as cancer, Acquired immunodeficiency syndrome(AIDS) and psoriasis.[5] Pomegranate peel contains tannins by 22_28% authority galactose returns the therapeutic value for pomegranate an tioxidant materials such as phenolic compounds, [6, 7] and in a study by 2 to study the inhibitory effect showing that the aqueous extract was the least on the pumping diameter area towards the bacteria of diseases isolated from the

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intestines and stomach in humans while the alcoholic extract Gave a significant effect of all types of bacteria at concentration of 15%. In the present and future, the resistance of bacteria of many antibiotics is an insurmountable problem recognized as a big public health threat affecting humans worldwide. Multidrug-resistant organisms (MRO) have emerged not only in the hospital environment but are now often identified in community settings, suggesting that reservoirs of antibiotic-resistant bacteria (ARB) are present outside the hospital because of differences in their genes.[8] The number of people deaths due to this problem by the World Health Organization (WHO) estimated at 10 million in 2050.[8] The development of multi-drug resistance in pathogenic bacteria against commonly treatment; it has become necessary to use therapeutic alternatives to antibiotics a search for new therapeutic agents from other sources.[9] Despite found numerous experiments in the use of medicinal plants and herbs in traditional medicine. The scientific studies and research suggest the possibility of discovery, diagnosis and identification of antibacterial active plant compounds, they can lead to the discovery of new therapeutics (nature-based products) in the future.[10] The study aimed to investigate comparative of the inhibitory effect of Aqueous and alcoholic extract is existing chemical compounds of *Ziziphus spina-christi*, *Punica granatum* L. and *Eucalyptus camaldulensis* against Multidrug-resistant pathogenic bacteria.

MATERIALS AND METHODS

Collection of the plant material

Fresh free an abnormal state and disease found in *Eucalyptus camaldulensis* leaves, *Ziziphus spina-christi* leaves, and *Pomegranate* Peel that collected to study from local farms in holy Karbala, Iraq. Medicinal plant parts collected were labeled and stored at 5°C in clean and sterile plastic bags for two days. Collected leaves and peel were separated and washed thoroughly in tap water, rinsed in distilled water and shade dried for eight days in the open air and then grinding using pestle and mortar, reduced to powder using Laboratory grinder for 6 minutes at high speed and then stored in airtight closed bottles for two days. sixty grams of all the dried and powdered samples were stored to prepare water and alcohol extracts.[11]

Preparation of herbal medicine extraction

Aqueous extracts

Method of[12] is used in the preparation with some changes: using 15 grams of powder plant and mixed with 300 mL of distilled water by electric mixer for 5 minutes, then the extract was mixed by device magnetic stirrer for 1 hour per plant sample, after that the samples for maceration for 24 hours and filtered by using layers of the gauze after all this, centrifugation was carried out with centrifuge of 3000 circles per minute for 10 minutes.

Alcoholic extracts

- *Eucalyptus camaldulensis* leaves and *Ziziphus spina-christi* leaves: 15 grams of the sample were weighed and placed in soxhlet with ethanol (95% for sider concentration and 70% for *Eucalyptus*) for 24 hours.[13]
- *Pomegranate* Peel: 15 grams of sample was weighted with 300 ml of ethanol (70%) and then mixed by a magnetic stirrer for two hours, the sample was soaked for 24 hours and filtered and centrifuged at 3000 C/min for 5 minutes.[14]

Preparation 100 mg/ml concentrations Medical plant extracts

For the preparation 100 mg /mL concentrations of Aqueous and Alcoholic plant extracts were taken 1 gram of the powder extract and dissolved in 10 mL distilled water. The solution sterility by Microfiltration using membrane filters especially with a diameter of 0.22 µm.[15]

Bacterial strains and culture conditions

Twenty clinical isolates of pathogenic bacteria isolated and identified in Karbala Public Health Laboratory of the Ministry of Health Used in this study, which includes (*Staphylococcus aureus HL*, *Staphylococcus aureus HM, streptococcus pyogenes, Pseudomonas aeruginosaHL*, *Pseudomonas aeruginosaHM, Pseudomonas aeruginosa ZZ, Pseudomonas spp, Klebsiella spp, Shigella spp, Enterobacter spp, Salmonella spp, Salmonella spp, Yersinia enterocolitica*, *Listeria monocytogenus, Listeria spp, Brucella meletensis, Brucella abortus, Escherichia coli, Escherichia coli, serrratia mercescens*. The bacterial strains were cultured aerobically on nutrient agar (NA) plates (*HiMedia*) at 37 Celsius for 24 hours. For the antibacterial activity test, the bacteria were aerobically cultured in nutrient broth (NB) (Sigma-Aldrich, Germany) at 37 Celsius and 200 rpm in Shaker Incubator (Lab tech) for 24 hours, and then suspended in sterile saline at a density equivalent to that of the 0.5 McFarland standard. Bacterial suspensions with a concentration of 105 colony-forming units per milliliter (CFU/mL) were used in antibacterial activity test of each plant extracted.[16,17]

Antibacterial medical plant extracts activity test

Antibacterial activity of the different Aqueous and Alcoholic medical plant extracts was assayed by agar well diffusion method. Mueller-Hinton agar (*HiMedia)* were prepared and sterility by autoclave, and after leave to cool down to 45 degrees Celsius were poured into sterile Petri dishes. Inoculum was spread on Mueller-Hinton Agar plates by a sterile cotton swab moistened with the pathogenic bacterial suspension and leave to dry; 4 wells (5 mm diameter) were bored on the surface of the Mueller-Hinton agar on each plate.; 4 wells (5 mm diameter) were bored on the surface of the agar media on each plate. Two plate agar were applied for each bacterial isolate, the first plate agar for the alcohol extracts, and the second for the water extracts.The first three wells were filled with 50 µL of *Eucalyptus camaldolehsis* leaves, *Ziziphus spina-christi* leaves, and *Pomegranate* Peel alcohol extract at concentrations of 100 mg/mL and the second dish of water extracts with the same concentration. The fourth well was filled for each plate agar by the solvent used for extract preparation as a negative

control. The inoculated plates were left for an hour at room temperature to allow the diffusion of the Medicinal Plants extracts into the plate agar before the growth of the bacteria commenced. The ready plates were incubated at 37°C for 24 hours, The antibacterial activity of medicinal plants extracts were determined by measuring the diameter of the growth inhibition zone for pathogenic bacterial species.[18,19]

Standard antibiotics sensitivity test

Disk diffusion susceptibility tests (DDST) were performed against clinical bacterial isolates, according to The National Committee for Clinical Laboratory Standards (NCCLS) guidelines. The commercially Antimicrobial types used in tested for clinical isolates included ciprofloxacin (CIP 10 µg/disk), Azithromycin (AZM15 µg), Augmentin (amoxicillin/clavulanate potassium) (AUG 20 + 10 µg), Amoxicillin + Clavulanic Acid (AMC 20 + 10 µg), Tobramycin (TOB 10 µg), Ceftazidime (CAZ 30 µg), Vancomycin (VA 30 µg), Tobramycin (TM 5 µg), Ampicillin (AM 25 µg). The disks were prepared from bioanalyse, Turkey.[20,21]

RESULTS

Medicinal plants Used and their extracts

Figure (1) shows the types of plant extracts used in the study, which represent the water and alcohol extract of *Eucalyptus camaldolehsis* leaves, *Ziziphus spina-christi* leaves and *Pomegranate* Peel after extraction and before the drying process.

Effects of alcoholic and aqueous extract of medicinal plants against bacterial

The results of the observation of antibacterial activity alcoholic and aqueous extracts of the three plant extract at concentrations of (100 mg/mL) on pathogenic bacteria using Mueller-Hinton Agar well diffusion method showed that the Pomegranate Peel alcohol extract showed maximum zone of inhibition against *Yersinia enterocolitica* (30 mm) Table 1. The Aqueous extract of Ziziphus leaves showed the minimum rate of antibacterial activity on all the the twenty of pathogenic bacteria when compared to alcoholic and aqueous of *Eucalyptus*

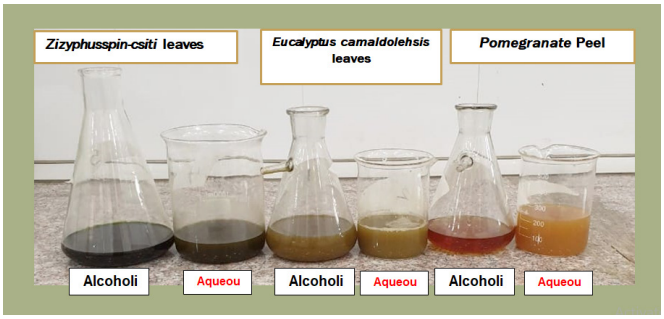


Figure 1: The aqueous and alcohol extract of *Eucalyptus camaldolehsis* leaves, *Ziziphus spina-christi* leaves and *Pomegranate* Peel.

Table 1: The diameters of inhibition zone obtained of well diffusion method from some Plants Extracts used in this study against pathogenic bacteria. (diameter inhibition mm).

		Mean of Inhibition zone (mm)					
		Eucalyptus camaldolehsis leaves		*Ziziphus spina-christi leaves*		*Pomegranate peel*	
No	*Pathogenic bacteria*	*Aqueous*	*Alcoholic*	*Aqueous*	*Alcoholic*	*Aqueous*	*Alcoholic*
1	*Staphylococcus aureus HL*	-	-	-	-	13	18
2	*Staphylococcus aureus HM*	-	23	12	20	21	22
3	*streptococcus pyogenes*	-	-	-	-	15	16
4	*Pseudomonas aeruginosaHL*	12	17	15	19	15	22
5	*Pseudomonas aeruginosaHM*	-	-	-	-	13	14
6	*Pseudomonas aeruginosa ZZ*	-	14	-	13	-	15
7	*Pseudomonas spp*	-	12	-	8	-	18
8	*Klebsiella spp*	-	-	15	17	22	17
9	*Shigella spp*	16	-	-	-	16	17
10	*Enterobacter spp*	14	22	-	15	17	23
11	*Salmonella sppT*	13	19	15	17	-	24
12	*Salmonella spp*	21	25	-	-	22	27
13	*Yersinia enterocolitica*	18	21	15	25	23	30
14	*Listeria monocytogenus*	16	18	-	-	18	23
15	*Listeria spp*	-	12	-	-	12	20
16	*Brucella meletensis*	-	15	-	-	-	15
17	*Brucella abortus*	-	13	-	-	22	25
18	*Escherichia coli H*	21	18	14	13	15	20
19	*Escherichia coli L*	18	24	12	16	12	15
20	*serrratia mercescens*	-	-	-	-	-	14

— : No inhibition zone observed

camaldolehsis leaves, *Pomegranate* Peel and alcoholic extract of Ziziphus leaves. Percentages effect of types alcoholic and aqueous extract of medicinal plants on antimicrobial activity showed A variation of the sensitivity of the studied bacteria to the types of plant that used and the water and alcohol extracts of the same extracts Table 2.

Antibiotic activity

Table 3 shows the rates of inhibition diameters of nine antibiotics discs for the growth of the 20 species of bacteria. The **a**ntibiotic (CIP) was more effective for the bacteria used in the study.

DISCUSSION

The observation in this study is of antibacterial activity alcoholic and aqueous extracts of the three medicinal plant extract against twenty various pathogenic bacteria by using MHA well diffusion method. Antimicrobial properties the valorization of different between three plant extracts against various bacterial isolate. This may be attributed medical plants contain multi-chemical component mixtures and different bioactive compounds contained and concentration according to type.[24,25] Antibacterial activity of alcoholic (ethanoll) extraction represented by inhibition zones diameters of *Pomegranate* Peel, *Eucalyptus camaldolehsis* leaves and Ziziphus leaves respectively showed strong activity comparison of aqueous extracts This may be due to the difference in active compounds in both extraction methods. The biochemical analysis of some studies have shown that the water extracts of medicinal plants contain compounds such as glycosides, alkaloids, saponines, tannins, and volatile oils., And alcohol extracts contain compounds (in addition to the mentioned compounds) to what contains plant extracts on Phenols, Resins, Flavonids, and Coumarins, because these compounds dissolve only with organic solvents.[26,27] The ethanolic extracts of *Pomegranate* Peel have shown an interesting activity against *Yersinia enterocolitica***,** *Salmonella spp*, and *Brucella abortus* with inhibition zones diameters of 30.0, 27.0 and 25.0 mm, respectively Similarly[28] Also, The highest value reached in diameters of inhibition zone by used water extracts 23mm against *Yersinia enterocolitica*, This was in agreement with[29] of determining their sensitivity towards water extracts. The results of the bacterial sensitivity test of antimicrobial discs are shown CIP, one of the *Fluoroquinolones* group were the most effective antibiotics against almost all the studied pathogenic bacteria and less resistance of (TOB) (AZM) (CAZ) (AMC) and full resistance of (TM) (AM) (AUG) respectively (30). Moreover. When comparing the results of the sensitivity of bacteria against plant extracts and antimicrobial discs shown excellence of *Ciprofloxacin* In inhibiting the growth of bacteria on *Pomegranate* Peel, *Eucalyptus*

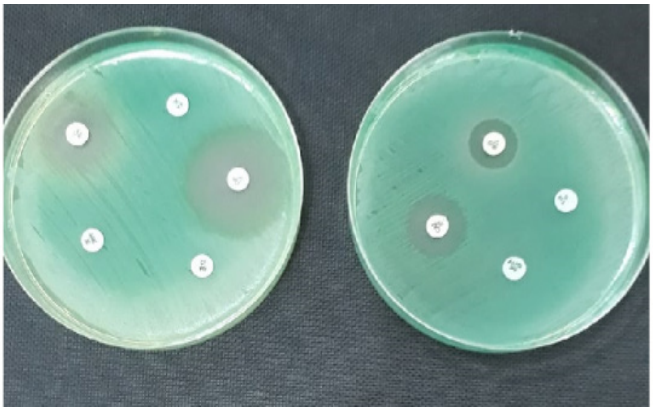
Table 2: Percentages of sensitivity and resistance to alcoholic and aqueous of Susceptibility percentage of three medicinal plants against pathogenic bacteria isolates according to (NCCLS) guidelines[22,23]

Type of Susceptibility	*Pomegranate Peel*		*Ziziphus spina-christi leaves*		*Eucalyptus camaldolehsis leaves*	
	Alcoholic	*Aqueous*	*Alcoholic*	*Aqueous*	*Alcoholic*	*Aqueous*
Suseptible	40%	25%	5%	0%	25%	10%
Intermediate	30%	10%	20%	0%	20%	10%
Resistance	30%	65%	75%	100%	55%	80%

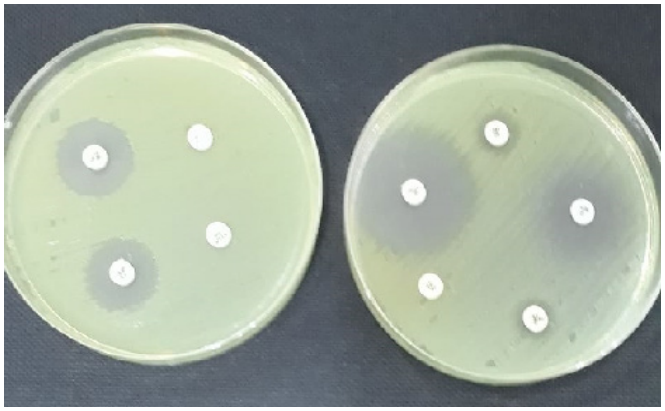
Table 3: Diameters of antibiotic discs inhibition zones (mm) against pathogenic bacteria.

Antibiotic / *No*	*Pathogenic bacteria*	*Mean of Inhibition zone (ml)*								
		CIP	*AZM*	*AUG*	*TOB*	*CAZ*	*VA*	*TM*	*AM*	*AMC*
1	*Staphylococcus aureus HL*	29	10	-	10	8	-	-	-	6
2	*Staphylococcus aureus HM*	8	-	-	-		4	-	8	-
3	*streptococcus pyogenes*	17	-	8	-		-	-	12	-
4	*Pseudomonas aeruginosaHL*	32	10	-	15	14	-	-	7	6
5	*Pseudomonas aeruginosaHM*	25	-	-	-		-	-	-	-
6	*Pseudomonas aeruginosa ZZ*	30	17	-	10	12	-	-	-	-
7	*Pseudomonas spp*	23	-	-	-		-	-	-	-
8	*Klebsiella spp*	28	15	18	-		-	-	-	-
9	*Shigella spp*	21	-	-	-		-	-	-	-
10	Enterobacter spp	30	-	-	-		-	-	-	-
11	Salmonella spp T	20	-	-	-		-	-	-	-
12	*Salmonella spp*	25	-	-	-		-	-	-	-
13	*Yersinia enterocolitica*	32	-	-	-		-	-	-	-
14	*Listeria monocytogenus*	30	-	-	20	6	17	-	-	17
15	*Listeria spp*	22	10	-	17	10	19	12	-	17
16	*Brucella meletensis*	27	-	-	9	12	16	-	-	15
17	*Brucella abortus*	18	-	-	6	17	22	-	-	10
18	*Escherichia coli H*	30	8	-	28	-	-	10	-	-
19	*Escherichia coli L*	35	19	-	13	8	-	8	-	-
20	*serrratia mercescens*	33	12	-	-	-	-	-	-	-

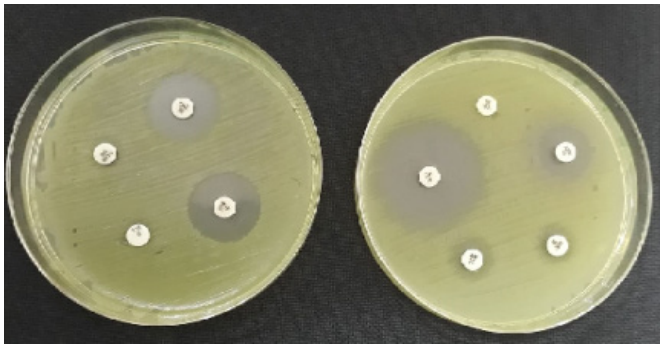
— : No inhibition zone observed



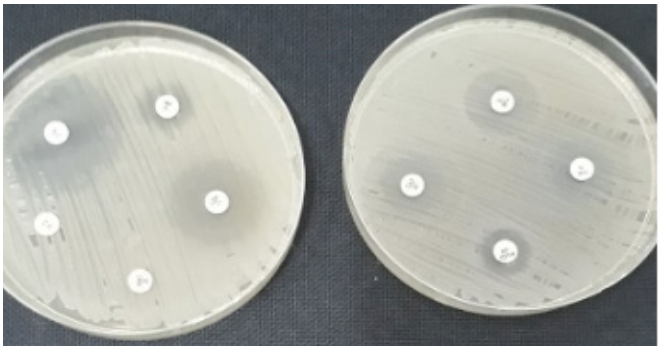
Pseudomonas aeruginosa ZZ



Staphylococcus aureus HL



Pseudomonas aeruginosaHL



Escherichia coli L

camaldolehsis leaves and *Ziziphus* leaves but efficacy of plant extracts inhibits bacteria more than some antibiotics be clear in *Staphylococcus aureus HM* and *Salmonella sppT* estimated (22mm) (24mm) respectively in alcoholic extract of *Pomegranate* Peel. Highly proven alcoholic extracts have been of *Eucalyptus camaldolehsis* leaves and *Ziziphus* leaves to most types of bacteria resistant to (TM) (AM) (AUG) This indicates the sensitivity of the bacteria to the direction of the active compounds and synergistic effect found in the plant extracts and their inability to resist them.[31,32] The some of phytochemicals compound may act of inhibiting microbial growth by action inducing phospholipoidal cellular membrane perturbations, interference with microbial metabolic processes, action of modulation of signal transduction and gene expression pathways.[33,34] Than progress shows that plant extracts were effective against many types of bacteria studied so we recommend that you do extensive studies on medicinal plants and try to extract them with more than one type of extract and compare the extract water, ethylene, methyl and other extracts for each of them for use as alternative treatments for antibiotics vital.[35]

CONCLUSION

The effectiveness of inhibit growth bacteria depend to type of plant extracts if proved Pomegranate Peel an interesting activity against pathognic bacteria compared with Eucalyptus and *Ziziphus* extracts. It has been shown that the extraction method has an effect in the extraction of activity compounds that have an antibacterial effect if considered alcoholic solvents like ethanol are more suitable than other solvents such as water in extraction. The differences in sensitivity and resistance of some of the bacterial isolates under study against the standard antibiotic types used and the types of medicinal plants differed by different extraction methods. It was found that there are bacterial isolates that are sensitive to plant extracts more than antibacterial agents such as *Staphylococcus aureus HM* and *Salmonella sppT.* Therefore, the use of processed products from plant extracts should be used as an alternative to antibiotics used to prevent the presence of multi drug-resistant bacteria. It is considered a problem of future medicine

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