



Evaluation of Antibacterial Activity between Medical Plant Extracts and Antibiotics against Bacterial Isolates from Tonsillitis

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Objectives: Acute tonsillitis is an inflammatory process of tonsil tissue and is usually infectious. Acute tonsillitis starts suddenly and usually disappears within one to two weeks. This study aims to isolate bacteria from patients with infectious tonsillitis and determine the antibacterial activity of *Mentha piperita* L, *Citrus aurantium*, banana peel extract and antibiotics including penicillin, ampicillin, oxacillin, meropenem, tobramycin, gentamicin, levofloxacin, and clarithromycin against bacteria.

Methods: Were Collected 60 clinical samples of sputum and throat swabs from patients with tonsillitis admitted to AL-Furat Alawsait Hospital in Najaf. The samples were cultured in blood, MacConkey agar, and incubated at 37°C for 24 hours, and then the Vitek2 system was used for diagnosis to detect G + ve and G-ve bacteria.

Results: It was found that out of 60 sputum samples and throat swabs, 15 cases were gram positive bacteria, while 45 gram negative bacteria. As revealed by the in vitro agar well diffusion method, it was found that the aqueous extracts of these plants, especially *Mentha piperita*, have strong antibacterial activity against a variety of pathogens. The results show that *Mentha* is very effective at all concentrations used in this study (125, 250, 500, 1000) mg / ml in all bacteria isolated from tonsillitis. The zone of greatest inhibition of *Mentha*, *Citrus anrantium* and banana peel extract was 30, 28, 25 mm at concentrations of 1000 mg / ml respectively. Used eight selected

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antibiotics, including (penicillin, ampicillin, oxacillin, meropenem, tobramycin, gentamicin, levofloxacin, and clarithromycin). To shows the susceptibility of all bacteria isolates used in this study, the best antibiotics to sensitive for all bacteria (Meropenem and Levofloxacin), while the resistance to oxacillin.

Conclusion: The selected commonly used aqueous extracts have been tested for their antibacterial activity against multi drug-resistant bacteria, and it is believed that their extracts can be used for multi drug-resistant bacteria that can cause nosocomial and community-acquired infections.

Keywords: *Tonsillitis; mentha piperita; citrus aurantium; banana peel extract; vitek 2 system; antibiotics.*

1. INTRODUCTION

The most common inflammation of the tonsils that infects children is called tonsillitis. Infection is spread through close contact with an infected person, usually with symptoms that appear 2 to 5 days after exposure, called coughing & sneezing [1]. Other symptoms are nasal congestion, bad breath, sore throat, runny nose, fever, headache, swelling of tonsils, swollen lymph nodes, redness and partial or complete pus coverage [2]. Mainly, Guidelines for the identification of tonsillitis swabs that can be used to diagnose the microorganisms of acute and recurrent and the correct treatment the diagnoses of disease through medical history and clinical examination, However using it may lead to wrong conclusions, A number of studies have shown that there are significant differences between the outer and central pathogenic flora of tonsils [3,4]. Possible pathogens include *S. aureus*, *S. pyogenes*, *C. diphtheriae*, β -hemolytic streptococci, *E.coli* & *Pseudomonas*. Most G + ve Streptococci are non spore cocci, non motile, and approximately 0.5 to 1.2 μ m in size, They grow in chains or in pairs, and are negative for catalase and oxidase test. The most common forms of Streptococcus pyogenes disease include respiratory tract infections and skin infections. Each form usually causes different strains in addition to the upper respiratory tract colonies, and has a highly toxic effect when passing through the hosts defense system [5].

Part of plants including (roots, stems, leaves, fruits) are prepared. Plant extracts are now becoming cheaper and cheaper due to their low cost, wide range of applications available immediately, and potential medicinal properties and some The ability of health. Used mostly in research. Grow in recognition. Some

researchers have shown that the use of medicinal plants is superior to common antibiotics used to treat patients with different diseases [6]. An important medicinal plant belonging to the Labiate family is called (peppermint) *Mentha piperita* L. [7]. Para-nonyl adrenaline is widely used in weight management products, and as a thermogenic agent and its main alkaloid component called citrus (*Citrus aurantium*) is found in Rutalesorder. Dried orange flower (called citrus) can also be used to relieve icing, which is used as an anticonvulsant in neurological diseases such as hysteria and neurasthenia. In addition, orange peel has a mild sedative, appetizing effect, and can repair the heart [8]. Due to its rich nutritional properties, bananas have also been used as medicines for a long time. In addition, bananas also have biological activity such as anti-diabetic, anti-diarrhea, anti-tumor, anti-mutagenic, anti-worm and anti-cancer effects antibacterial, and antioxidants [9]. Banana peel contains biologically active compounds, such as alkaloids, flavonoids, fluorobutane, tannins, glycosides and terpenoids. According to reports, this biologically active compound has pharmacological effects, especially as antioxidants, antidiabetics, anti-inflammatory drugs and antibiotics [10]. The role of these microorganisms in plants The biological control of many diseases shows that their research on plant/microbe interactions can be used in many applications [11].

This study aimed to isolate bacteria from patients with infectious tonsillitis and investigated the activity of three medicinal plants including (*Mentha piperita*, *Citrus aurantium*, banana peel extracts) compare with antibiotics against human pathogenic bacteria.

2. MATERIALS AND METHODS

2.1 Collection of Samples and Bacterial Isolates

From October 2020 to January 2021 (60) clinical samples collect of throat swabs from patients with different age admitted to AL-Furat Alawsait hospital in Najaf province. Culture the sample in blood, MacConkey agar and incubated for 24 hours at 37°C. Colonies or culture media were selected and identified according to standard bacteriological and biochemical criteria [12].

2.2 Diagnosis by Advanced Technique Vitek2 System

The test has been perfumed according to the manufacturers instructions. Recently, this kit is used for rapid detection of G+ve and G-ve bacteria.

2.3 Preparation of (*Mentha piperita*, *Citrus anrantium* and Banana Peel Extract)

Preparation of the extract Collect apparently healthy plants washed them thoroughly with tap water, and dry them for several days at room temperature in the dark. It was made into powder and then extracted from 15 grams of powder, placed in 100 ml of distilled water (cold water extract) in a conical flask, and the crude material are place at 2 hours. The extract was filtered and un dissolved particles are removed through filter paper. Then it was centrifuged at 3000 rpm for 10 minutes. The supernatant containing the plant extract was concentrated by evaporating the solvent using a vacuum drying oven at a temperature of about 80°C. The aqueous extract was stored in sample bottles at 40°C before using the published procedure with a slight standardized modification on our lap [13].

The banana peel is air-dried for two weeks, and then ground into a powder with a mechanical mixer. Dispense 9 grams of peel powder into 100 milliliters of distilled water. The mixture was vigorously intermittently stirred with a magnetic stirrer and then allowed to stand for 24 hours. The other method to extract Rumble

other steps of *mentha* and *citrus aurantium* or extract.

2.4 Antibacterial Activity Test

The antibacterial activity was measured using the disc diffusion method. The nutrient agar is inoculated with the given microorganisms by spreading the bacterial inoculum on the medium. A hole (8mm diameter) with four concentrations (125, 250, 500, 1000 mg/ml) against test bacteria is drilled into the agar and filled with plant extract include (*Mentha*, *Citrus anrantium* and Banana peels extract), Incubate the plate at 37°C for 24 hours. If there is an area of inhibition around the well, evaluate it in millimeters (mm).

2.5 Antimicrobial Susceptibility Test

For the confirmed bacterial isolates, a drug susceptibility test was carried out using the standard disc diffusion method. It was incubated overnight at 37 °C for 24h, According to the Clinical and Laboratory Standards Institute [14].

3. RESULTS

It was found that out of 60 sputum samples and throat swabs, 15 cases were gram positive bacteria including (10) 17% *Staphylococcus lugdunensis*, (5) 8% *Rothia mucilaginoso*, while 45 gram negative bacteria included (8) 13% *Pseudomonas oryzihabitians*, (12) 20% *Pseudomonas aeruginosa*, (5) 8% *Citrobacter freundii*, (7) 12% *Escherichia coli*, (7) 12% *Klebsiella pneumonia* ssp, (6) 10% *Enterobacter aerogenes*, Fig. 1.

This study only considers bacterial isolates obtained from clinical samples with pure and dominant growth. All these bacteria were identified based on the colony morphology and biochemical characteristics compared with the standard description in the Bergeys manual of determinative bacteriology. The final identification was performed using the VITEK-2 automated compact system with GP-ID and GN-ID cards, which contained 43 biochemical tests against Gram-positive and Gram-negative bacteria. The results are shown in (Tables 1 & 2).

Through the disc diffusion method, the antibacterial activity of different extracts including *Mentha*, *Citrus anrantium* and banana peel extracts against Gram-positive and Gram-negative bacteria was analyzed in vitro, and the diameter of the growth inhibition zone and its Subsequent concentration. as shown in Fig. 2.

The results of the antibacterial activity of the aqueous extract on all isolates; the observation results are shown in Fig. 2. The results show that *Mentha* is very effective at all concentrations used in this study (125, 250, 500, 1000) mg / ml in all bacteria isolated from tonsillitis. The zone of greatest inhibition of

Mentha, *Citrus anrantium* and banana peel extract was 30, 28, 25 mm at concentrations of 1000 mg / ml respectively.

At a concentration of 1000 mg / ml, the highest clearance zone was obtained from the aqueous extract against *Staphylococcus lugdunensis*, *Rothia mucilaginosa*, *Pseudomonas oryzihabitians*, *Citrobacter freundii*, *Klebsiella pneumonia ssp*, *Enterobacter aerogenes*, with a diameter of 30 mm. The lowest zone of inhibition at this concentration was (25.28) mm against *Pseudomonas aeruginosa* and *Escherichia coli* respectively, Fig. 3.

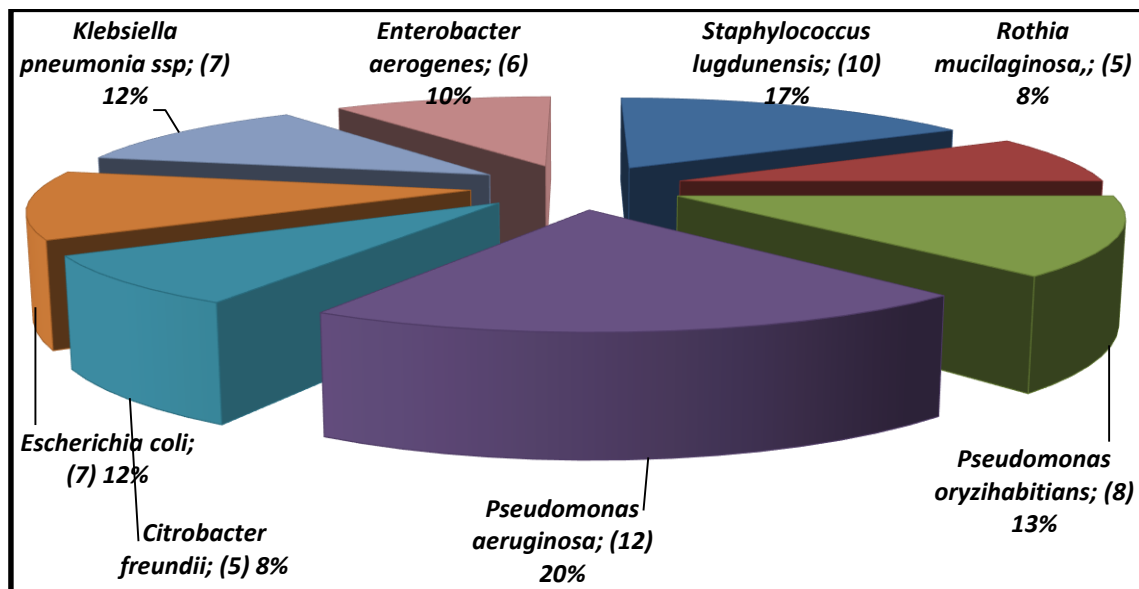


Fig. 1. Number and Percentage of the Microorganism

Table 1. Diagnosis of gram positive bacteria with VITEK-2 compact system

N	Biochemical test	Gram positive Bacteria	
		<i>Staphylococcus lugdunensis</i>	<i>Rothia mucilaginosa</i>
1	AMY	-	-
2	APPA	-	+
3	LeuA	-	+
4	AlaA	-	+
5	dRIB	+	-
6	NOVO	-	-
7	dRIB	-	-
8	OPTO	+	+
9	PIPLC	+	-
10	CDEX	-	-
11	ProA	-	+
12	TyrA	-	+
13	ILATk	-	-
14	NC6.5	+	-
15	O129R	+	-

N	Biochemical test	Gram positive Bacteria	
		<i>Staphylococcus lugdunensis</i>	<i>Rothia mucilaginosa</i>
16	dXYL	-	-
17	AspA	-	-
18	BGURr	-	-
19	dSOR	-	-
20	LAC	-	-
21	dMAN	-	-
22	SAL	-	-
23	ADH1	+	-
24	BGAR	-	-
25	AGAL	-	-
26	URE	-	-
27	NAG	+	-
28	dMNE	+	+
29	SAC	-	+
30	BGAL	-	-
31	AMAN	-	-
32	PyrA	+	+
33	POLYB	+	(-)
34	dMAL	+	+
35	MBdG	-	+
36	dTRE	+	+
37	AGLU	-	+
38	PHOS	-	-
39	BGUR	-	-
40	dGAL	-	-
41	BACI	+	-
42	PUL	-	-
43	ADH2S	-	-

Table 2. Diagnosis of gram negative bacteria with VITEK-2 compact system

N	Biochemical Test	Gram negative Bacteria					
		<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	<i>Citrobacter freundii</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i> ssp	<i>Enterobacter aerogenes</i>
1	APPA	-	-	-	-	-	-
2	H2S	-	-	+	-	-	-
3	BGLU	-	-	-	-	+	+
4	PROA	-	+	-	-	-	+
5	SAC	-	-	+	+	+	+
6	ILATK	-	+	-	-	+	+
7	GLYA	-	-	-	-	-	+
8	O129R	+	+	+	-	+	+
9	ADO	-	-	-	-	+	+
10	BNAG	-	-	-	-	-	+
11	DMAL	+	-	+	+	+	+
12	LIP	-	-	-	-	-	-
13	DTAG	-	-	-	-	-	+
14	AGLU	-	-	-	-	-	-
15	ODC	-	-	+	+	-	+
16	GGAA	-	-	-	-	-	-

N	Biochemical Test	Gram negative Bacteria					
		<i>Pseudomonas</i> <i>habitans</i>	<i>Pseudo</i> <i>monasae</i> <i>ruginosa</i>	<i>Citroba</i> <i>cterfreu</i> <i>ndii</i>	<i>Escheri</i> <i>chia</i> <i>coli</i>	<i>Klebsiell</i> <i>pneumoni</i> <i>a ssp</i>	<i>Enteroba</i> <i>cteraero</i> <i>genes</i>
17	PYRA	+	-	+	-	+	+
18	AGLTP	-	+	-	-	-	-
19	DMAN	-	+	+	+	+	+
20	PLE	-	-	-	-	+	+
21	DTRE	+	-	+	+	+	+
22	SUCT	-	+	+	+	-	+
23	LDC	-	-	-	-	+	+
24	IMLTA	-	+	-	-	-	-
25	IARL	-	-	-	-	-	-
26	DGLU	+	+	+	+	+	+
27	DMNE	+	+	+	+	+	+
28	TYRA	-	-	+	-	-	+
29	CIT	-	+	-	-	+	+
30	NAGA	-	-	-	-	-	+
31	IHISA	-	-	-	-	-	-
32	ELLM	-	-	+	-	-	-
33	DCEL	-	-	-	-	+	+
34	GGT	-	+	+	-	-	+
35	BXYL	-	-	-	-	+	+
36	URE	-	-	+	-	+	+
37	MNT	-	+	-	-	+	+
38	AGAL	-	-	+	-	+	+
39	CMT	+	+	+	+	+	-
40	ILATA	-	+	-	-	-	-
41	BGAL	-	-	+	+	+	+
42	OFF	-	-	+	+	+	+
43	BAIAP	-	+	-	-	-	-
44	DSOR	-	-	+	+	+	+
45	5KG	-	-	-	-	-	-
46	PHOS	-	-	+	-	+	+
47	BGUR	-	-	+	+	-	-

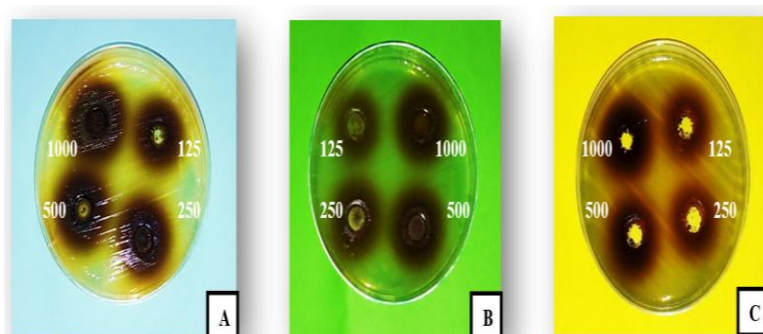


Fig. 2. inhibition zone of four concentration (125, 250, 500, 1000 mg/ml) against test bacteria (A): *Mentha*, (B): *Citrus anrantium*,(C): Banana peels extract

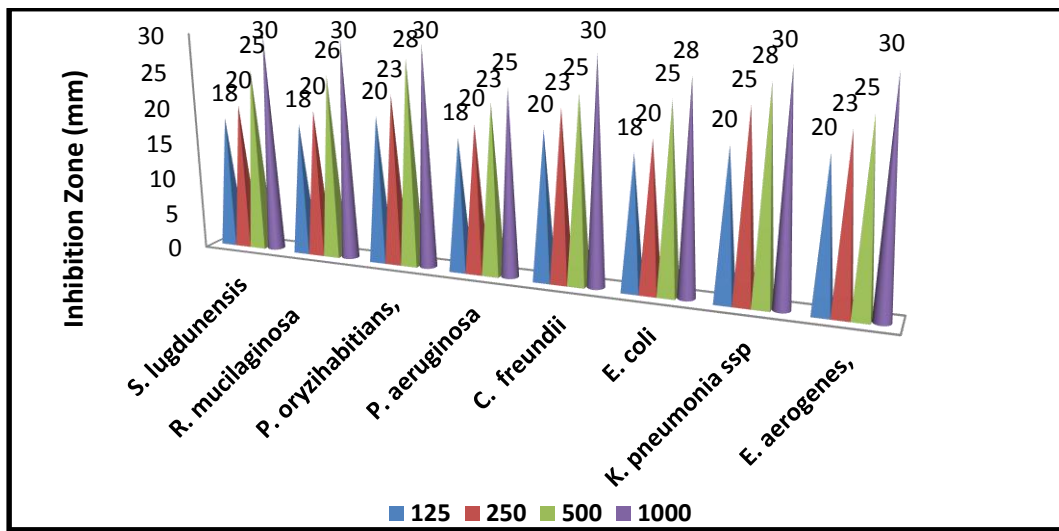


Fig. 3. The diameter of inhibition zones of different concentration (125,250,500,1000 mg/ml) of aqueous *Mentha* extract against test bacteria

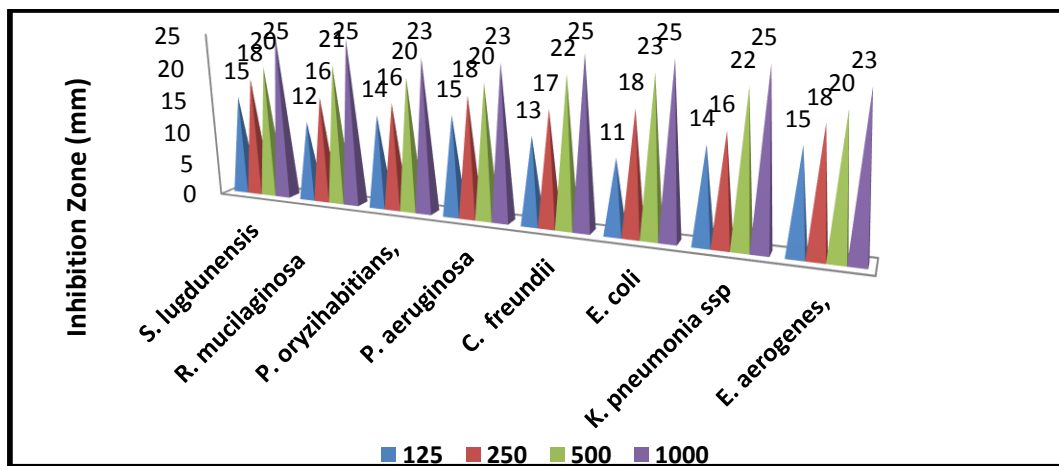


Fig. 4. The diameter of inhibition zones of different concentration (125,250,500,1000 mg/ml) of aqueous *Citrus anrantium* extract against test bacteria

The results are shown in Fig. 4. the highest antibacterial activity was observed with aqueous extract of *Citrus anrantium* against *Staphylococcus lugdunensis*, *Rothia mucilaginosa*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumonia ssp*, with 25 mm at a concentration of 1000 mg / ml, while *Pseudbitomonas oryziha*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, zone of inhibition of 23 mm at some concentration.

While Banana peel aqueous extract has the highest antibacterial activity, zone of inhibition was 28 mm against *Enterobacter aerogenes*, 26 mm against *Staphylococcus lugdunensis*, 25 mm against *Pseudomonas aeruginosa*,

Klebsiella pneumonia ssp, at the concentration (1000 mg / ml). The lowest zone of clearance in this experiment was 23-20 mm against *Pseudomonas oryzihabitans*, *Escherichia coli* and *Rothia mucilaginosa*, *Citrobacter freundii* respectively at some concentration, Fig. 5.

(Table 3) shows the antibiotic susceptibility of the bacterial isolates used in this study. Used eight selected antibiotics, including penicillin, ampicillin, oxacillin, meropenem, tobramycin, gentamicin, levofloxacin, and clarithromycin. the best antibiotics for the treatment of all bacteria (Meropenem and Levofloxacin), while the resistance to oxacillin.

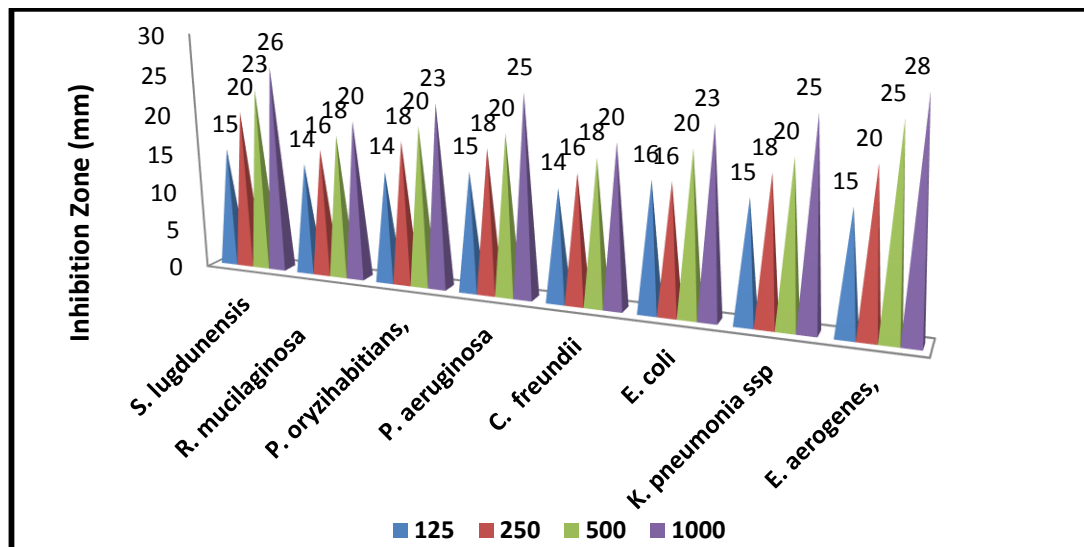


Fig. 5. The diameter of inhibition zones of different concentration (125,250,500,1000 mg/ml) of aqueous Banana peels extract against test bacteria

Table 3. Antibiotics susceptibility of Clinical Bacterial Isolates

Bacteria	P	AM	OX	MEM	TOB	GEN	LEV	CLR
Antibiotic								
<i>S. lugdunensis</i>	R	R	R	S	R	S	S	R
<i>R. mucilaginosa</i>	S	S	R	S	S	S	S	S
<i>P. oryzihabitian</i>	R	R	R	S	S	S	S	R
<i>P. aeruginosa</i>	R	R	R	S	R	R	S	R
<i>C. freundii</i>	S	R	R	S	S	R	S	R
<i>E. coli</i>	R	R	R	S	R	R	S	S
<i>K. pneumonia ssp</i>	S	R	R	S	S	R	S	S
<i>E. aerogenes</i>	S	S	R	S	R	S	S	S

P: Penicillin, AM: Ampicillin, OX: Oxacillin, MEM: Meropenem, TOB: Tobramycin, GEN: Gentamicin, LEV: Levofloxacin, CLR: Clarithromycin

4. DISCUSSION

Some tonsillitis infections may be caused by viruses, while others may be caused by fungal invasion. Among adults, only 5-10% of such complaints are related to bacterial infections and 15-20% of complaints in children [15]. These findings may explain its surrounding environments immunity to pathogens in young children, the main symptoms of tonsillitis include sore throat and dysphagia [16], In this study, less than one third of the patients with these discomforts were because children rarely complained of sore throats or difficulty swallowing. In the study it was infants, considering that almost one third of patients complained of difficulty swallowing or sore throat, This result is consistent with [17]. Different bacteria isolated from tonsillitis have

been studied based on bacteriological studies. Another study disagrees [18] According to bacteriological studies, the main bacteria are β -hemolytic streptococcus (51.4%), followed by coagulase-positive staphylococci (12.5%) and pneumococcus (9.7%). There is only one case of *Corynebacterium diphtheriae*. We use polar solvents and aqueous solvents to extract active ingredients from plant leaves. The research results show that aqueous and polar solvents can effectively resist common bacterial infections. The use of peppermint leaves contains many effective compounds such as menthyl acetate, limnone, menthone, menthol and furfuran [19]. The current work is similar to [20], showing that peppermint compounds have powerful antibacterial activity, and that peppermint leaf extract must contain effective active ingredients responsible for eliminating

bacterial pathogens. In a study [21] The antibacterial activity of citrus peel oil was evaluated. It was observed that the peel oil of Zhonghua C and Platycodon grandiflorum showed more or less similar inhibition of G+ve & G-ve bacteria. Compared with our research on certain test organics and solvents, these results show that citrus extracts and extracts show relatively similar results. The foregoing results prove the pharmacological properties of citrus, which can be used as an effective prodrug to reduce the adverse reactions and inflammation of patients with urinary tract infection [22]. Another study showed that citrus can effectively inhibit bacteria isolated from burn infections [23]. Several studies have shown the antibacterial activity of banana peels [24]. This result agrees with [25,26] on the effect of these plant on pathogenic bacteria isolate from clinical specimen. The banana peel extract (*M. paradisiaca*) was evaluated for its antibacterial activity against human pathogens, and it was found that the banana peel extract showed inhibitory effects on *Escherichia coli*, *Proteus* and *Staphylococcus aureus*, This result is another result similar to the effect of plant extracts on bacteria. Isolated from the upper respiratory tract [27]. This result is inconsistent with [28]. Studies on the antibacterial activity of banana peel extracts in clinical isolates show that it has the highest antibacterial activity against *Moraxella catarrhalis* and *Staphylococcus aureus*, followed by *Streptococcus pyogenes*, *Enterococcus aerogenes* and *Klebsiella pneumoniae* have no effect on *Candida albicans* and *Escherichia coli*.

The sensitivity of the isolated bacteria to different antibiotics and chemotherapeutic drugs indicates that Gram-positive bacteria are more sensitive to antibiotics than Gram-negative bacteria. Most strains are sensitive to the antibiotics penicillin, ampicillin, gentamicin, cefotaxime, erythromycin, chloramphenicol, cefotaxime, cephalixin, and amikacin. [29] The most effective antibiotic is called penicillin, which is used to treat tonsillitis caused by bacteria. Of the 9 coagulase-positive staphylococci, 3 developed drug resistance. In many bacteria, the incidence of drug resistance is increased due to the production of β -lactamase by the bacteria, which leads to the lysis of antibiotic activity and may be absorbed by sensitive strains of drug-resistant transfer factors during the recombination process. Follow-up of patients with tonsillitis showed that

the recurrence of the disease was 70.3%. and the disease-free cases were 27.6%. 2.1% of patients had peritonsillar abscesses and received antibiotic treatment with incision and drainage. The results of antibiotic resistance are consistent with our series of studies [30]. [31] Recurrence of acute tonsillitis has been observed. Its low sensitivity and specificity prove that it may be a predictor of bacterial tonsillitis. This is consistent with other studies that show that using individual signs to predict possible bacterial tonsillitis is unreliable. Similar to another study on the effect of antibiotics on pathogenic bacteria [32]. Similar to another study on the comparative effects of medicinal plants and antibiotic on pathogenic microorganisms [33]. [34] In this study, it was found that Gram-negative coliforms (*Proteus*, *Pseudomonas* and *Klebsiella*) are abnormally present. Due to poor personal hygiene, they may have transferred from the anal area to the throat because of their Not a traditional pathogen of the upper respiratory tract.

5. CONCLUSIONS

In this study, the aqueous extract of *Mentha piperita* was showed promise antibacterial activity against the most prevalent bacteria in tonsillitis infection compare with *Citrus aurantium* and banana peel extracts, the effects of *Mentha piperita* extract on Gram-positive bacteria does greater than Gram-negative bacteria detected in this study, the Result obtains through this work have scientifically verified the application of the plant in the treatment of this infection, most drugs prescribed under generic names that encourage the rational use of drugs. In most cases, a culture and sensitivity test was performed to identify the organism and prescribed appropriate antibacterial agents to indicated proper operation.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company

rather it was funded by personal efforts of the authors.

CONSENT

Written informed consent was obtained from all participants prior to the administration of the questionnaire and sample collection.

ETHICAL APPROVAL

Ethical approval for the work was obtained from the Ethics and Research Committee of the hospital.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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