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Susceptibility of *Staphylococcus spp.* to the Plant Crude Extract

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ABSTRACT

The study aimed to investigate whether the extract from the *Thuja orientalis* plant had any antibacterial properties against fifteen MRSA isolates in a group of thirty-seven *Staphylococcus aureus* isolates. To determine bacterial resistance, an antibiotic susceptibility test was conducted on five different drugs. Methicillin showed the highest level of bacterial resistance (100%), whereas Vancomycin had the lowest level of resistance (6.6%). The results of the examination of the *Thuja orientalis* plant extract clearly showed that it had a significant suppressive effect on all *Staphylococcus aureus* isolates.

Keywords- *Thuja orientalis*, *Staphylococcus aureus*, MRSA, Antibacterial activity.

I. INTRODUCTION

Staphylococcus aureus is a type of bacteria commonly found on human skin and in the nasal cavity [16]. It is a Gram-positive cocci and can also be present in the oropharynx [14]. About thirty percent of people carry *S. aureus* without showing any symptoms [16]. However, it can also act as a pathogenic bacteria and cause various infectious diseases in humans, from skin infections like impetigo to more severe conditions like osteomyelitis and endocarditis [16]. Antibiotics are used to treat S. aureus infections, but antibiotic-resistant strains have emerged, making treatment more challenging [12].

Since many dangerous germs are resistant to the common medicines used in clinics, antibiotic resistance poses a severe hazard. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a specific source of concern. Penicillin and methicillin are among the antibiotics that MRSA has become resistant to; certain strains have also gained resistance to daptomycin and vancomycin, further complicating treatment [4] [17]. Antibiotic resistance is spreading rapidly, while the development of new antibiotic compounds is not keeping up. This could mean that the age of antibiotics is coming to an end and a new era without antibiotics is beginning. In response, scientists are working on innovative therapies that do not rely on conventional antibiotics and are also striving to better understand how bacteria adapt to antibiotic resistance. Resistant to methicillin one well researched and clinically significant strain of *S. aureus* is resistant to antibiotics. Methicillin resistance has been reported in up to 60% of clinically isolated strains of *S. aureus* [15].

Since ancient times, medicinal plants have been utilized because of their advantageous impacts on the treatment and control of various disorders. As a result of reported cost-effectiveness, fewer side effects, lack of bacterial resistance, and easy availability, Medicinal plants have become much more reliable and popular all around the world even at the start of the twenty-first century [7].

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Thuja orientalis L., a member of the cupressaceae family, is significant due to its several traditional use in India. It is frequently referred to as *Thuja* or *Morepankhi*. According to folkloric research, *Thuja orientalis* L. has a number of medicinal uses. It's a decorative plant. It is indigenous to northwest China and has spread to Iran, Florida, Russia, Korea, Japan, and India. It is also grown in parks, gardens, backyards, and other places throughout the world. Leaves of *Thuja orientalis* L. include essential oil It is a hazardous substance. α-thujone has anthelmintic and insecticidal properties that help treat parasitic worms. They interfere with brain neuronal signals, hence ingesting essential oils can be fatal [11]. Leaf extract from *Thuja orientalis* L. is traditionally used as a febrifuge, stomachic, diuretic, astringent, emollient, antitussive, haemostatic, and antibacterial [9], the plant has a flat stalk, scale-like leaves, and flattened fan-shaped leaves that are growing with resin glands.

The present research aimed to screen the extract of *Thuja orientalis's* antibacterial effectiveness against isolates and assess its potential use in, treating infections brought about by these multi-drug resistant clinical isolate

II. MATERIAL AND METHOD

2.1 Sample Collection

Thirty-seven *Staphylococcus aureus* isolates were obtained from private hospitals and clinical laboratories in Baghdad city. 37 presumed S. aureus strains were supplemented with 15 well-defined MRSA isolates for analysis. All samples were subjected to biochemical tests and cultural identification.

2.2 Bacterial identification

The suspected *Staphylococcus aureus* isolates were identified using a number of morphological, physiological, and biochemical tests. Gram staining, Mannitol salt agar, Staph 110, Coagulase test were among the tests conducted.

Bergey's Manual of Determinative Bacteriology was referenced for all tests [5].

2.3 Antibiotic Susceptibility Testing

The Kirby-Bauer method was applied to ascertain the susceptibility of *Staphylococcus aureus* isolates to different antibiotics. After growing pure colonies on nutrient agar, the colonies were moved into the brain-heart infusion broth and incubated for four hours at 37ºC to standardize, and obtain turbidity equal to the standard of McFarland tube (0.5) which was previously prepared, to be used as turbidity standard. A portion of the bacterial culture was transferred by using a sterile cotton swab, carefully spread on the Mueller- Hinton agar medium, and, left for 10 min. Subsequently, antibiotic susceptibility screening was performed using the disc diffusion method. Antibiotic discs (Mastdiscs, U.K) used include (Methicillin, Gentamicin, Oxacillin, Penicillin and Vancomycin) effective against gram-positive bacteria. Using sterile forceps, the disks were positioned on the agar medium and pressed firmly to guarantee contact with the agar. The plates were then inverted and incubated for eighteen hours at 37ºC [18].

Inhibition zones that developed around the discs were measured by millimeters (mm) by using a metric ruler mentioned by CLSI [6]. Isolates were classified as susceptible, intermediate, or resistant to a given medication, as shown in Table 1.

Table 1: Standard inhibition zones (CLSI, 2020) Id

			Diameter of inhibition zone by (mm)		
Id	**Antibiotics**	Conc. μg/disk	Susceptible	Intermediate	Resistant
1	Methicillin	30	≥29	22-28	≤21
2	Gentamicin	10	≥15	13-14	≤12
3	Oxacillin	30	≥22	-	≤21
4	Penicillin	10	≥29	-	≤28
5	Vancomycin	30	≥23	12-22	≤11

2.4 Preparation of plant-extract

The plant extract was obtained from a local market. Extract was prepared by dissolving (50 g) of the plant powder in (500) ml of boiled distilled water at 100ºC, then after leaving for ten minutes, it was filtered through Watman papers number 1, then was poured in glass dishes and left in the incubator at 37°C until the powder was dried after that was kept in the fridge until use [3]. Three concentrations (25, 12.5 and 6.25 mg\ml) of the plant extract were tested against *Staphylococcus aureus* isolates in Muller Hinton agar by making wells in the plates. The plates were streaked with *Staphylococcus aureus* isolates and 50µl of plant extract was put in the wells, then plates were incubated for 18 hours at (37°C) in order to test the plant extract effect against the growth of isolates.

III. RESULTS AND DISCUSSION

3.1 Identification of Bacteria

All of the possible isolates that produced colonies like those of *Staphylococcus aureus* were sub-cultured in order to evaluate their physiological, morphological, and biochemical characteristics. All isolates of bacteria tested were aerobes, Gram-positive and negative for the Motility test.

All isolates could grow on staph 110 medium because of their potency to resist the high concentration

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of 7.5% Nacl also The Coagulase test yielded a positive result, and on Mannitol Salt Agar, Mannitol Fermenters showed up as yellow colonies. (Figure-1).

Figure 1: *Staphylococcus aureus* on Mannitol salt agar

3.2 Evaluation the Antibiotic Susceptibility of Bacterial Isolates

Susceptibility of *Staphylococcus aureus* isolates to different antibiotics was as shown in Figure-2. The disc diffusion test was performed as a quick screening method to indicate the susceptibility of isolates to antibiotics.

Results of antibiotic susceptibility of our isolates are summarized in Table-2. According to the results of the Kirby-Bauer method, the highest percentage of resistance was against Methicillin (100%), while the lowest bacterial resistance was against Vancomycin (6.6 %) as shown in Figure-3.

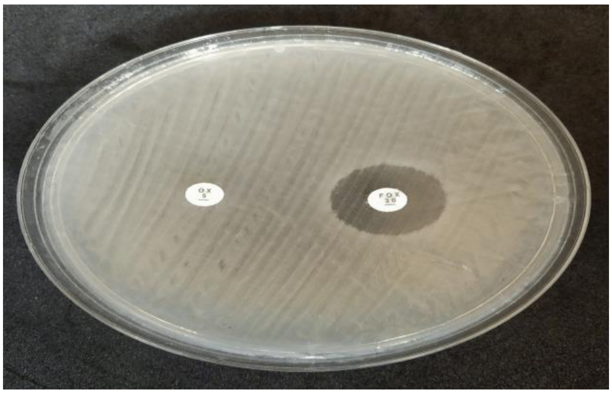

Figure 2: Antibiotic susceptibility test shows the resistance of Staphylococcus aureus against different antibiotics

Table 1: Results of antibiotic susceptibility test for Staphylococcus aureus against different antibiotics.

No.	Antibiotics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Methicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
2	Gentamicin	R	I	S	S	R	I	S	R	I	I	S	R	S	R	I
3	Oxacillin	S	R	R	R	R	S	R	R	R	R	R	S	R	S	R
4	Penicillin	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R
5	Vancomycin	I	S	S	S	I	S	I	I	S	S	R	S	S	I	S

S: Susceptible I: Intermediate R: Resistance

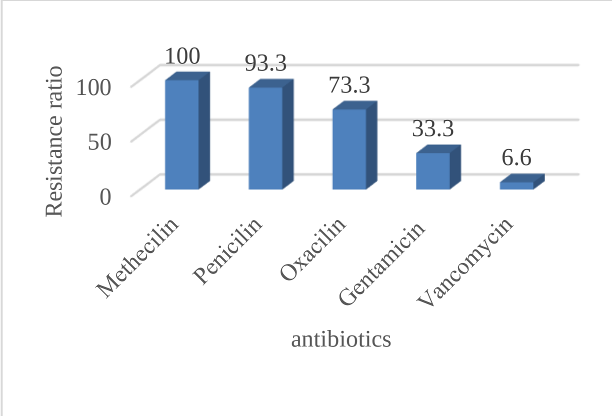

Figure 3: Resistance percentage of Staphylococcus aureus against Methicillin, Gentamicin, Oxacillin, Penicillin and Vancomycin.

The sensitivity test was done on MRSA strain only which was 15 out of 37 strains, for this investigation, 15 MRSA isolates were obtained from clinical samples. Results of antimicrobial susceptibility testing showed that methicillin and penicillin had the highest levels of resistance among the five drugs tested (100%, 93.3%) Respectively, Oxacillin (73.3 %) and the lowest to Gentamicin (33.3 %) and Vancomycin (6.6 %).

The results showed that Methicillin and Penicillin were the more effective antibiotics against S. aureus with a 100% effect, this was identical to the reported results of (Gurung et al., 2020; Mirzaie et al., 2020), as they found (98-100%) of S. aureus isolates were resistant to both antibiotics. Our results differed from (Amoako et al., 2020) results, as they reported that (55.83%) of S. aureus isolates were resistant to Penicillin.

The resistance of S.aureus toward Oxacillin was (73.3%), this was nearly close to that of (Mirzaie et al., 2020), as they found that 68% of isolates were Oxacillin resistant.

While the resistance against Gentamicin was (33.3%), these results were different from the results of [2] [10], they found that the bacterial resistance to Gentamicin was (8.33 %, 10.2 %) respectively, but similar to the results of [13], they reported that Gentamicin resistance was 40%.

Finally, Vancomycin showed the lowest resistance (6.6%). These results were similar to the results of [2] [10] [13] who found that the bacterial resistance toward Vancomycin was (0.0%).

3.3 The Impact of Plant Extracts on Bacterial Growth

The effect of plant extract on the growth of *S. aureus* was tested by making wells in the Muller Hinton agar plate, and then *S. aureus* was streaked on the agar plates, and the plant extract was put in the wells. Different concentrations of the plant extract were tested (25, 12.5, and 6.25 mg\ml). All the concentrations were effective against the S. aureus isolates, it showed a growth inhibition zone, indicating that the plant extract inhibited the growth of *S. aureus* (figure 4).

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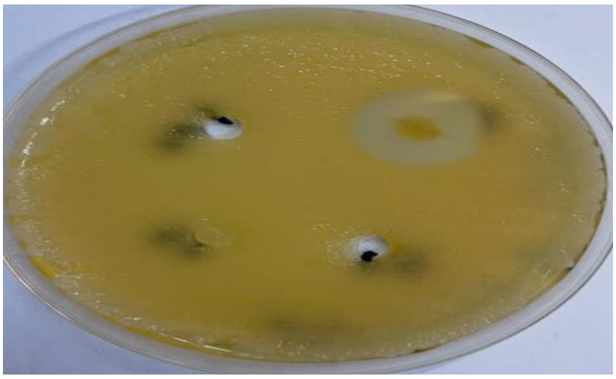

Figure 4: Effect of plant extracts on S. aureus growth.

The results of the presented study showed similarities with other results of previous Iraqi studies that were conducted to determine the antimicrobial activity of *T. orientalis*, and it was effective in inhibiting microbes against some pathogenic microorganisms including *S. aureus* [1]. Another study done in Saudi Arabia showed a moderate effect of *T. orientalis* extract [8]

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