Study the effect of olive extract or refined oligonucleus on *Staphylococcus aureus* Isolated from different clinical cases

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Abstract

The present study included the collection of 100 clinical samples obtained from patients in Ramadi women’s and children's hospital, Hit General Hospital and some private laboratories in Ramadi city, including Bacterimia samples, Urine, abscesses, heart inflammation, wounds, rhinitis, osteoporosis, otitis. Laryngitis, sputum distributed (35, 20, 5, 6, 7, 5, 8, 7, 4, 3) samples respectively to investigate the presence of *staphylococcus aureus*.

Sensitive *S. aureus* has been distinguished from resistance by antibiotics Metecilin, Oxacilin and Cefoxitine. All isolates showed 100% resistance to Metecilin and Oxacilin and 62.5% to Cefoxitine.

The results also showed that the alcoholic extract of olive leaves contained tannins, glycosides, soaps, flavonoids, phenols, resins and amino acids. While it does not contain alkaloids using gas chromatography - mass spectrometry results showed that the extract contains many chemical compounds that are of biological importance in the inhibition of *S. aureus* bacteria.

The olive leaf alcoholic extract of *Olea Europaea* showed a stronger antimicrobial activity than the aqueous extract against *S. aureus*, and the minimum inhibitory concentration of the extract was 100 mg / ml.

**Keywords: clinical cases; extracts; health; toxicity.**

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1-Introduction

*S. aureus* is a public health cause that causes serious infections and is often life-threatening in the community and hospital. It is more difficult to eliminate with the use of antibiotics because of its high resistance to these antibiotics (¹). Bacterial infections range from simple warts such as warts, Imptigo, boils and abscesses to acute infections such as Bacteremia and Toxic shock syndrome (²). Many countries in the world resort to the use of drugs of plant origin to treat many diseases and injuries external and internal
caused by bacteria and extracted from different parts of the plant because of the high resistance to antibiotics and alternatives to antagonists that have become out of service and the fact that these plant extracts are harmless to humans and useful\(^3\). As the result were made\(^4\) The effect of olive leaf extract on S. aureus bacteria may be due to the phenolic compounds present in olive leaves. Researchers have been able to detect the most powerful phenols that have the effect of high concentration within the olive leaves call it Oleuropein substance was considered as an active ingredient in this tree\(^5\). Enables \(^6\) Detection of plant compounds and infection of the strongest belonging to it The group that smelled Luteolin, 0.5 \(^{-}\) Apigenine, Apirin, Chrysoeriol, Quercetin, either \(^7\). They have been able to isolate other existing phophonic compounds exists in olive leaves such as; Luteolin-7 \(\cdot\) Rutin, glucoside The researcher referred to the compound F-0 \(-\) 5-glucoside Luteolin is the largest flavonoid compounds in the plant Another aspect \(^8\) pointed to the existence Anthocyanine, which is considered to be an effective flavonoids Against free radicals, existing citrus fruits are included in plant leaves which pointed out \(^9\) Oleuafricein acid Which is a mixture of Uroslic Acid and Acid Oleanolic acid Eleonolic acid is a compound metabolites Hypersensitivity. Other phenolic compounds found in this genome are included: The ability of olive leaf extract to elimination of microorganisms and pathogenic parasites. Many current studies have tended to use natural plants, including olive \(Olea europaea\) to eliminate pathogenic bacteria as an alternative to some types of antibiotics. Therefore, our current study aimed to use the leaf extract of olive plant as an effective treatment for bacteria, \(S\) \(aureus\) produces biofilms as well as inhibitors of some genes of virulence factors in these bacteria.\(^{10\text{–}12}\)

2-Materials and methods

Collect samples and diagnose Staphylococcus aureus

100 samples were collected from bacteremia cataracts, skin pores and abscesses, heart inflammation, wounds, rhinitis, osteomyelitis, otitis, laryngitis and sputum by using sterile swabs, \(S.\ aureus\) isolates were diagnosed using routine laboratory methods (phenotypic and biochemical) and Vitek-2 to confirm the diagnosis. Isolated bacteria under the head tested for allergies to 10 antibiotics

Antibiotic Sensitivity Test

Detecting sensitivity for antibiotics (HARLEY & PRESCOTT ET AL, 1996):

Olive leaf collection

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The leaves of the plant were taken and washed thoroughly to remove dirt and suspended dirt and then dried at room temperature with constant daily stirring to prevent rot, then ground well using an electric mill and turned into a fine powder kept in the refrigerator at 4 °C until use.

**Preparation of alcoholic extract of leaves of the olive plant**

Weigh 25 grams of vegetable powder and put in a glass flask with a capacity of 500 ml and add 500 ml of ethanol alcohol, leave the mixture in the shaking incubator Shaking incubator for 24 hours and temperature

The solution was filtered using gauze, then distributed in a centrifuge tubes at a speed of 3000 r / min for 30 minutes. Discard the supernatant and place in a glass petri dish in a drying oven. After drying at 37 °C, scrape the extract and store in the refrigerator at a temperature 4 °C until use.

**Preparation of aqueous extract of leaves of the olive plant**

The aqueous extract was prepared by mixing 25 g of olive tree leaf powder in 500 ml of distilled water. Leave the mixture in the shaking incubator for 24 hours at 37 °C. Then filter the solution using gauze, and then distributed the filtrate in the centrifuge tubes at a speed of 3000 r / min for 30 minutes. discard the supernatant and place the filtrate in glass petri dishes in a drying oven at 37 °C for 48 hours after which the extract was skimmed and stored in the refrigerator at 4 °C until use.

**Estimate percentage of plant extract:**

The percentage of previously prepared alcoholic extract was estimated as reported in (3).

Percentage of the extract = Weight of extract /Weight of plant powder X 100

**Chemical statements of active compounds found in olive leaf leaves:**

For the purpose of chemical detection of some of the active chemical compounds of olive leaf extract, the following were performed:

1- Detection of Tannins: add drops of Iron chloride solution to 0.5 ml of aqueous extract and when you notice the color dark green or blue indicates the presence of Altatinat (4).

2- Detection of Alkaloids: Add several drops of reagent Darkendov to 1 ml of extract extracted with 0.1 of hydrochloric acid concentrate and when the precipitate orange, this indicates the presence of alkaloids (5).

3- Detection of Phenols Phenols: 1 ml of Iron chloride 1% concentration in distilled water is added to the extract and when the reagent color turns from green to green blue, this indicates the presence of phenolic compounds (5).

4- Detection of Resins: Add 50 ml of ethyl alcohol at a concentration of 95% to 5 g of vegetable powder and then left in a water bath at 100 m for 2 minutes, then add to the filter 100 ml of distilled water with hydrochloric acid concentration of 4% and when a clear turbidity indicates the presence of resins (6).
Detection of Glycosides: Add 1 ml of the extract in a test tube and add 2 ml of Benedict solution and shake the solution well after the tube was placed in a boiling water bath for 5 minutes after the cooled tube was observed the emergence of red precipitate, indicating the presence of compounds Glycosides \(^7\).

Detection of Siphoning: shake extract in a test tube that the emergence of dense foam for a long time indicates the presence of soap \(^6\).

Flavonoids detection: 10 g of the extract is dissolved in 5 ml of ethyl alcohol concentration of 95% and then filtered solution and then add 10 ml of ethyl alcohol concentration of 50% to 10 ml of solution of potassium hydroxide 50% and when mixing an equal amount of the first and second solutions and note the color is yellow On the presence of flavonoids \(^8\).

Detection of amino acids Amino acids: Add drops of solution of Alnhydrin 0.2% to the extract in the test tube and then left to boil for two minutes until the appearance of purple or yellow to indicate the presence of amino acids \(^9\).

**Diagnosis of chemical compounds in olive plant extract using MASS / GC technology**

Chemical compounds of olive leaf alcohol extract were diagnosed with gas chromatography device connected to mass spectrometer (Shimadzu GC-MS-QP2010Ultra)

**Antibacterial activity test for alcoholic extract of olive leaves**

The Agar dilution method was followed by drilling wells \(^{10}\).

**Determination of cellular toxicity of plant extract**

The cytotoxicity of vegetable alcoholic extract was estimated according to the sources \(^{11}\).

**Determination of Minimum Inhibitory Concentration of Olive Leaf Extract MIC**

A double dilution method was used to prepare the concentrations of the extract under study \(^{12}\) in order to calculate the MIC of the extract using a microtitration plate containing 96-wall under sterile conditions.

**Synergistic test for antibiotics and olive leaf extract**

Synergism test between antibiotics and olive leaf extract against Staphylococcus aureus isolates was carried out by two replicates for each isolate using antibiotic discs and saturated with the lowest inhibitory concentration on Muller Hinton Agar medium.

**3-Results and discussion**

**Diagnosis of S. aureus**
The results of laboratory diagnosis of bacterial colonies growing on the blood agar media and mannitol salt agar showed that 59 isolates belong to Staphylococcus including 55 isolates belonging to Staphylococcus aureus and 54 isolates were resistant to anti-methicillin (MRSA) while one isolate was sensitive to it.

**Antibiotic Sensitivity Test**

The results of the sensitivity test for s.aureus isolates under study against 10 antibiotics showed a variation in their resistance as they were 100% resistant to methicillin. Either antihistamines (Ciprofloxacin, Vancomycin, Tetracyclin, Erythromycin, Trimethoprim/sulfamethoxazole, Ciftraxone, Gentamycin were down by (%91, %51, %76, %80, %27 %36, %58) respectively, as for Amikacin and Rifampin were sensitive %100.

**Specific chemical reagents for active compounds in plant extract**

The percentage of plant extract was calculated according to the above equation and the percentage of alcohol extract was 8%. The results of the chemical statements of the active compounds found in the raw plant extract showed that the leaves of the olive plant contain many active compounds which are considered as secondary metabolism products. The results of the chemical disclosure of the olive leaf extract showed that the extract contains the following compounds: Tannins, glycosides, Saponins, flavonoids, phenols, resins, amino acids and alkaloids.

**Table (1) Chemical qualitative detection of active compounds in olive leaf extract**

<table>
<thead>
<tr>
<th>Effective compounds</th>
<th>Olive leaf leaves</th>
<th>Detection result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>alcoholic</td>
<td>The name of the reagent used</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
<td>Iron Chloride</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-ve</td>
<td>Darkendov reagent</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+ve</td>
<td>Kid reagent</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ve</td>
<td>Mercurous chloride</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+ve</td>
<td>Nenhydrin solution</td>
</tr>
<tr>
<td>Phenols</td>
<td>+ve</td>
<td>Iron Chloride</td>
</tr>
<tr>
<td>Resins</td>
<td>+ve</td>
<td>HCl 4%</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
<td>Ethyl alcohol</td>
</tr>
</tbody>
</table>

+ ve= Exists, –ve = does not exist
The results of this study are identical to the researcher (15) that the extract of olive leaves contains Titanins, glycosides, Saponin, flavonoids, phenols, resins, amino acids and not alkaloids.

Also, the results of our study are consistent with the results of (14) in terms of containing the alcoholic extract of olive leaves on phenols and their role in their antioxidant activity.

Also our results were contrary to the results of (15) for the absence of amino acids in his study on olive leaf extract and the appearance of alkaloids that did not appear in our current study but similarity in terms of the presence of resins, turbinates, phenols and Saponin.

The secondary plant metabolic compounds are important therapeutic alternatives that have become competitive with chemical treatments used in the treatment of many diseases, which would inhibit the work of microorganisms and their ability to grow, where the olive plant was selected because of its medical benefit against many diseases, as well as its deadly effect On some microorganisms because they contain the active compounds leading to that function (15).

**Diagnosis of chemical compounds in alcoholic extract using GC-MS technique.**

Figure (1) shows the results of the gas chromatography analysis - mass spectrometry of the alcoholic extract of olive leaves, where 12 compounds (Table 2), their molecular formulas, their molecular weight, the percentage of their area and retention time (min) were detected for each of these compounds. The designation of compounds was approved by the International Union of Pure and Applied Chemistry (IUPAC).

The results of the gas-mass spectrometry show the presence of many compounds of biological importance in the inhibition of microorganisms, including phenols that most studies indicate their role in the inhibition of bacteria and this was confirmed (15) in his study that proved the effectiveness of the phenolic compound against Staphylococcus aureus.

![Figure (1) Results of Gas Chromatography-Mass Spectrometry Analysis of Olive Leaf Extract.](http://doi.org/10.36295/ASRO.2020.23923)
Table (2) Types of Chemical Compounds Diagnosed with GC-MS for Olive Leaf Extract

<table>
<thead>
<tr>
<th>No.</th>
<th>R.T</th>
<th>Name (IUPAC)</th>
<th>Chemical Formula</th>
<th>M.W  g/mol</th>
<th>Peak Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.991</td>
<td>4-Methyl-N-acetyl</td>
<td>C₇H₈N₂O₃</td>
<td>168</td>
<td>5.09</td>
</tr>
<tr>
<td>2</td>
<td>8.943</td>
<td>2,3-dihydro 3,5-Dihydroxy-6-methyl-</td>
<td>C₆H₁₀O₄</td>
<td>144</td>
<td>2.16</td>
</tr>
<tr>
<td>3</td>
<td>10.328</td>
<td>2-Furaldehyde, 5- hydroxymethyl</td>
<td>C₆H₁₂O₃</td>
<td>126</td>
<td>16.02</td>
</tr>
<tr>
<td>4</td>
<td>11.520</td>
<td>4-Hydroxy-6-methoxy-2H-chromen-2-one</td>
<td>C₁₄H₁₂O₄</td>
<td>192</td>
<td>1.81</td>
</tr>
<tr>
<td>5</td>
<td>16.457</td>
<td>4-Pyridinecarboxaldehyde, 1-oxide</td>
<td>C₆H₈NO₂</td>
<td>123</td>
<td>11.38</td>
</tr>
<tr>
<td>6</td>
<td>16.991</td>
<td>3,4,4a,5,6,7,8,9-Octahydro-2H-</td>
<td>C₁₇H₁₆O</td>
<td>164</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>benzo[a]cyclohepten-2-one</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>17.215</td>
<td>Arachic acid</td>
<td>C₂₀H₄₀O₂</td>
<td>312</td>
<td>1.55</td>
</tr>
<tr>
<td>8</td>
<td>17.619</td>
<td>Methyl-3,4,4a,5,6,7,8,9-octahydro-2H-</td>
<td>C₁₃H₁₄O</td>
<td>178</td>
<td>4.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>benzo[a]cyclohept</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>17.733</td>
<td>3,5,6-trimethylpyrazine</td>
<td>C₁₅H₁₇N₃O₃</td>
<td>287</td>
<td>4.61</td>
</tr>
<tr>
<td>10</td>
<td>19.412</td>
<td>Hexadecanoic acid</td>
<td>C₂₂H₄₄O₄</td>
<td>256</td>
<td>18.33</td>
</tr>
<tr>
<td>11</td>
<td>21.181</td>
<td>Tetradecenoic acid</td>
<td>C₁₄H₂₆O₂</td>
<td>226</td>
<td>20.73</td>
</tr>
<tr>
<td>12</td>
<td>21.358</td>
<td>Nonadecanoic acid</td>
<td>C₁₉H₃₈O₂</td>
<td>298</td>
<td>3.70</td>
</tr>
</tbody>
</table>

**Determination of cellular toxicity of alcoholic extract of leaves of olive plant**

The alcohol extract of the leaves of the olive plant showed no toxicity of human red blood cells in concentrations (200, 100, 300) mg / ml.

**Effect of Olive Leaf Extract on Growth of Staphylococcus aureus**

The results of our current study showed that (100%) all isolates of Staphylococcus aureus were sensitive to olive leaf extract in concentrations (100, 200 and 400) mg / ml and the total rate of inhibition diameters (26, 18, 26) mm respectively, where the inhibition diameters of the alcoholic extract is higher than the inhibition diameters of the aqueous extract, so the alcoholic extract was taken in our study.
The results showed that the minimum inhibitory concentration (MIC) of olive leaf extract was at a concentration of (1/4) mg / ml.

**Effect of interaction of olive leaf extract with antibiotics against S. aureus isolates**

The results of the present study in Table (3) showed that the interaction of alcoholic olive leaf extract with antibiotics had an effect on increasing the isolation sensitivity to antibiotics if the rate of antidepressant diameters (GN, SXT, CRO, AK, VA, E, TE, RA, CIP, OX) with the extract was (15.5, 27.30) (24.5, 23, 22.5, 12, 26.5, 27.5, 26 mm. and when it was alone (20.5, 21.5, 20.0, 24, 23.5, 22. 0, 23.5, 26) mm respectively.

**Table 3: Effect of olive leaf extract with antibiotics on MRSA**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antibiotic</th>
<th>Inhibition of the extract at a concentration of 100 mg/m</th>
<th>Inhibition of antibiotic (mm)</th>
<th>Interaction of extract with antibiotic (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIP</td>
<td>25.5</td>
<td>23.5</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>OX</td>
<td>25.5</td>
<td>26</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>AK</td>
<td>25.5</td>
<td>Zero</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>SXT</td>
<td>25.5</td>
<td>21.5</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>CRO</td>
<td>25.5</td>
<td>20</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>GN</td>
<td>25.5</td>
<td>20.5</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>VA</td>
<td>25.5</td>
<td>24</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>25.5</td>
<td>Zero</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>25.5</td>
<td>23.5</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>TE</td>
<td>25.5</td>
<td>22</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

The results showed that there was a difference in the sensitivity of Methicillin-resistant Staphylococcus aureus isolates to antibiotics on the one hand, and their sensitivity when using antibiotics mixed with plant extract on the other. Theories that explain the ability of natural compounds to change the isolation sensitivity of antibiotics are intertwined with natural compounds, including the theory that some compounds have the ability to inhibit the mechanism of extracellular antagonist pumping (16th), supported this theory when note that some plants produce antimicrobial compounds that act as inhibitors of multiple resistance.
Conclusions:
The highest resistance to all S. aureus isolates was Methicillin and the lowest resistance was Ceftraxone; and Alcohol extract of olive leaves was found to be able to inhibit the growth of S. aureus bacteria at a concentration of 100 mg mm.

References


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