Antibacterial activity of Oak and Oak gall extracts
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ABSTRACT

Objective: The objective of this study was to evaluate the antibacterial activity of methanol extracts of oak, oak gall and whole fruit against number of Gram positive bacteria and Gram negative bacteria.

Methods: The fresh fruits extracts (Oak, oak gall and whole fruit) were separately prepared with methanol by standard procedure. The samples were stored in refrigerator at 4°C until use. The bacterial samples were taken from Microbiology laboratory at the University of Raparin, Ranya-Kurdistan Region-Iraq. The antibacterial activity of different extracts of oak, oak gall and whole fruit was determined by using disc diffusion method.

Results: The results of current study showed that all methanol extracts have effect on the growth of tested bacteria, except Gram negative bacteria. The study demonstrated that there was more significant effect on growth of gram positive bacteria (Staphylococcus sp, Streptococcus sp, Bacillus sp and Micrococcus sp) based on the inhibition zone diameter.

Conclusion: This study indicates that all extracts mainly whole fruit have high antibacterial activity, and may serve as natural alternatives antibiotic against variety of bacterial infections caused by multi drug resistance Gram positive bacteria. More studies are require to isolate the active antibacterial agents in oak and oak gall so that they could use in pharmaceutical purposes.

Keywords: Extracts, oak (Quercusbranti), oak galls (Quercusinfectoria), antibacterial activity

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INTRODUCTION

Herbal medicines with their active ingredients are regarded as origin of modern medicine [13]. The major source of medicines in both developing and industrialized countries is medicinal plants. The plants have therapeutic importance in the traditional Asian system of medicine are approximately 2000 species [14]. The oldest forms of medical treatment in human history are medicinal plants, and one of the indications of the modern pharmaceutical industry such as, medicines, health products, diet supplements, cosmetics are increasing globally. According to the World Health Organization (WHO), the plant components are provided approximately 11% of the medicines [8]. In addition, 25% of recent drugs have plant origins. There are many studies estimate that 75% people of the developing countries use medicinal plants for therapeutic purpose [6].

The therapeutic characteristics of herbal medicines are very beneficial in healing from bacterial and fungal diseases, because they contain phytochemicals with antioxidant activity and combat them for hundred years ago[1]. Microbial infections have been treated by a variety of antimicrobials [15]. However, increasing antibiotic resistance and their adverse effects on the human health over the past decades is a big problem in the world [5]. This made the researchers do a study on plants to discover new drugs as an alternative remedy [5,6].

Oaks tree (Quercusbranti) consists of tree and their fruits, grows in Iraq, Iran, Syria and Turkey [2]. Oak gall (Quercusinfectoria) cultivates in west Azerbaijan, Greece, and Kurdistan [6]. Oaks have approximately 600 species in the world [3]. The Oak and oak apple extractions of has the high rates of phenolic, tannin and flavonoids content [7]. A chemical compound of phenolic has broad ranges of medicinal characteristics, such as anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effect. In the late era, oak and oak gall used in the treatment of microbial infections such as impetigo, eczema, haemorrhages, chronic diarrhea and dysentery [6]. The plant of Quercusinfectoria and Quercusbranti in Kurdistan are mostly used as therapeutic plant. Oak and oak apple have effects on the gram positive and negative microbes as a natural resource [4]. They could be considered broad spectrum medicinal plant agents in the traditional medicine [1]. Furthermore, they can be used as antiseptic for burns, for the treatment of genital, and anal mucosal inflammation [5]. The objective of current study...
was to assess the antibacterial activity of methanol extracts of oak and oak gall against Gram-positive bacteria include *Staphylococcus sp*, *Micrococcus sp*, *Streptococcus sp*, *Bacillus sp*, and Gram-negative bacteria such as *Klebsiella sp* and *Escherichia coli*.

**MATERIALS AND METHODS**

**Preparation of Oak and Oak gall extracts**

The galls of *Quercus infectoria* and *Quercus branti* were collected from the Kurdistan, Iraq. The fresh oak and oak gall, whole fruit(oak and oak gall) were separated manually and rinsed with distilled water (D.W). The collected plants separately were dried in oven at 50 °C for 48 hours and grounded into fine powder using an electric blender. The powder (20 g) of oak, oak gall, and whole fruit extracts were separately placed in beaker and extracted with 100 ml of 99% methanol at room temperature for 24 hours. The clear extracts were filtered, and sterilized in oven. The samples were kept in refrigerator at 4°C.

**Bacteria and cultures**

The test microorganisms (*Staphylococcus sp*, *Micrococcus sp*, *Streptococcus sp*, *Bacillus sp*, *Klebsiella sp*, and *Eschericia coli*) were identified and taken from Microbiology laboratory at the University of Raparin, Ranya-Kurdistan Region-Iraq. The bacterial samples were sub-cultured and incubated at 37 °C for a day. The organisms were inoculated on Muller Hinton Agar. Sterile paper disks (5 mm diameter) were soaked with (20 μg /ml) of oak and oak apple extracts separately. Then the extraction discs were placed on the surface of agar plates of tested bacteria and incubated at 37 °C for 24 hours [16].

**Determination of antibacterial activity**

The antimicrobial activity of oak, oak gall and whole fruit extracts were performed by Kirby-Bauer method. The antibacterial activity was estimated by measuring the diameter of inhibition zone (DIZ) around disc of extractions.

**Statistical analysis**

All statistical analyses were performed statistical Package for Social Science (SPSS) V20. The results were expressed as mean and standard deviation. Statistically, the significant was calculated by ANOVA test (LSD). The mean differences were significant at the 0.05 level [17].

**RESULTS**

The results for methanolic oak, oak apple, whole fruit (oak and apples) extracts on tested bacteria have shown some variation based on diameter of inhibition zone (DIZ) values as showed in (tables 1-4). Gram-positive bacteria include *Staphylococcus sp*, *Streptococcus sp*, *Micrococcus sp* and bacillus sp were sensitive for all extracts. While, Gram-negative bacteria include *Klebsiella sp* and *E. coli* was resistance to all extracts.

Table 1: The Means and standard error for different types of methanolic extraction with control on *Staphylococcus sp*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>Mean Std. Error±</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak extract</td>
<td>30</td>
<td>*17.0000 .57735</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oak–gall extract</td>
<td>30</td>
<td>*22.0000 .57735</td>
<td>106.714</td>
<td>0.001</td>
</tr>
<tr>
<td>Whole fruit(Oak and oak- gall) extract</td>
<td>30</td>
<td>*29.0000 .57735</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30</td>
<td>*7.3333 1.45297</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Methanolic extraction of oak, oak gall and whole fruit extracts have significantly affected on the \textit{Staphylococcus sp}, statistical values were P.value <0.05. The symbol (*) means that the means values are significant at level of 0.05.

Table 2: The Means and standard error for different types of methanolic extraction with control on \textit{Streptococcus sp}.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>Mean ± Std. Error</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak extract</td>
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<td>120.750</td>
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<tr>
<td>Oak –gall extract</td>
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<td>*6.0000 .57735</td>
<td></td>
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<tr>
<td>Whole fruit(Oak and oak- gall) extract</td>
<td>30</td>
<td>*11.0000 .57735</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30</td>
<td>*23.3333 .88192</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Methanolic extraction of oak, oak gall and whole fruit extracts have significantly affected on the \textit{Streptococcus sp}, statistical values were P.value <0.05. The symbol (*) means that the means values are significant at level of 0.05.

Table 3: The Means and standard error for different types of methanolic extraction with control on \textit{Micrococcus sp}.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>Mean ± Std. Error</th>
<th>F</th>
<th>P-value</th>
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</thead>
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<tr>
<td>Oak extract</td>
<td>30</td>
<td>*17.6667 .66667</td>
<td>21.651</td>
<td>0.001</td>
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<tr>
<td>Oak gall extract</td>
<td>30</td>
<td>*25.0000 .57735</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole fruit(Oak and oak gall) extract</td>
<td>30</td>
<td>*23.3333 .88192</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30</td>
<td>*18.6667 .88192</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Methanolic extraction of oak, oak gall and whole fruit extracts have significantly affected on the \textit{Micrococcus sp}, statistical values were P.value <0.05. The symbol (*) means that the means values are significant at level of 0.05.
Table 4: The Means and standard error for different types of methanolic extraction with control on *Bacillus sp*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>Mean (Std. Error±)</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak extract</td>
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<td>*17.3333</td>
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<td></td>
</tr>
<tr>
<td>Oak gall extract</td>
<td>30</td>
<td>*21.0000</td>
<td>44.333</td>
<td>0.001</td>
</tr>
<tr>
<td>Whole fruit(Oak and oak gall)</td>
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<td>*17.6667</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30</td>
<td>*24.0000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Methanolic extraction of oak, oak gall and whole fruit extracts have significantly affected on the *Bacillus sp*, statistical values were P.value <0.05. The symbol (*) means that the means values are significant at level of 0.05.

Note: *Eschericia coli* and *Klebsiella sp* were resistant to all extractions.

**DISCUSSION**

The change in the inhibition zone of bacterial growth around the oak, oak apple, whole fruit (oak and gall) extracts discs are given in tables [1-4]. There were significant variations in the antibacterial activities of extracts. Our results indicated that all extracts affect the growth of Gram-positive bacteria. The current study is agreed with the study that showed that oak and oak gall extracts have strong antibacterial activity against Gram-positive bacteria [4]. Statistically, there was significant variation between values. For *Staphylococcus sp*, the mean values for oak, oak gall, and whole fruit were 17mm, 22mm, 29mm respectively, as shown in (table 1). While, for *Streptococcus sp*, the mean values for oak, oak gall, and whole fruit were 15mm, 6mm, 11 mm respectively, as given in (table 2). This supported by several studies that showed oak, and oak gall extracts were more effective on *Staphylococcus sp* than *Streptococcus sp* [6,11]. For *Micrococcus sp*, the mean values for oak, oak gall, whole fruit were 17 mm, 25mm, 23mm respectively, as shown in (table 3). Similarly, it has been stated that the oak and oak gall extracts have strong activity against *Micrococcus sp* [11]. Our results reported that oak and oak gall have strong antibacterial activity against *Bacillus sp* and the mean values for oak, oak gall, and whole fruit was 17 mm, 17mm,17 mm respectively, as shown in (table 4). There are several studies also showed that *Bacillus sp* were sensitive for oak and oak gall extracts [4,6].

The mean value of oak extracts on *Staphylococcus sp, Bacillus sp, and Micrococcus sp* was the same (17 mm), while it was 15 mm on *Streptococcus sp* as given in (tables 1-4). This means our result showed oak extracts have higher antibacterial activity against *Staphylococcus sp, Micrococcus sp, and Bacillus sp* compare to *Streptococcus sp*. Similar results were recorded in previous studies that demonstrated Gram-positive bacteria include *Staphylococcus sp, Micrococcus sp* and *Bacillus sp* were more sensitive than *Streptococcus sp* to the oak extracts [4,10,11].

The oak gall extracts were also more effective against *Bacillus sp, Staphylococcus sp, and Micrococcus compare to Streptococcus sp*. The mean value of oak gall extracts on *Bacillus sp Staphylococcus sp, and Micrococcus sp* were 21mm, 22mm, 25mm respectively, while it was 6mm on *Streptococcus sp*. This is supported by other studies showed that oak gall has high antibacterial activity on *Staphylococcus sp, Micrococcus sp and Bacillus sp* [6,12,13]. From our results as showed in (tables 1-4), both extracts of oak and oak gall separately showed similar effect against gram-positive bacteria. This agrees with numerous previous studies that exhibited Gram-positive bacteria were similarly sensitive to both oak and oak gall extracts [6, 11, 13]. In this study, among the extracts, whole fruit (oak and oak apple) extract were recorded the highest antibacterial activity against most of the tested Gram-positive bacteria. This is due to the presence high amount of antimicrobial compounds such as phenol and tannin in combination therapy. The all extracts were active against tested Gram-positive bacteria. This is may be because of Gram positive bacteria don’t have outer membrane [14].

CONCLUSION

The current study suggests that all extracts mainly the whole fruit extracts have a high antibacterial activity against most gram positive bacteria. Further studies are necessary to investigate the full clinical therapeutic agents in the oak and oak gall. This could be a suitable as natural alternatives antibiotic against infections by multidrug-resistant gram positive bacteria.

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REFERENCES