The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents

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**ABSTRACT**

Objective: To evaluate the antibacterial potential of aqueous and acetone extracts of galls of *Quercus infectoria* by determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values.

Materials and Methods: The extracts from the galls of *Q. infectoria* at 10 mg/ml were screened against three Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* and *Bacillus subtilis*) and three Gram-negative bacteria (*Escherichia coli* NCTC 12079 serotype O157:H7, *Salmonella typhimurium* NCTC 74 and *Pseudomonas aeruginosa* ATCC 27853). The MIC of the extracts were then determined using the twofold serial microdilution technique at a concentration ranging from 5 mg/ml to 0.0024 mg/ml. The MBC values were finally obtained from the MIC microtiter wells which showed no turbidity after 24 hrs of incubation by subculturing method.

Results: Out of the six bacterial species tested, *S. aureus* was the most susceptible. On the other hand, the extracts showed weak inhibitory effect against *S. epidermidis*, *B. subtilis*, *S. typhimurium* and *P. aeruginosa* while there was no inhibition zone observed for *E. coli* O157. The MIC values of the extracts ranged from 0.0781 mg/ml to 1.25 mg/ml whereas the MBC values ranged from 0.3125 mg/ml to 2.50 mg/ml. The MBC values of aqueous extract against *S. aureus* and *S. typhimurium* were higher than their MIC values. The MBC value of acetone extract against *S. aureus* was also higher than its MIC value. Interestingly, however, the MIC and MBC values of acetone extract against *S. typhimurium* were the same (1.25 mg/ml).

Conclusion: The aqueous and acetone extracts displayed similarities in their antimicrobial activity on the bacterial species and as such, the galls of *Quercus infectoria* are potentially good source of antimicrobial agents.

**KEY WORDS:** MIC, MBC, *Staphylococcus aureus*, tannin

**Introduction**

*Quercus infectoria* Olivier (Fagaceae) is a small tree native of Greece, Asia Minor and Iran. The galls arise on young branches of this tree as a result of attack by the gall-wasp *Adleria gallae-tinctoria.* The galls are locally known as *manjakani* in Malaysia, and are used in combination with other herbs as drinking remedy by women after childbirth to restore the elasticity of the uterine wall. *Majuphal*, as it is widely known in Indian traditional medicine have been used as dental powder and in the treatment of toothache and gingivitis. The galls of *Q. infectoria* have also been pharmacologically documented to possess astringent, antidiabetic, antitremorine, local anaesthetic, antiviral, antibacterial, antifungal, larvicidal and anti-inflammatory activities. The main constituents found in the galls of *Q. infectoria* are tannin (50-70%) and small amount of free gallic acid and ellagic acid.

As a result of indiscrimate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics. There is a need to develop alternative antimicrobial drugs. One approach is to screen local medicinal plants which represent a rich source of novel antimicrobial agents. The present study was carried out to investigate the antibacterial properties of the galls of *Q. infectoria* extracted by two solvents of different polarity.
Materials and Methods

Plant materials

The galls of *Q. infectoria* used in this study were obtained from the local market and were identified based on its physical characteristics. The galls were crushed to small pieces using pestle and mortar and powdered in an electric grinder.

Preparation of acetone extract

The acetone extract was prepared by immersing 100g of the dried material in 500 ml acetone for 24 h at room temperature. The mixture was then filtered and the process was repeated using the remaining residue with 300 ml acetone. The two filtrates were added and concentrated under reduced pressure using a rotary evaporator. The resulting pellet was finally pounded to dryness under hot air-dryer to produce a powdery crude acetone extract.

Aqueous extraction

In the preparation of aqueous extract, the powdered material was dissolved in distilled water for 24 h at 45°C and centrifuged at 3000 rpm at 4°C. The supernatant was then filtered and the whole process repeated using the remaining residue with 300 ml distilled water. The filtrates were combined and freeze-dried at -50°C under vacuum for 12 h to produce a fine crystal-like crude aqueous extract. The extracts were stored in air-tight jars at 4°C until further use.

Preparation of extract solution

The extracts were dissolved in sterile distilled water to a final concentration of 10 mg/ml for disc diffusion assay and a 5 mg/ml concentration for broth microdilution technique. The choice of dose concentration was based on our previous finding in which a crude antifungal extract isolated from sea cucumber species was capable of inhibiting the growth of filamentous fungi at 10 mg/ml. All the extracts were sterilized by passing through a 0.45 µm membrane filter.

Microorganisms

The bacterial strains used in this study were *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* NCTC 12079 (serotype O157:H7), *Salmonella typhimurium* NCTC 74 and local clinical isolates of *Staphylococcus epidermidis* and *Bacillus subtilis*. All the bacterial strains were grown and maintained on nutrient agar slants. The inoculum size of each test strain was 10^6 bacteria/ml for disc diffusion assay which was standardized by adjusting the optical density of the bacterial suspension to a turbidity corresponding to spectrophotometric absorbance = 0.08 (OD_{620} = 0.08) at 620 nm.

Screening for antibacterial activity

The disc diffusion method was used to evaluate the antibacterial activity. Mueller Hinton agar was prepared in the plates as the media for the test microorganisms. Sterile filter paper discs (Whatman No. 1, 6 mm) were impregnated with 100 µl of each of the extracts (10 mg/ml) to give a final concentration of 1 mg/disc and left to dry under the laminar flow cabinet overnight. The bacterial inoculum was spread evenly onto the surface of the Mueller Hinton agar plates using a sterile cotton bud before the extract discs were positioned on the inoculated agar surface. Each extract was assayed in triplicate. Sterile distilled water served as negative control. Gentamicin (10 µg/disc) was used as standard to confirm that all the microorganisms tested were inhibited by the antibiotic. All the plates were incubated for 24 h at 37°C. The antibacterial activity was interpreted from the size of the diameter of zone inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the discs.

Determination of MIC and MBC values

The minimum inhibitory concentration (MIC) of the extracts was determined for *S. aureus* and *S. typhimurium* using the twofold serial microdilution method with saline at a final concentration ranging from 5 mg/ml to 0.0024 mg/ml. The tested extracts were added to sterile Mueller Hinton broth into microtiter plates before the diluted bacterial suspension (final inoculum of 10^8 bacteria/ml) were added. Each extract was assayed in triplicate. The bacterial suspensions were used as positive control and extracts in broth were used as negative control. The MIC values were taken as the lowest concentration of the extracts in the wells of the microtiter plate that showed no turbidity after 24 hours of incubation at 37°C. The turbidity of the wells in the microtiter plate were interpreted as visible growth of the microorganisms. The minimum bactericidal concentration (MBC) was determined by subculture of the well showing no apparent growth in a sterile agar plate. The least concentration showing no visible growth on agar subculture was taken as MBC value.

Results

Determination of MIC and MBC

The MIC values of the aqueous and acetone extracts from the galls of *Q. infectoria* against *S. aureus* and *S. typhimurium* are shown in Table 1. The MIC values of the aqueous and acetic extracts were determined.

Table 1

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>S. aureus ATCC 25923</th>
<th>S. typhimurium NCTC 74</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous acetone</td>
<td>Aqueous acetone</td>
<td>Positive negative</td>
</tr>
<tr>
<td>5.0000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.5000</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1.2500</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0.6250</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0.3125</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.1563</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.0781</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.0391</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.0195</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>0.0098</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.0049</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.0024</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- Absence of growth, Positive Control: Bacterial suspensions and saline; + Presence of growth, Negative Control: Extracts and broth

Antibacterial Activity of the Galls of *Quercus infectoria*
etone extracts were the same (0.0781 mg/ml) against S. aureus, whereas the MIC values of aqueous and acetone extracts against S. typhimurium were 0.6250 mg/ml and 1.25 mg/ml, respectively. The lower MIC values of both the extracts against S. aureus in comparison to S. typhimurium suggests that S. aureus showed greater sensitivity towards the extracts of the Q. infectoria galls.

Table 2 shows the result of MBC of the aqueous and acetone extracts from the galls of Q. infectoria against S. aureus and S. typhimurium. The MBC value of the aqueous extract against S. aureus was higher (0.3125 mg/ml) compared to its MIC value of 0.0781 mg/ml. This was also observed for S. typhimurium in which the MBC values of the aqueous extract was also higher (2.5 mg/ml) compared to its MIC value i.e. 0.6250 mg/ml. As for the acetone extract against S. aureus, although its MBC value was also higher (0.1563 mg/ml) than its MIC value of 0.0781 mg/ml, it is interesting to note that the MIC and MBC values of the acetone extracts against S. typhimurium were the same (1.250 mg/ml). This means that the acetone extracts of the galls of Q. infectoria, may be considered bactericidal for S. typhimurium. As for S. aureus, both the aqueous and acetone extracts were bacteriostatic agents. The aqueous extracts, however, displayed consistent bacteriostatic activity against both bacterial species.

Discussion

Our study showed that the extracts from the galls inhibited the Gram-positive bacteria better than Gram-negative. Generally, plant extracts are usually more active against Gram-positive bacteria than Gram-negative bacteria. Our findings were also supported by other researchers who reported that the crude powder of the galls of Q. infectoria was found to be active against S. aureus and B. subtilis while both the methanol and aqueous extracts were active against S. epidermidis. In the present study, S. aureus, S. epidermidis and B. subtilis were also inhibited by the acetone extract. In addition to these bacterial strains, P. aeruginosa was also found to be susceptible to both the extracts tested. This is in accordance with a study that one of the most susceptible bacteria to the effect of the ethanol extract from the galls of Q. infectoria was P. aeruginosa. The range of MIC values for both S. aureus and S. typhimurium correlated well with the results obtained using the disc diffusion method. The MIC values for both extracts against S. aureus are lower when compared with Gram-negative bacteria. This shows that the Gram-positive bacteria is more susceptible to the effect of the extracts from the galls of Q. infectoria with respect to its Gram-negative counterpart. The MBC values were higher than the MIC values of the extracts against both the bacteria tested except for acetone extract from the galls of Q. infectoria. The MIC and MBC values of acetone extract were the same against S. typhimurium while the MBC values of both the extracts were 2-4 times greater than their MIC values for S. aureus. This suggests that the bioactive compound in the extracts of the galls of Q. infectoria, was bacteriostatic against S. aureus rather than bactericidal as reported previously.

It is well known that tannin is a phenolic compound which is soluble in water, alcohol and acetone, and gives precipitates with protein. The similarity in the antimicrobial activity of both the aqueous and acetone extracts suggest that these extracts may have high total tannin content. The antimicrobial activity seemed to depend on the contents of tannin in the plant extracts.

High amounts of tannin present in the galls of Q. infectoria implied that tannin may be the active compound which may be responsible for the antibacterial activity in this study. Tannin in plant extracts was found to possess antibacterial activity.

In conclusion, the extracts of the galls of Q. infectoria have high potential as antibacterial agent. This finding provides an insight into the usage of the galls of Q. infectoria in traditional treatment of wounds or burns associated with bacterial infections.

References


Table 2

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Extracts</th>
<th>Concentration of the extracts (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
<td>5.0000 2.5000 1.2500 0.6250 0.3125 0.1563 0.0781</td>
</tr>
<tr>
<td>S. aureus ATCC 25923</td>
<td>-</td>
<td>+ +</td>
</tr>
<tr>
<td>Acetone</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium NCTC 74</td>
<td>-</td>
<td>+ ND ND ND ND</td>
</tr>
<tr>
<td>Aqueous</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Acetone</td>
<td>-</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND =Not done because the microtiter well at the tested concentration showed the presence of growth as shown in Table 1.

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