Potential use of *Quercus infectoria* gall extracts against urinary tract pathogenic bacteria

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**ABSTRACT**

Galls of *Quercus infectoria* have been traditionally used by Malaysian women as a remedy after childbirth and also to prevent various infections including urinary tract infection (UTI). This study aimed to evaluate the *in vitro* antibacterial activity of *Q. infectoria* gall extracts against several bacterial pathogens of the urinary tract. Methanol and aqueous extracts of *Q. infectoria* galls were screened against 4 Gram-positive bacteria (*Staphylococcus saprophyticus* ATCC 49907, *Streptococcus agalactiae* ATCC 13813, *Streptococcus pneumoniae* ATCC 27336, and *Enterococcus faecalis* ATCC 29212) and 4 Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 1706, *Proteus mirabilis* ATCC 12453 and *Proteus vulgaris* ATCC 49132) using disc diffusion method. The minimum inhibitory concentrations (MIC) were determined using the two-fold serial micro dilution technique at concentrations ranging from 0.01 mg/ml to 10 mg/ml. Minimum bactericidal concentration (MBC) were determined by sub culturing the wells which showed no turbidity after overnight incubation at 37°C on the agar plate. Both extracts displayed similar inhibitory effects against each tested bacteria at a concentration of 5 mg/disc except for *P. vulgaris*, *E. coli* and *K. pneumoniae*. The extracts were also considered bactericidal against the tested bacteria (undetermined for *S. pneumoniae*) based on calculated MBC/MIC ratio. Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed that the major compound in both extracts was pyrogallol. These data suggested that *Q. infectoria* galls are potentially effective as an antibacterial agent for the prevention and treatment of UTI.

**Keywords:** *Quercus infectoria* gall extracts, Urinary tract pathogens, Antibacterial activity.

**INTRODUCTION**

Urinary tract infections (UTIs) are considered to be the most common type of bacterial infections affecting the urinary tract\(^1\). Annually, it is estimated that one billion women around the world suffer from UTI\(^2\). Women are likely to suffer from UTI and a higher risk occurs during pregnancy or following childbirth necessitating an appropriate antimicrobial therapy\(^3\). Acute uncomplicated UTI are infections of the lower urinary tract in healthy, non-pregnant and adult women. Pathogens that commonly contribute to the infection include *Escherichia coli*,...
Staphylococcus saprophyticus, Klebsiella pneumoniae, Proteus vulgaris, and Enterococcus spp. [4]. Resistance towards antibiotic is significantly increasing [2] and alternative method using medicinal herbs is one of option to overcome the problems. There are many claims about the beneficial effects of dietary supplements and natural products for the prevention of UTI [5]. Medicinal plants have been used in traditional health world wide as they are considered to be less toxic and free from side effects compared to the synthetic drugs [6]. Quercus infectoria galls, locally known as “Manjakani” is one of the popular medicinal plants used to treat various ailments. It is a deciduous, small tree or shrubs that grow only up to the height of 2 metres and is mainly found in Asia, Greece, and Iran [7]. The galls of Q. infectoria are round-shaped abnormal growth found arising on young branches of the oak tree due to the attack by the gall-wasp Adleria gallae-tintoria [8]. Pharmacologically, the galls have been documented to possess astringent and antibacterial properties [9-10]. The astringent properties are mainly derived from tannin, the main compound constituting 50-70% of Q. infectoria galls [11]. Tannin has displayed their antibacterial action by several studies [9, 12]. Galls of Q. infectoria also contain some sugar, starch [13] as well as gallic acid and ellagic acid [14]. In recent times, microorganisms developed resistance to many antibiotics due to the indiscriminate use of antimicrobial drugs in the treatment of infectious diseases [15]. Therefore, development of alternative antimicrobial agents is required, and local medicinal plants are considered the important sources of novel anti-microbial agents [16]. Extract of Q. infectoria galls contains natural antimicrobial properties [12] that are potentially effective in preventing the bacterial infection around the reproductive and urinary part of humans [17]. In the present study, methanol and aqueous extracts of Q. infectoria gall have been tested and compared for their antibacterial activities towards the commonly isolated urinary tract pathogens.

METHODOLOGY

Plant material
Q. infectoria Olivier gall was purchased from the local herbal shop in Kota Bharu, Kelantan and authenticated based on their physical appearances which were globular in shape, 0.8 cm to 2.5 cm in diameter, green-yellow in colour; odour is slight, strongly pungent taste and tuberculated surface [18]. The galls were washed with distilled water, left dry at room temperature before they were crushed and ground prior to the extraction.

Preparation of gall extracts
The methanol extract was prepared by immersing 100 g of the Q. infectoria gall powder in 500 ml of absolute methanol (Merck) for 72 hours at 50°C in water bath. The mixture was filtered using Whatman filter paper No 1 and the filtrates were concentrated under reduced pressure using a rotary evaporator at a temperature of 55°C. The resulting pellet was finally pounded to dryness at 50°C for 48 hours to produce a powdery and brown crude methanol extract.

The aqueous extract was prepared by immersing 100 g of the gall powder in 500 ml of sterile distilled water for 72 hours at 50°C in water bath. The mixture was then pre-filtered using coffee filter and then filtered using Whatman filter paper No 1. The filtrates were concentrated under reduced pressure using a rotary evaporator at a temperature of 80°C. The resulting pellet was freeze-dried at -50°C under vacuum until a fine crystal-like crude aqueous extract was obtained. The crude extracts were stored in airtight jars at 4°C until further use.

The extracts were dissolved in sterile distilled water to a final concentration of 100 mg/ml for disc diffusion technique and 20 mg/ml for broth micro dilution technique with slight modification [12]. The mixture was left to dissolve on rotary mixer. Prior to antimicrobial activity assays, the diluted extract solutions were sterilized through membrane filter size 0.2 µm. Blank discs (Oxoid) were impregnated with the desired volume of each extract solution to a final concentration of 5.0 mg/disc and allowed to dry in sterile condition.

Microorganisms and preparation of inoculum
Eight ATCC strains of bacterial species were selected in this study which comprised of a few Gram-positive bacteria (S. saprophyticus ATCC 49907, S. agalactiae ATCC 13813, S. pneumoniae ATCC 27336 and E. faecalis ATCC 29212) and Gram-negative bacteria (K. pneumoniae ATCC 1706, E. coli ATCC 25922, P. mirabilis ATCC 12453 and P.
vulgaris ATCC 49132). The Gram-positive and Gram-negative bacteria were subcultured and maintained onto blood agar (Difco) and MacConkey agar (Difco) respectively. The suspension of each strain was prepared using colonies from an overnight culture plate at a concentration of $10^7$ cells/ml or McFarland equivalent of 0.5 (standard test inoculums) for disc diffusion test and microbroth dilution assay.

**Antibacterial activity**

The procedures described for disc diffusion test and determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were according to the standard antimicrobial susceptibility method provided by Clinical and Laboratory Standards Institute.[19] The disc diffusion test was performed by streaking the standard test inoculum onto the surface of Mueller Hinton agar (MHA) or MHA with 5% sheep’s blood (for *S. pneumoniae* and *S. agalactiae*) using a sterile cotton swab. Extract discs were positioned on the inoculated agar surface along with negative and positive control within 15 minutes of inoculation. Disc impregnated with sterile distilled water was used as negative control while the standard antibiotic disc was used as positive control. The test was done in triplicate. All plates were incubated at 37°C for 18-24 hours. The antibacterial activity was observed from the size of the inhibition zone diameter surrounding the disc measured in millimeters (mm).

The MIC values of each extract against the bacterial strains were determined using two-fold serial micro dilution of extracts in Mueller Hinton broth (MHB) with concentration ranging from P 0.01 mg/ml to 10 mg/ml. For *S. pneumoniae* and *S. agalactiae*, MHB supplemented with 5% of Laked Horse Blood (Oxoid SR0048C) (MHBB) was used instead. The diluted bacterial suspensions (final inoculums: $5 \times 10^5$ bacteria/ml) were added to each well and incubated at 37°C for 20-24 hours. Each test was assayed in triplicate. The bacterial suspension was used as positive control and MHB or MHBB was 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 20-24 hours of incubation at 37°C. Subsequently, wells with no turbidity were subcultured onto blood agar plates for MBC determination.

**Phytochemical analysis**

The phytochemical qualitative tests were carried out on the methanol and aqueous *Q. infectoria* gall extracts to detect the presence of tannin, saponin, flavonoids and alkaloid using standard procedures[20-21] for determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were according to the standard antimicrobial susceptibility method provided by Clinical and Laboratory Standards Institute.[19] The disc diffusion test was performed by streaking the standard test inoculum onto the surface of Mueller Hinton agar (MHA) or MHA with 5% sheep’s blood (for *S. pneumoniae* and *S. agalactiae*) using a sterile cotton swab. Extract discs were positioned on the inoculated agar surface along with negative and positive control within 15 minutes of inoculation. Disc impregnated with sterile distilled water was used as negative control while the standard antibiotic disc was used as positive control. The test was done in triplicate. All plates were incubated at 37°C for 18-24 hours. The antibacterial activity was observed from the size of the inhibition zone diameter surrounding the disc measured in millimeters (mm).

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**Statistical Analysis**

Data entry and statistical analysis were performed using IBM SPSS software version 20. Student’s t-test was used for statistical comparison of zone sizes between methanol and aqueous extracts and p-values < 0.05 were regarded as significant.

**RESULTS**

Table 1 shows the results of antibacterial activity of both extracts against urinary tract bacterial species obtained by screening disc diffusion test. The antibacterial activity of most species was moderately observed at optimal concentration of 5 mg/disc. Methanol extract exhibited inhibitory effects on both Gram-positive and Gram-negative bacteria species while aqueous extract inhibited the growth of most tested bacteria except *E. coli* and *K. pneumoniae*. Among all bacteria, both extracts displayed largest inhibitory zone sizes against *S. saprophyticus*. The results of MIC and MBC were summarized in Table 2. The MIC values ranged from 0.31 mg/ml to 2.50 mg/ml for methanol extract and from 0.16 mg/ml to 10.00 mg/ml for aqueous extract. The
MBC/MIC ratio for both extracts against all tested bacterial species were less than or equal to 4 except *S. pneumoniae*. MIC determination was not feasible for *S. pneumoniae* due to the intense background colour of the extract and MHBB that masked microbial growth observation.

Preliminary qualitative phytochemical screening for both methanol and aqueous gall extracts showed positive detection of tannins (Table 3). Following phytochemical screening, GC-MS analysis showed two major compounds scanned by the NIST05a.L database. Figure 1 shows the active principle, retention time (RT), molecular weight and molecular structure of the compound. 1, 2, 3 - Benzenetriol (pyrogallol) in both methanol and aqueous extracts of *Q. infectoria* gall was identified in high percentage which accounted for 81.66% and 100% of the total respectively.

**Table 1: Antibacterial activity of *Q. infectoria* gall extracts by disc diffusion test**

<table>
<thead>
<tr>
<th>Species</th>
<th>Inhibition zone diameter (mm±SEM)*</th>
<th>Positive control (10 µg/disc)</th>
<th>Methanol extract (5.0 mg/disc)</th>
<th>Aqueous extract (5.0 mg/disc)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. mirabilis</em> ATCC</td>
<td>20.67±1.15</td>
<td>14.33±0.57</td>
<td>13.33±0.57</td>
<td>0.101</td>
<td></td>
</tr>
<tr>
<td><em>P. vulgaris</em> ATCC</td>
<td>18.67±0.57</td>
<td>17.00±0.00</td>
<td>15.67±0.57</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> ATCC</td>
<td>18.33±0.57</td>
<td>12.33±0.57</td>
<td>-</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae</em> ATCC</td>
<td>17.00±0.00</td>
<td>9.33±0.57</td>
<td>-</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td><em>S. saprophyticus</em> ATCC</td>
<td>26.00±1.00</td>
<td>18.00±0.00</td>
<td>17.00±1.00</td>
<td>0.158</td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em> ATCC</td>
<td>17.67±0.57</td>
<td>13.67±1.15</td>
<td>12.67±1.15</td>
<td>0.348</td>
<td></td>
</tr>
<tr>
<td><em>S. agalactiae</em> ATCC</td>
<td>18.67±0.57</td>
<td>11.33±0.57</td>
<td>11.00±0.00</td>
<td>0.373</td>
<td></td>
</tr>
<tr>
<td><em>S. pneumoniae</em> ATCC</td>
<td>21.00±0.00</td>
<td>12.00±0.00</td>
<td>11.33±0.57</td>
<td>0.116</td>
<td></td>
</tr>
</tbody>
</table>

*Mean values of three determinations, each from different plates
* Student’s t-test for comparison of zone sizes diameter between methanol and aqueous extracts

ATCC: American Type Culture Collection

- No inhibition

**Table 2: MIC and MBC values of *Q. infectoria* gall extracts against tested bacterial species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Methanol Extract</th>
<th>Aqueous Extract</th>
<th>MBC/MIC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/ml)</td>
<td>MBC (mg/ml)</td>
<td>MBC/MIC ratio</td>
</tr>
<tr>
<td><em>P. mirabilis</em> ATCC</td>
<td>0.63</td>
<td>0.63</td>
<td>1</td>
</tr>
<tr>
<td><em>P. vulgaris</em> ATCC</td>
<td>0.31</td>
<td>0.63</td>
<td>2</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC</td>
<td>1.25</td>
<td>2.50</td>
<td>2</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> ATCC</td>
<td>5.00</td>
<td>5.00</td>
<td>1</td>
</tr>
<tr>
<td><em>S. saprophyticus</em> ATCC</td>
<td>0.63</td>
<td>0.63</td>
<td>1</td>
</tr>
<tr>
<td><em>E. faecalis</em> ATCC</td>
<td>0.63</td>
<td>1.25</td>
<td>2</td>
</tr>
<tr>
<td><em>S. agalactiae</em> ATCC</td>
<td>0.63</td>
<td>2.50</td>
<td>4</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> ATCC</td>
<td>ND</td>
<td>2.50</td>
<td>ND</td>
</tr>
</tbody>
</table>

ATCC: American Type Culture Collection

ND: Not determined
Table 3: Phytochemical screening of *Q. infectoria* gall extracts

<table>
<thead>
<tr>
<th>Compound</th>
<th>Methanol Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+: detected, -: not detected

**DISCUSSION**

UTI is among the most common bacterial infections that lead patients to seek medical care. Besides antibiotic consumption, other alternative measures such as the use of herbal plant has been one of the promising therapeutic approach to human disease \(^{(22)}\) as well as providing safe, cheap and effective antibacterial treatment \(^{(23)}\).

Various solvents are available and can be used to extract the active compounds from this plant. Aqueous solvent was used in this study because water is a universal solvent which was used traditionally to extract most plant products with antimicrobial activity \(^{(24)}\). Organic extract of plants was reported to have high antimicrobial activity \(^{(7, 25)}\) and relatively low toxicity to the test organisms \(^{(26)}\). Therefore we compared both extracts to establish their potential inhibitory effects on the commonly isolated species of uropathogens.

In this study, it is interesting to observe that the growth of *S. saprophyticus* was largely inhibited by both extracts. Methanol extract of *Q. infectoria* galls has also been reported to have higher inhibition on microorganisms \(^{(15, 27)}\). We found them ethanol extract displayed relatively better inhibitory activity towards all tested urobacterial species and significantly
inhibited the growth of the Gram-negative bacteria compared to the aqueous extract. For the aqueous extract, a relatively weak antibacterial activity was observed for both *E. coli* and *K. pneumoniae*. The MIC values of *E. coli* and *K. pneumoniae* were comparatively higher (10.0 mg/ml) than the MIC observed in other Gram-negative and Gram-positive bacteria. *E. coli* and *K. pneumoniae* are known as highly resistant [28, 29] as compared to the Gram-positive bacteria due to the difference in cell wall content and arrangement [30]. Previous research studying on antibacterial activity of *Q. infectoria* gall extract had also reported that Gram-positive bacteria were relatively more susceptible compared to Gram-negative bacteria [12].

MBC is one of various in vitro microbiological techniques to determine the bactericidal activity of antimicrobial agents. Microbiological definition of bactericidal activity has been taken arbitrarily as a ratio of MBC to MIC of 4 or less [31, 32]. In this study, the MBC values of the extracts ranged from 0.31 mg/ml to 10.00 mg/ml. Both extracts were regarded as bactericidal against all tested bacterial species based on the calculated MBC/MIC ratio. Previous study also reported similar findings when the extracts were tested against Gram-positive bacteria [17]. Therefore *Q. infectoria* gall extracts might be potentially considered as a bactericidal agent. A cidal activity would be preferable [33] to treat urinary bacterial infections.

The phytochemical screening analysis in this study has identified tannins in both extracts. Tannins which is the main component found in *Q. infectoria* [11] are soluble in polar compound and water [34] thus it is possible that the active constituents were available in both extracts. Tannin may prevent bacteria from adhering to cells because it is believed that the hydrolysable tannins contain structures similar to the bacterial-binding receptors found on the surface of urinary tract cells and thereby preventing adherence of the bacteria to the cell surface receptors [35]. Without binding to the cells, the bacteria cannot multiply and this is apparently necessary to cause a bacterial infection. The effect of tannins on microbial metabolism can be measured by their action on membranes which they can cross the cell wall, composed of several polysaccharides and proteins, and bind to its surface [36].

Pyrogallol is known as one of hydrolysable tannin [37] and has been found as the major compound of *Q. infectoria* galls in this study. The presence of hydroxyl groups and alpha-beta double bonds in a phenolic compound (Figure 2) plays an important role towards the antimicrobial activity [39]. Those activities were possibly triggered by the presence of three hydroxyl groups in the structure which eventually affects the biosynthesis of cell wall and cell membrane [40]. Changes in the permeability of cell membrane could cause a decrease in cell volume, thus abnormalities may attribute to cell membrane alterations [41].

However, the use of tannins or pure bioactive compounds in elucidating maximum antimicrobial activity is doubtful because it has been found that whole herbal extracts are more effective than isolated Phytochemicals due to a synergistic effect among the phytochemical components [42].

In conclusion, extracts of *Q. infectoria* galls hold antimicrobial potential, which might be further explored in the treatment and control of some bacterial infections. It is intriguing to note that crude extracts of this medicinal plant showed good antimicrobial activity against the most commonly isolated species in UTI. Further studies are needed to develop a standardized *Q. infectoria* gall extract and to understand its mechanism of antibacterial activity.
ACKNOWLEDGEMENTS
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