Studies on the Antibacterial Activity of Quercus Infectoria Galls

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Abstract—Extract of Quercus infectoria galls was examined for its antimicrobial activity against a wide variety of pathogenic bacteria. The antimicrobial activity of Q. infectoria was examined using different solvents of varying polarity and efficacy was compared. Antimicrobial activity was carried out by the disc diffusion method. Crude extracts of the solvents varied in zones of inhibition. All the Gram-positive bacteria and Gram-negative bacteria were susceptible to all aqueous and solvent extracts of Q. infectoria galls. The antimicrobial activity of the methanol extract was superior to all other extracts. Ethanol and aqueous extracts showed strong antimicrobial effect against all the tested organisms. Chloroform and hexane extracts of Q. infectoria were found to be low active against most of the tested organisms. Compared to the commercial antibiotics, all the extract exhibited a good antimicrobial activity. Scanning electron microscopy (SEM) was used to determine the effect of the extracts on morphological changes of test microorganisms.

Keywords—antimicrobial activity; Quercus infectoria galls; pathogenic bacteria; morphology; scanning electron microscopy

I. INTRODUCTION

The evolution of bacterial resistance to currently available antibiotics has necessitated the research for novel and effective antimicrobial compounds. It is known that local plants have medicinal properties and this has made traditional medicine cheaper than modern medicine. Globally, plant extracts are employed for their antimicrobial, antifungal and antiviral activities. In particular, the antimicrobial activity of plant extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, and alternative medicine. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it.

Quercus infectoria Olivier (Fagaceae) is a small tree or a shrub mainly present in Greece, Asia Minor, Syria and Iran. The galls of Quercus infectoria have also been pharmacologically documented to possess astringent, antibacterial [1], antifungal [2], larvicidal [3], antidiabetic [4], local anaesthetic [5], antiviral [6], and anti-inflammatory [7] 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 [8-9]. Tannins are commonly defined as water–soluble polyphenolic compounds ranging in molecular weight from 500 to 3000 Daltons that have the ability to precipitate proteins [10].

Due to the fact that this plant is very useful, as found by the mentioned researches, there is a need to find out more about the potentiality of this plant as an antimicrobial agent. The present study is designed to assess the potency of different solvent extract of Q. infectoria galls on Gram-positive and Gram-negative bacteria. We also investigate the effect of the extracts on the morphological changes of the test pathogen bacteria by using scanning electron microscope.

II. MATERIAL AND METHOD

A. Test microorganisms

Tested pathogenic bacteria comprised of Escherichia coli ATCC 25922, Bacillus subtilis, and Staphylococcus aureus ATCC 25923. The bacteria were maintained by subculturing periodically on nutrient agar and were preserved at 4 °C prior to use.

B. Culture medium

The nutrient agar was prepared by dissolving 5 g peptone, 1.5 g beef extract, 1.5 g yeast extract, 5 g NaCl and 20 g agar in 1000 ml distilled water and boiling the solution. The pH was adjusted to 6.4–6.8 and sterilized by autoclaving at 15 psi pressure (121 °C) for 20 min. Sterilized petriplates were prepared with an equal thickness of nutrient agar. Test bacteria were grown overnight at 37 °C, 120 rpm in 10 ml nutrient broth. This broth was used for seeding the agar plates.

C. Plant materials

The galls of Q. infectoria were obtained commercially from a Thai traditional drug store. All samples were washed with distilled water, cut into small pieces and dried at 60 °C overnight. They were crushed in a mechanical mortar before extraction (Fig 1).

D. Extraction conditions

Extracts of dried plant materials were prepared by using solvents of varying polarity. The dried plant materials of 5 g each were extracted by maceration in solvents (25 ml) for 5 day at room temperature in dark. After the plant materials were successively extracted with methanol, ethanol, hexane,
chloroform, and distilled water separately, the extract was filtered through Whatman filter paper IV. The solvent was then distilled under reduced pressure in a rotary evaporator until it became completely dry. The weight of the solid residue was recorded and taken as yield of crude extracts. Extracts were stored at -20°C and were freshly dissolved in 10% dimethyl sulfoxide (DMSO, Merck, Germany) before use. The corresponding concentration was expressed in term of mg of extract per ml of solvent (mg ml⁻¹).

E. Antibacterial assay

Antimicrobial activity was tested by the disc diffusion method. The 50 µl of the extracts was impregnated onto a small disc of filter paper (diameter 6.0 mm) and placed on top of the seeded medium. Each disc containing 5 mg ml⁻¹ extract were tested against E. coli, S. aureus, and B. subtilis. The antibacterial assay plates were incubated at 37°C for 24 h. The control experiment was carried out to compare the diameter zone of clearing from the extracts and already standardized antibiotics. The antibiotics used were Tetracycline, and Kanamycin. The standard discs of the antibiotics tetracycline (10 µg per disc) and kanamycin (10 µg per disc) served as positive antibacterial control. The diameter of the zones of inhibition around each of the discs (disc diameter included) was taken as measure of the antibacterial activity. The diameters of the zones of inhibition by the samples were then compared with the diameter of the zone of inhibition produced by the standard antibiotic disc used. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

F. Morphological observations by scanning electron microscope (SEM)

Cells of each strain at a logarithmic phase in nutrient agar were treated with the extracts for 12 h. The bacterial cells treated with 10% DMSO were used as control. The cells were collected by centrifugation and washed with the sodium phosphate buffer. The samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, at 4°C overnight, and postfixed in 1% osmium tetroxide in the phosphate buffer for 1 h at room temperature before processing for scanning electron microscope.

III. RESULTS AND DISCUSSION

A. Yield of extracts

The solvents with their increasing order of polarity were used for the extraction of Q. infectoria galls. The percent yields of the extracts are shown in Fig. 1. The percentage yield of extracts for Q. infectoria was: methanol (52), ethanol (42), water (30) hexane (2), and chloroform (2). Methanol extracted the most material followed by ethanol and aqueous extracts. The extracts of chloroform and hexane were similar quantities.

B. Antimicrobial test

The antimicrobial activity of five solvent extracts of Q. infectoria galls was studied by the disc diffusion method and the results are shown. (Table 1, Fig. 2-4). All the solvent extracts showed significant inhibitory activity. Inhibition was observed against all pathogenic bacterial strains. Methanol, ethanol and aqueous extracts showed higher activity than chloroform and hexane extracts and produced inhibition zones ranging from 19.0 to 25.3 mm in diameter at a concentration of 5 mg ml⁻¹. Methanol extract displayed excellent activity against Gram-positive B. subtilis (25.3 mm), S. aureus (22.0 mm) and Gram-negative E. coli (20.3 mm). Ethanol extracts also showed strong activity against Gram-positive B. subtilis (24.0 mm), S. aureus (20.0 mm) and Gram-negative E. coli (19.0 mm). Aqueous extracts also showed strong activity against Gram-positive B. subtilis (21.0 mm), S. aureus (18.0 mm) and Gram-negative E. coli (15.7 mm). Chloroform and hexane extracts showed mild to moderate activity against all of the tested bacteria. Chloroform extracts were found to be active against Gram-
positive *B. subtilis* (13.0 mm), *S. aureus* (12.7 mm) and Gram-negative *E. coli* (10.3 mm). Hexane extracts were found to be active against Gram positive *B. subtilis* (12.0 mm), *S. aureus* (11.3 mm) and Gram-negative *E. coli* (10.0 mm). The results were compared with those of Tetracycline, and Kanamycin as a standard antibiotic. On overall consideration, of alcoholic extract were higher as compared to those of other extracts extracted from *Q. infectoria* galls. In general, higher activity was observed against Gram-positive bacteria.

TABLE I. **ANTIBACTERIAL ACTIVITIES OF DIFFERENT EXTRACTS OF Q. INFECTORIA GALLS**

<table>
<thead>
<tr>
<th>Solvent</th>
<th><em>E. coli</em> Diameter of inhibition zone (mm) ± S.D.</th>
<th><em>S. aureus</em> Diameter of inhibition zone (mm) ± S.D.</th>
<th><em>B. subtilis</em> Diameter of inhibition zone (mm) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>20.3 ± 0.8</td>
<td>22.0 ± 0.7</td>
<td>25.3 ± 0.4</td>
</tr>
<tr>
<td>Ethanol</td>
<td>19.0 ± 0.7</td>
<td>20.0 ± 0.7</td>
<td>24.0 ± 0.7</td>
</tr>
<tr>
<td>Distilled water</td>
<td>15.7 ± 0.4</td>
<td>18.0 ± 0.7</td>
<td>21.0 ± 0.7</td>
</tr>
<tr>
<td>Chloroform</td>
<td>10.3 ± 0.9</td>
<td>12.7 ± 0.4</td>
<td>13.0 ± 0.7</td>
</tr>
<tr>
<td>Hexane</td>
<td>10.0 ± 0.7</td>
<td>11.3 ± 0.8</td>
<td>12.0 ± 0.7</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>19.0±0.6</td>
<td>28.9±0.4</td>
<td>20.5±0.6</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>18.6±0.8</td>
<td>18.7±0.5</td>
<td>26.3±0.5</td>
</tr>
</tbody>
</table>

*Mean value of three determinations, each from a different plate*

Results of this study demonstrated that the Gram-positive bacteria were more susceptible to the extracts than Gram-negative bacteria such as *E. coli* exhibited more resistant than *S. aureus* and *B. subtilis* when they were tested with *Q. infectoria* extract. The reason would be that lipopolysaccharide (LPS) layer of Gram-negative bacteria in outer membrane having high hydrophobicity and acts as a strong barrier against hydrophobic molecules [11]. It can pass through cell wall of Gram-positive bacteria easier than the Gram-negative bacteria because cell wall of the Gram-positive contained peptidoglycan and lack of outer membrane [12]. High amounts of tannin present in the galls of *Q. infectoria* implied that tannin is the active compound for the antibacterial activity in this study [12-13]. Tannin is a phenolic compound which is soluble in water, alcohol and acetone, and gives precipitates with protein [11, 13]. The similarity in the antibacterial activity of both the alcohol and aqueous extracts suggests that the extracts may have high total tannin content.
C. Scanning electron microscopic observation at 24h

The effects of the methanol extracts of *Q. infectoria* galls on the surface morphology of Gram-positive and Gram-negative bacteria during its logarithmic phase of growth were showed in Fig. 5-7. The spectrum of antimicrobial activity on the surface morphology of individual cells and bacterial populations was visualized.

The effects of the methanol extracts on the surface morphology of *E. coli* were generally similar. Untreated organisms (Fig. 5a) appeared rod-shaped. Exposure to the extract resulted in only occasional morphologic defects characterized by tubular outpouchings from the cell wall (Fig. 5b).

The effects of the methanol extracts on the surface morphology of *S. aureus* during its logarithmic phase of growth were similar under the experimental conditions utilized. Untreated staphylococci (Fig. 6a) appeared to be smooth and spherical in grapelike clusters. Exposure to the extracts resulted in the appearance of small bleb-like structures on the surface of occasional cells; irregular spherical structures lying free or appearing to extrude from cells were also observed (Fig. 6b).

The effects of the methanol extract on the surface morphology of *B. subtilis* were generally similar. Untreated organism (Fig. 7a) appeared rod-shaped. Exposure of this organism to the extract resulted in morphological abnormality. Formation of spherical forms and collapse resulted in structures of the treated cells.
IV. CONCLUSION

The results obtained from this study reveal that *Quercus infectoria* galls have antimicrobial activity against Gram-positive and Gram-negative bacteria. In this study, it was observed that the potency of this medicine plant was enhanced by the type of solvent used, indicating that some of the active materials in *Q. infectoria* dissolve well in alcohol and water than in hexane or chloroform. All extracts from the galls inhibited the Gram-positive bacteria better than Gram-negative bacteria. This study corresponds with the results of other researchers who observed that the alcohol extracts of *Q. infectoria* was found to be more active against all bacteria studied during present work. The present studies demonstrate visually the nature and spectrum of the effect of the extracts on the surface morphology of specific bacteria. The utilization of the scanning electron microscope may help to correlate these various morphologic forms with biochemical alternations occurring within the cell wall.

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