

## Antibacterial Activity of The Galls of *Quercus infectoria*

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**ABSTRACT** In this study, the galls of *Quercus infectoria* were finely ground and extracted successively with petroleum ether, ethyl acetate and ethanol. Successive extraction with acetone followed by methanol was also carried out in addition to preparation of aqueous extraction. The extracts were screened against 3 Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *S. epidermidis* and *Bacillus subtilis*) and 4 Gram-negative bacteria (*Escherichia coli* O157:H7, *Salmonella typhimurium* NCTC 74, *S. enteritidis* NCTC 5188 and *Pseudomonas aeruginosa* ATCC 27853). The antibacterial testing was carried out by using the disc diffusion method. All extracts showed moderate activity against all the Gram-positive bacteria; methanol extract exhibiting the highest inhibition zone diameter of 15.84 mm  $\pm$  0.56 mm against *Staphylococcus aureus*. Among the Gram-negative bacteria tested, *E. coli* O157:H7 appeared to be the most resistant towards the extracts.

**ABSTRAK** Dalam kajian ini, biji dari *Quercus infectoria* telah dikisar halus dan diekstrak secara berturut-turut dengan petroleum eter, etil asetat dan etanol, dan secara berasingan menggunakan aseton dan metanol. Di samping itu, ekstrak akueus juga disediakan. Kesemua ekstrak tersebut diuji terhadap tiga bakteria Gram-positif (*Staphylococcus aureus* ATCC 25923, *S. epidermidis* dan *Bacillus subtilis*) serta empat bakteria Gram-negatif (*Escherichia coli* O157:H7, *Salmonella typhimurium* NCTC 74, *S. enteritidis* NCTC 5188 dan *Pseudomonas aeruginosa* ATCC 27853). Ujian antibakteria dijalankan dengan menggunakan kaedah resapan disk. Semua ekstrak menunjukkan aktiviti sederhana terhadap semua bakteria Gram-positif; ekstrak metanol menunjukkan diameter zon perencatan yang paling tinggi iaitu 15.84 mm  $\pm$  0.56 mm terhadap *Staphylococcus aureus*. Di antara bakteria Gram-negatif yang dikaji, *E. coli* O157:H7 didapati yang paling resistan terhadap ekstrak tersebut.

(*Quercus infectoria*, antibacterial, galls, tannin)

### INTRODUCTION

The galls of *Quercus infectoria* or locally known as 'biji manjakani' arise on young branches of this tree (family Fagacea) as a result of attack by the gall-wasp *Cynips gallae-tinctoria* [1,2]. Galls contain hydrolysable tannins or tannic acid as the principal constituent from 50 % to 70 % [3,4,5]. It is termed gallotannic acid to distinguish it from other varieties of tannic acid [3]. They also contain 2% - 4% gallic acid, ellagic acid, starch, sugar and resin [3,4,5]. Galls of *Quercus infectoria* are also known commercially as Aleppo galls or Turkey galls because they are collected in Asiatic Turkey, especially in the province of Aleppo [3]. They are also referred to as oak galls, nutgalls, Majuphal (India) and in Malaysia, the galls are widely used in Malay traditional medicine as pessary particularly after

childbirth to stimulate the contraction of the vaginal muscles as well as to treat vaginal infections [6]. The galls are usually applied as a local astringent in the form of ointment [3] for burns and dermatitis [5]. It is also a hemostatic agent [5] and useful in cases of anal fissures and hemorrhoids [2]. The gall has been widely used in Indian traditional medicine for various ailments such as diarrhea, dysentery, internal hemorrhages, gonorrhoea, leucorrhoea, menorrhagia, [1]. Traditional usage of the galls of *Q. infectoria* in treatment of various diseases has prompted scientists to investigate the claims of its effectiveness and other therapeutic potential. It was reported that the methanol extract from the galls of *Q. infectoria* significantly reduced blood sugar levels in rabbits [8]. Tannin and other related compounds such as polygalloylglucoses have been isolated from these galls and

characterized [9] and the antimicrobial properties of tannins have been studied [10]. The antiviral activities of these galls have also been investigated. It was found that the methanol and water extracts of the galls have inhibitory effects on hepatitis C virus protease [11]. In another study to identify the major compound possessing hypoglycemic activity, hexagalloylglucose which was isolated from the methanol extract of the galls of *Q. infectoria* significantly inhibited  $\alpha$ -glucosidases [12]. Molluscicidal activity was evaluated and the acetone extract and gallotannin of these galls have presented high activity against *Bulinus truncatus* [13]. Redwane *et al.* (2002) studied the efficacy of extracts and fractions of *Q. infectoria* against the larvae of *Culex pipiens* and reported the extracts have interesting larvicidal activity [14].

The present study reports on the preliminary screening for antibacterial activity of five extracts from the galls of *Q. infectoria* against seven bacterial strains.

## MATERIALS AND METHODS

### Plant materials

The galls of *Q. infectoria* were obtained from the local market. The galls were identified and verified by Associate Professor Dr. Kamarudin Mat Salleh from Faculty of Science and Technology, Universiti Kebangsaan Malaysia and voucher specimens were deposited in the Herbarium at the Universiti Kebangsaan Malaysia. The galls were crushed to small pieces using pestle and mortar before powdered using an electric grinder.

### Preparation of extracts

The dry plant material was extracted using six solvents with different degree of polarity; petroleum ether (Fisher Scientific), acetone (J.T. Baker, USA), ethyl acetate (Searle Company Hopkin & Williams), ethanol (Chemical Industries, Malaysia), methanol (Scharlau Chemie S.A., Barcelona, Spain) and distilled water. For each gram of dry material, 5 ml of the respective solvent was used (1:5). The preparations of all the organic extracts were carried out by immersing the dried plant material (100 g) in their respective extractant solvents (500 ml) for 24 hr at room temperature. In the preparation of aqueous extract, the powdered material was dissolved in distilled water for 24 hr at 45°C.

### Successive extraction

The ground powder (100 g) of the galls of *Q. infectoria* was successively extracted with petroleum ether, ethyl acetate and ethanol. Another 100 g of the powdered material was successively extracted with acetone followed by methanol. The filtrate from each extractant solvents were concentrated under reduced pressure using a rotary evaporator (Buchi rotavapor R-114) at 45°C and the resulting pellet was pounded to dryness under hot air-dryer.

### Aqueous extraction

In the preparation of an aqueous extract, the mixture of the powdered plant material in distilled water 100 g of the dried material was centrifuged (centrifuge model Jouan MR22, Jepsen & Jessen Marketing) at 3000 rpm for 2 mins at 4°C. The supernatant was then filtered and the whole process was repeated using the remaining residue with 300 ml distilled water. The filtrate was combined and then freeze-dried using Heto LyoLab 3000 freeze-dryer at -50°C under vacuum for 12 hrs to produce a fine crystal-like extract.

The extraction yield was recorded as a percentage of quantity of initial powdered plant material (100 g) used. 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 and sterilized by filtration through a 0.45  $\mu$ m membrane filter (UNIFLO 25/0, 45 RC Dassel, Germany).

### Microorganisms

Bacterial strains used (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and local clinical isolates of *Staphylococcus epidermidis* and *Bacillus subtilis*) were obtained from the Department of Biomedical Sciences, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia. The three bacterial strains *Escherichia coli* (NCTC 12079 serotype O157:H7), *Salmonella typhimurium* (NCTC 74) and *Salmonella enteritidis* (NCTC 5188) were kindly supplied by Dr. Noraziah Mohamed Zin from the Department of Biomedical Sciences, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia. All the bacterial strains were grown and maintained on nutrient agar slants (Merck, Germany). The

inoculum size of each test strain was standardized by adjusting the optical density of the bacterial suspension to a turbidity corresponding to about 0.08 at 620 nm ( $OD_{620} = 0.08$ ) using UV-160A Visible Recording Spectrophotometer.

#### Antibacterial activity test

The disc diffusion method [15] was used to evaluate antibacterial activity. Mueller Hinton agar (Merck, Germany) was prepared in the plates as the media agar for the test microorganisms. Sterile filter paper disc (Whatman No. 1, 6 mm) were impregnated with 100  $\mu$ l of each of the extracts (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 and a standard antibiotic disc, Gentamycin (10  $\mu$ g/disc) was used as a positive control and incubation was done for 24 hr at 37°C. Inhibition zones were recorded as the mean diameter measured to the nearest millimeter (mm) of growth-free zones using a vernier caliper.

#### RESULTS AND DISCUSSIONS

The yields of all the extracts from galls of *Q. infectoria* based on a dry weight basis are presented in Table 1. No yield was obtained when petroleum ether was used as an extraction solvent. Acetone produced the highest percentage yield of 48.6 %, followed by water (40.1%), ethyl acetate (25.8 %), methanol (14.1 %) and ethanol (9.6%). The major compounds of the galls of *Q. infectoria* are gallic acids and tannins [12] and according to Scalbert [10], tannins are water soluble polyphenols. Our results of extraction yield is in line with Pan *et al.* [16] who reported that a higher extraction yield of polyphenols was obtained with acetone than with methanol, water or ethanol.

Table 2 and Figure 1 shows the results of the antibacterial assay (1 mg of crude extract per disc) against a range of microorganisms. All the five extracts from the galls of *Q. infectoria* showed high antibacterial activity against Gram-positive microorganisms and some Gram-negative microorganisms such as *Salmonella typhimurium* NCTC 74, *S. enteritidis* NCTC 5188 and *P. aeruginosa* ATCC 27853. In general, extracts

from the galls of *Q. infectoria* inhibited the Gram-positive better than the Gram-negative bacteria. This is in accordance with Lin *et al.* [17] who reported that plant extracts from the family Vitaceae are more active against Gram-positive bacteria than Gram-negative bacteria.

Out of all the five extracts screened for their antibacterial activity, the methanol extract was the only one showing positive activity against all the microorganisms studied. This is supported by a study [17] that the plant extracts by methanol gives a more consistent antimicrobial activity compared to those extracted by other solvents. Fatima *et al.* [18] demonstrated that in comparison to water extract, the methanolic extract of *Q. infectoria* appeared to be more active against *Staphylococcus epidermis* and *Streptococcus mutans*. In our study, *Staphylococcus epidermis* was not only susceptible to the methanol extract but was also equally inhibited by the water extract of the galls of *Quercus infectoria*. However, the most susceptible microorganisms to the effect of the methanol extract was *Staphylococcus aureus* ( $15.84 \pm 0.56$  mm) even though it was still slightly lower compared to the positive control (Gentamycin 10  $\mu$ g/disc) with an inhibition zone of 18.2 mm. This correlates with reports [20, 21] that crude plant preparations generally exhibit lower antimicrobial activity than pure antibiotic substances such as Gentamycin.

Acetone, ethyl acetate, ethanol and aqueous extracts seemed to show little variation ( $10.8 \pm 1.53$  mm to  $14.94 \pm 1.53$  mm) against *Salmonella typhimurium* NCTC 74, *S. enteritidis* NCTC 5188, *P. aeruginosa* ATCC 27853 and all the Gram-positive strains tested. Among the bacterial strains used in the study, *E. coli* O157:H7 appeared to be the most resistant towards acetone, ethyl acetate, ethanol and aqueous extracts tested. The methanol extract exhibited minimal activity against *E. coli* O157:H7 ( $8.13 \pm 1.53$  mm). The extract exhibiting the highest antibacterial activity based on the number of bacterial strains susceptible to the effect of the extracts tested and by the diameter of zones of inhibition, was the one derived from the methanol extract of the galls of the *Q. infectoria*.

Our findings demonstrate that the methanol extract of the galls of *Q. infectoria* generally exhibits a high degree of antibacterial activity with equal strength against all the tested

microorganisms. The active agents which are responsible for the antibacterial property of these extracts could well be either gallotannin or ellagitannin which belong to a class of hydrolysable tannins [10]. In another study using a different plant extract [22], it was concluded that ellagitannins are the principal components responsible for its antimicrobial properties. The two main components of the gallotannin isolated from the Turkish galls of *Q. infectoria* were pentagalloylglucose and hexagalloylglucose [9]. Further study to elucidate the chemical composition of the most active fractions is now in

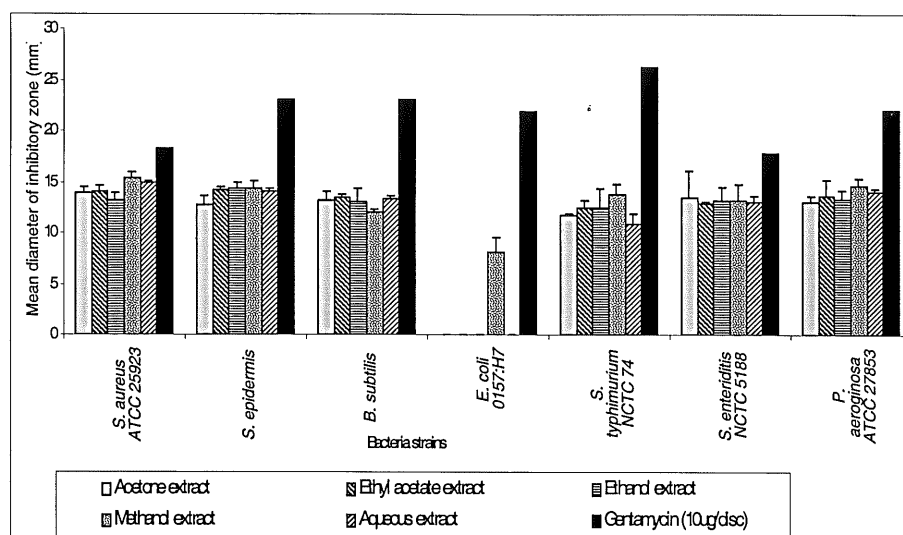
progress. A number of mechanisms has been proposed to explain tannin antimicrobial activity; polyphenols inhibit microorganism growth through iron deprivation or direct action on microbial metabolism through inhibition of oxidative phosphorylation [10]. It was also postulated that the antimicrobial activity of polyphenols may result from their interaction with bacterial enzymes and proteins [19]. However, further study need be undertaken to confirm the mechanism of antimicrobial action of the extracts of the galls of *Q. infectoria*.

**Table 1.** Percentage yield from extraction of powdered galls (100 g) of *Quercus infectoria* in six extractant solvents.

| Method of extractions | Extraction solvents | Percentage yield (%) |
|-----------------------|---------------------|----------------------|
| Successive extraction | Petroleum ether     | 0                    |
|                       | Ethyl acetate       | 25.8                 |
|                       | Ethanol             | 9.6                  |
|                       | Acetone             | 48.6                 |
| Aqueous extraction    | Methanol            | 14.1                 |
|                       | Water               | 40.1                 |

**Table 2.** The mean diameter of inhibitory zone (mm ± S.D.) of five extracts (acetone, ethyl acetate, ethanol, methanol and aqueous) of the galls of *Quercus infectoria* at 1mg/disc against seven bacterial strains. The positive control was Gentamycin (10 µg/disc).

| Bacteria strains                           | Galls of <i>Quercus infectoria</i> extracts |              |              |              |              | Positive control |
|--|---|--------------|--------------|--------------|--------------|------------------|
|  | Acetone                                     | EtoAc        | EtOH         | MeOH         | Water        |                  |
| <i>Staphylococcus aureus</i> (ATCC 25923)  | 13.85 ± 0.61                                | 14.01 ± 0.65 | 13.23 ± 0.68 | 15.84 ± 0.56 | 14.94 ± 0.21 | 18.2             |
| <i>Staphylococcus epidermidis</i>          | 12.73 ± 0.95                                | 14.20 ± 0.25 | 14.30 ± 0.61 | 14.27 ± 0.74 | 14.10 ± 0.21 | 23.1             |
| <i>Bacillus subtilis</i>                   | 13.20 ± 0.82                                | 13.49 ± 0.31 | 13.87 ± 1.23 | 12.02 ± 0.34 | 13.36 ± 0.26 | 23.1             |
| <i>Escherichia coli</i> (O157:H7)          | -   | -            | -            | 8.13 ± 1.53  | -            | 21.9             |
| <i>Salmonella typhimurium</i> (NCTC 74)    | 11.67 ± 0.16                                | 12.52 ± 0.66 | 12.44 ± 1.96 | 13.66 ± 1.11 | 10.8 ± 1.04  | 26.2             |
| <i>Salmonella enteritidis</i> (NCTC 5188)  | 13.46 ± 2.60                                | 12.95 ± 0.07 | 13.24 ± 1.22 | 13.22 ± 1.58 | 12.98 ± 0.68 | 17.8             |
| <i>Pseudomonas aeruginosa</i> (ATCC 27853) | 12.96 ± 0.56                                | 13.61 ± 1.62 | 13.42 ± 0.83 | 14.64 ± 0.74 | 14.13 ± 0.23 | 22.0             |



**Figure 1.** The mean diameter of inhibitory zone (mm) of five extracts (acetone, ethyl acetate, ethanol, methanol and aqueous) of the galls of *Quercus infectoria* at 1 mg/disc against seven bacterial strains. The positive control was Gentamycin (10 µg/disc).

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