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Quantification of Gallic Acid and Tannic Acid from *Quercus infectoria* (Manjakani) and their Effects on Antioxidant and Antibacterial Activities

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ABSTRACT

Effects of different types of solvent on the antioxidant and antibacterial activity of *Quercus infectoria* extract have not been well documented. Therefore, extraction process was conducted using conventional Soxhlet extraction with six different types of solvent (100% methanol, ethanol, acetone, water and 70% methanol, and ethanol). High performance liquid chromatography was implemented to identify gallic acid and tannic acid in the extracts. Water extracts contained the highest concentration of both gallic acid and tannic acid compared to other types of solvent; 51.14 mg/g sample and 1332.88 mg/g sample of gallic acid and tannic acid. Meanwhile, antioxidant and antibacterial activity were tested using DPPH free radicals scavenging and disc diffusion assay. Results demonstrated that water extracts gave the highest antioxidant activity (approximately 94.55%), while acetone extract gave the largest inhibition zone for disc diffusion assay (19.00mm respectively). The results also revealed rich sources of gallic acid and tannic acid in Q.infectoria which might provide a novel source of these natural antioxidant and antibacterial activity.

Keywords: Bioactive compound, solvent, antioxidant activity, antibacterial activity, gallic acid, HPLC, tannic acid, and *Q. infectoria.*

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INTRODUCTION

Quercus infectoria has been extensively used as a medicinal plant since ancient time because it is reported to contain large amounts of bioactive constituents such as tannin, gallic acid, syringic acid, ellagic acid, β-sitosterol, amentoflavone, hexamethyl ether,

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isocryptometrin, methyl betulate, methyl oleanate, hexagalloyglucose, etc. (Dar *et al*., 1976; Ikram & Nowshad, 1977; Hwang *et al*., 2000). The main constituents found in the galls of *Quercus infectoria* are tannin (50-70%), free gallic acid and ellagic acid (Evans, 1996; Wiart & Kumar, 2001). Tannic acid and gallic acid, which are derivatives of tannins, have been reported to have antioxidant activity and the ability to become antimicrobial (Everest & Ozturk, 2005), antibacterial (Hamid *et al*., 2005) and antifungal agent (Yamunarani *et al*., 2005). Extraction of bioactive compounds from medical plants has enabled demonstration of their physiological activity by medical researchers.

Quercus infectoria gall (QI), which is widely known as manjakani, is a small tree native to Greece, Asia Minor and Iran. It is also popularly known as oak tree. Galls arise on young branches of this tree because of attack by gall wasp called Adleriagallae-tinctoria. In Malaysia, it is known as a herbal drink to cure uterine wall of women after their childbirth. In India, it is better known as Majuphal and has been used as dental powder and in the treatments of toothache and gingivitis. In Asian countries, it has been used for centuries as a traditional medicine for inflammatory disease (Galla, 1911; Kaur *et al*., 2004). Hemorrhoids caused by inflammation of the skin can be treated by applying powdered *Quercus infectoria* in the form of ointment on the skin. Its potential medical and cosmeceutcal areas have greatly induced researchers to study its scientific values in further detail.

Most works on *Quercus infectoria* have been done on identification and isolation of its biological and pharmacological properties (Asghari *et al*., 2011; Basri *et al*., 2005; Kaur *et al*., 2005). However, none of the study has reported on the effects of different solvents on antioxidant and antibacterial activities. Therefore, the objectives of the present study were to identify and quantify gallic acid and tannic acid from the gall extracts using six different types of solvents (100% methanol, 70% methanol, 100% ethanol, 70% ethanol, 100% acetone and water) by using HPLC, and to evaluate the antioxidant and antibacterial activities using DPPH free radicals scavenging and disc diffusion method.

MATERIALS AND METHODS

Materials

Methanol (MeOH 100% and 70%), ethanol (EtOH 100% and 70%), Acetone 100% and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Gallic acid and Tannin acid were purchased from Sigma Aldrich (M) Sdn Bhd Chemicals.

Plant Material

The galls of *Quercus infectoria* were purchased from a local herbal market in Johor Bahru, Malaysia. The galls were crushed into fine powder and washed under tap water to remove undesired particles. After that, all the samples were dried in an oven at 50ºC.

Extraction Preparation

5 g of powdered galls were weighted and placed in the timber, while 150ml of methanol (100%) was placed at the bottom of the apparatus. The extraction process was done for 6 hours to achieve a complete extraction process..The extraction yield was put in the rotary evaporator at 400C to remove the solvent. All the steps were repeated using 70% methanol, 100% ethanol, 70% ethanol, acetone and water.

High Performance Liquid Chromatography (HPLC)

1. Determination of Gallic Acid

Determination of active constituents from the extracted compounds was done using high performance liquid chromatography as described by Pin *et al*. (2006) with slight modification. Waters 600E System Controller combined with Waters 996 Photodiode Array Detector was used and C18 column was selected as stationary phase. Meanwhile, 0.1% orthophosphoric acid (H_3PO_4) was consumed as solvent A and 100% and 100% acetonitrile (100%) as solvent B. Then, the flow rate of mobile phase was adjusted at 1 ml/min at 280nm and every injection was set until 10 µL was achieved.

2. Determination of Tannic Acid

The identification of tannic acid from the extracts was done according to Asghari *et al*. (2011), with slight modification. High performance liquid chromatography was performed by reversed-phase HPLC on a C18 column using a binary gradient elution consisting of an aqueous methanol eluents at low pH as mobile phase. The gradient system consisted of solvent A (25ml acetic acid and 975ml distilled water) and solvent B (99.8% methanol) pumped at 1mL/min. The gradient started with 100% solution A and ended with 100% solution B at 30 min. The column temperature was maintained at 30°C. The sample peaks were identified by comparing with standard solution of tannic acid at 280nm. The percentage of the tannin acid was calculated using the appropriate calibration curves.

Antioxidant Activity

This assay was carried out according to the method proposed by Miliauskas *et al*. (2004) with a slight modification. Extract solution was prepared by dissolving 0.025 g of the dry extract in 10ml of methanol to give a final concentration of 2.5mg/ml. After that, 77µL of the extract solution was mixed with 3ml of 6 x 10^{-5} M methanolic solution of DPPH. The mixture was placed in the dark for 30 minutes at room temperature and the decrease in the absorption was measured at 517nm by using a spectrophotometer. Radical scavenging activity of the samples was calculated by using following formula:

$$\text{DPPH quenched (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \qquad [1]$$

Antibacterial Assay

The extracts from the galls of *Quercus infectoria* at 50mg/ml were screened against two-gram positive bacteria (*Staphylococcus aureus* and *Bacillus Subtilis*) and two-gram negative bacteria (*Escherichia Coli* and *Pseudomonas aeruginosa*). 50µL of bacteria suspension from the culture suspension was applied to the nutrient agar plate. Then, it was swabbed to the entire surface of the agar by using a sterile hockey stick. After that, sterile filter paper disc (Whatman No.1, 6mm) was impregnated with 20µl of each of the extracts (50mg/ml). Then, streptomycin (10µg/ disc) was used as standard to confirm that the entire microorganism tested was inhibited by the antibiotic and sterile distilled water used as the negative control. All the plates were incubated for 24 hours at 37°C. Then, the antibacterial activity was interpreted from the size of diameter of zone inhibition measured to the nearest millimetre (mm), as observed from the clear zone surrounding the disc (Basri & Fan, 2005). The inhibition zone was measured after 24 hours.

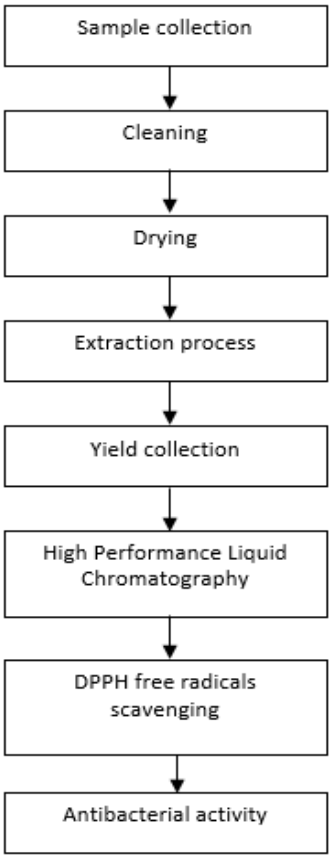

Fig.1: The flowchart of research activity

RESULTS AND DISCUSSION

Yield of Extracts

Table 1 shows the percentage yield of the *Quercus infectoria* extract using different types of solvents. The highest extraction yield was found with water extract (76%±0.084) and with a slight difference, followed by 70% methanol (74%±0.050) and 70% ethanol (71%±0.047). The findings indicated that *Quercus infectoria* was most soluble in polar solvents and most components were hydrophilic or water-soluble. On the other hand, 100% acetone resulted in lowest extraction yield suggesting that polar compounds in a biological plant were easier to extract with more polar solvents, whereas the less polar solvent was unable to extract the polar bioactive compounds. Tian *et al*. (2009) have reported similar findings, whereby the highest yield of *Galla chinensis* was obtained from aqueous solvent.

TABLE 1: Yield of Quercus infectoriaextract based on different types of solvent

Types of solvents	Yield of extract (% g/g sample)	Synder's solvent polarity index[c]
100% methanol	70±0.054[b]	6.6
70% methanol	74±0.050[b]	7.32
100% ethanol	52±0.147[a]	5.2
70% ethanol	71±0.047[b]	6.34
100% acetone	46±0.149[a]	5.4
100% aqueous	76±0.084[b]	9.0

Each bar represents means ± SD of three replicates. Different letters on the bars indicate groups are significantly different from each other according to Tukey's test ($p<0.05$).
[C] Synder's solvent polarity index cited from Markom *et al*. (2007).

From the ANOVA analysis, 100% ethanol and acetone were statistically significant compared with other solvents ($p<0.05$). Even though 100% ethanol and aqueous contain hydroxyl group which can create hydrogen bonding with the solute, aqueous solvent is proven to be more efficient in extracting the solute due to its higher polarity and shorter chain (Pin *et al*., 2006). These characteristics enhance its ability to extract polar compounds which thus explains the significant differences observed between the yields of 100% ethanol water.

The results above indicated that the mixture of the organic solvents (70% methanol and ethanol) produced a higher extraction yield compared to pure solvent (100% methanol and ethanol) alone, while the pure solvent of methanol gave a higher yield compared to absolute ethanol. As can be obtained from Table 1, the polarity of the mixture (70% methanol and ethanol) is higher compared to pure solvents (100% methanol and ethanol), which explains the higher yield obtained from those solvents. This finding is compatible with the previous findings documented by Markom *et al*. (2007) who found that the addition of water in ethanol significantly increased the extraction yield of *Phyllanthus niruri* Linn.

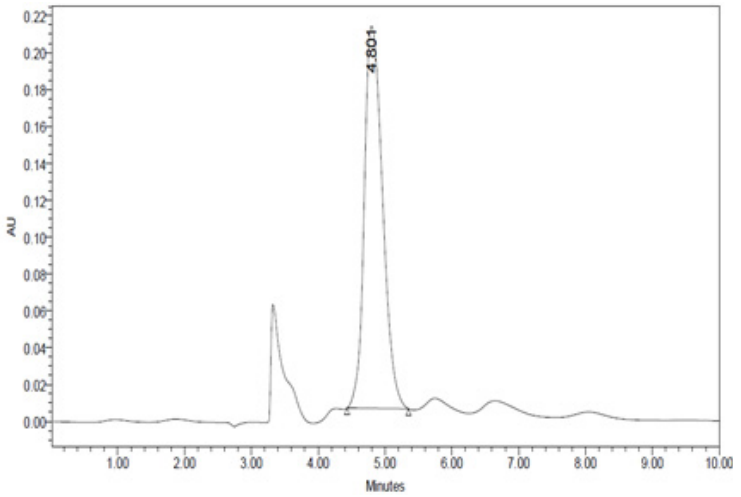

Fig.2: HPLC chromatogram of standard Gallic acid

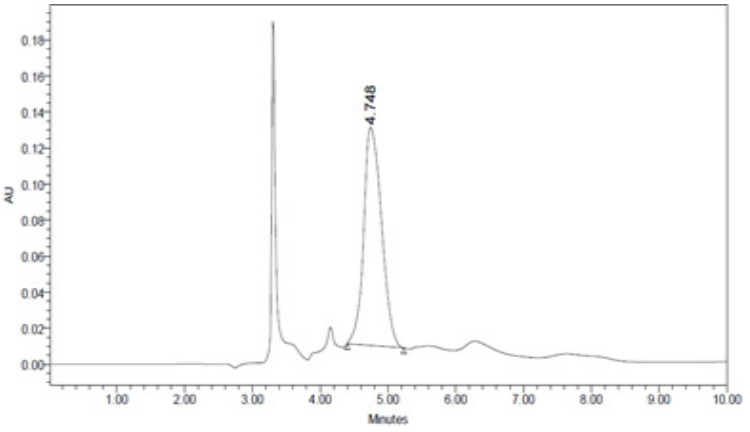

Fig.3: The HPLC chromatogram of Gallic acid from *Q. infectoria* galls extract

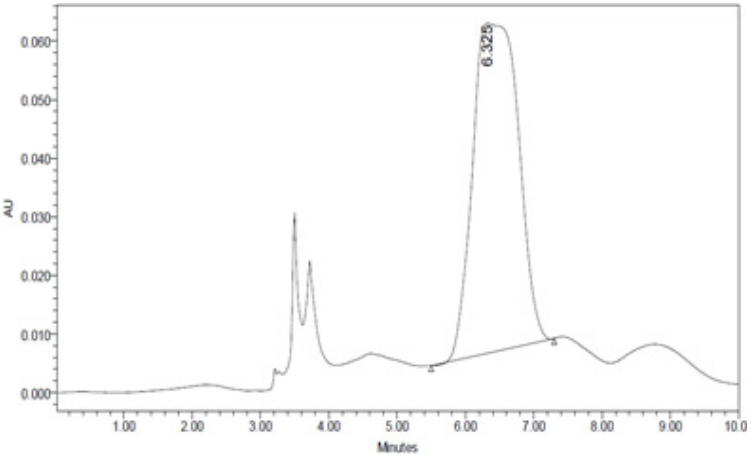

Fig.4: The HPLC chromatogram of standard Tannic acid

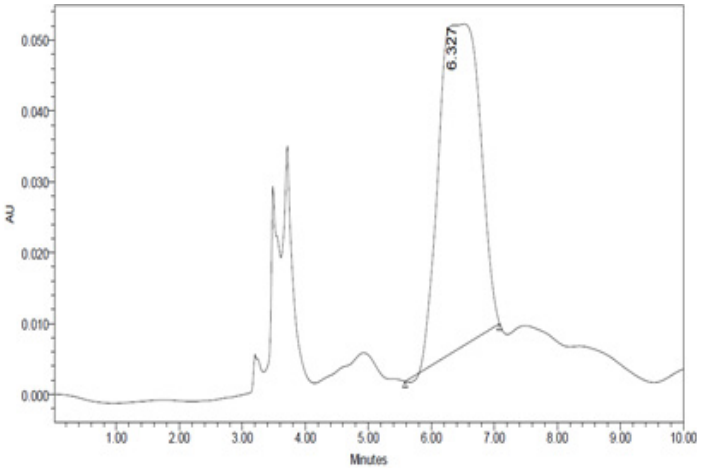

Fig.5: The HPLC chromatogram of Tannic acid from *Q. infectoria* galls extract

Identification and Quantification of Gallic Acid and Tannic Acid by HPLC Assay

The concentrations of the bioactive compounds were determined by using the peak area from the calibration curves (Song & Barlow, 2006) and are shown in Table 1. Fig.2 to Fig.5 show the chromatogram of standard and sample of gallic acid and tannic acid. HPLC analysis produced the similar trend in both gallic and tannic acid where 100% aqueous> 70% ethanol> 70% methanol> 100% methanol> 100% ethanol> 100% acetone. Aqueous extracts contain the highest concentration of gallic acid (101 mg/g sample) and tannic acid (2975 mg/g sample) compared to the other solvents used for the extraction process. This result indicates that aqueous solvent is a better extraction solvent compared to other solvents for the extraction of both gallic acid and tannic acid from the *Quercus infectoria* extracts.

A previous study reported lower concentrations of gallic acid and tannic acid from ethanolic extract of *Quercus infectoria*, which are 87 mg/g and 199 mg/g, respectively (Kaur *et al*., 2008). These different results might be contributed by the different solvent and solid to solvent ratio used.

Scavenging Antioxidant Activity

DPPH is a potent tool to ascertain the antioxidant capacity of the extracted compound. Fig.6 depicts the DPPH free radical scavenging by the *Quercus infectoria* extracts using different types of solvent.

In Fig.6, water extract (94%) gives the highest DPPH scavenging activity with a slight difference from 70% methanol (94%) and 100% methanol (93%). In addition, all the extracts showed higher scavenging activities compared to the control (BHA). The finding documented previously on the contradictive result revealed that the ethanol extract of *Galla chinensis* gave a higher reduction activity compared to the aqueous extract (Tian *et al*., 2009). The different findings are due to the ability of the solvent to extract the different bioactive compounds for different plants. From the ANOVA, types of solvents do not give significant differences in the scavenging of free radicals ($P > 0.05$).

This finding is compatible with that of Jain *et al*. (2011) who found that *Quercus infectoria* possesses antioxidant activity by scavenging free radicals about 90%, while Kaur *et al*. (2008) reported lower antioxidant activity, which is about 71%. The variation in the findings is due to the different concentrations and solvents used. In addition, the different origins of the raw material might have influenced the growth of the plant itself because different soil compositions would also yield different vegetative traits (Devkota & Jha, 2009).

The observation showed that the extract from 70% methanol and ethanol gave higher antioxidant activities compared to the absolute solvent. Turkmen *et al*. (2006) reported the same finding whereby 50% and 80% of solvent mixtures exhibited considerably higher DPPH radicals scavenging activity compared to the pure solvent. The high antioxidant activity of *Quercus infectoria* is due to the presence of gallic acid and tannic acid, which are proven to possess antioxidant activity (Govindarajan *et al*., 2005; Robert *et al*., 1999).

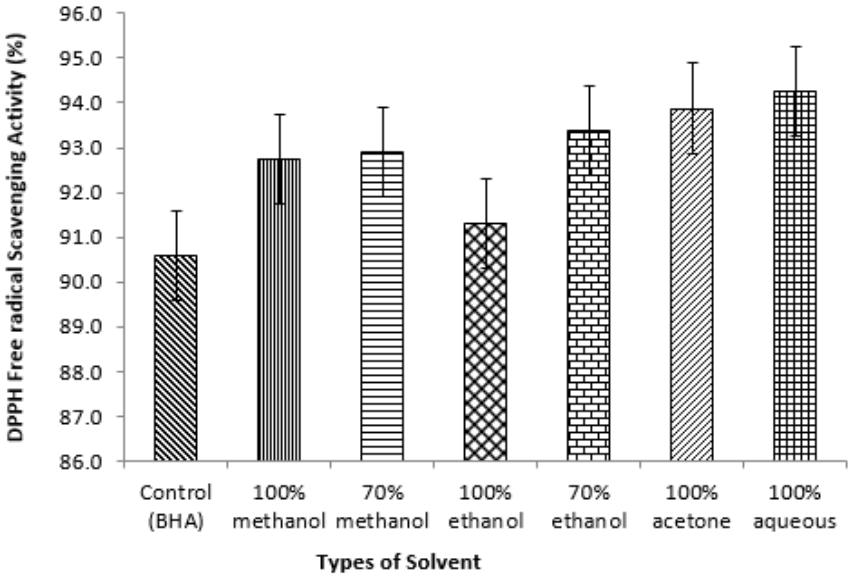

Fig.6: The DPPH scavenging activity of different types of solvent

Antibacterial Activity

The antibacterial activity of *Quercus infectoria* gall extracts using different solvents was studied by using disc diffusion method and the results are tabulated in Table 2 and Fig.7. All the extracts showed inhibitory effects that are not significantly different ($P>0.05$) against each bacterial species tested. After 24 hours, the largest inhibition zone (19±0.14mm) was shown by the extract of 100% acetone against *B.subtilis* and it was comparable with the commercial antibiotics (19±0.07.mm). However, other samples also showed inhibition zone that varied from 12±0.21mm to 18±0.21mm. *B. subtilis* was found to be the most susceptible towards all extracts. The smallest inhibition zone was exhibited by 100% acetone extract against *P. aeruginosa*.

TABLE 2 : Concentrations of Gallic Acid and Tannic Acid from Quercus infectoria galls extract

Types of solvent	Gallic acid (mg/g sample)	Tannic acid (mg/ g sample)
100% Methanol	51	1332
70% Methanol	71	1823
100% Ethanol	37	954
70% Ethanol	99	2512
100% Acetone	34	949
100% Aqueous	101	2975

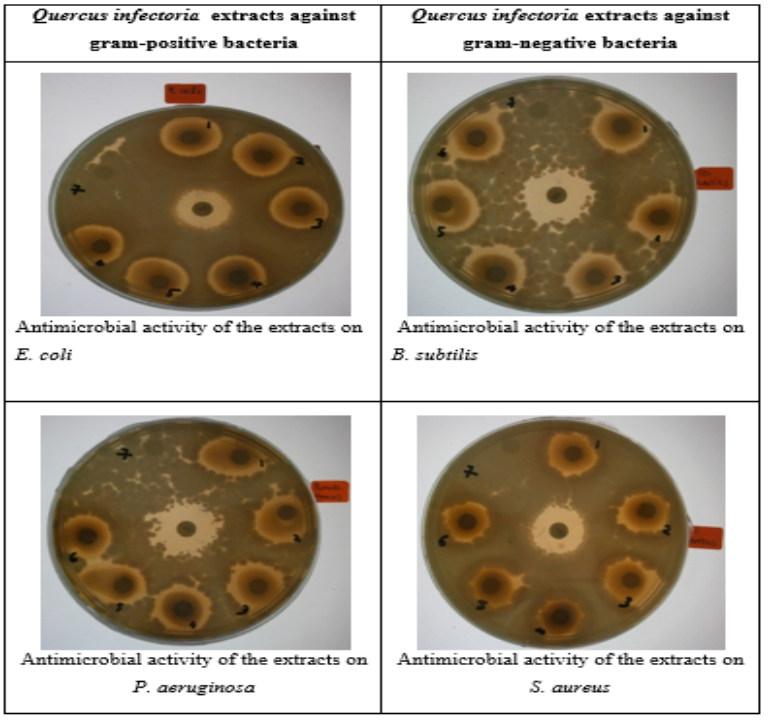

Fig.7: The antimicrobial activity of the extracts on *E. coli*: Disc diffusion test for the effect of *Quercus infectoria* against *E.coli* grown on nutrient agar medium. 1:100% methanol, 2:70% methanol, 3:100% ethanol, 4:70% ethanol, 5:100% acetone, 6:100% distilled water, 7: Negative control.

From Table 3, the antimicrobial properties of alcoholic and acetone extract were found to be superior compared to aqueous extract for those selected bacteria. This finding is in accordance with the result of a previous research which reported that the plant extracts using organic solvent exhibited more antibacterial activity compared to the extract using aqueous as a solvent (Turkmen *et al*., 2006; Parekh *et al*., 2005). Similarly, Basri and Fan (2005) have also documented the potential of aqueous and acetone extracts of galls as antibacterial agents. The most interesting finding is that the inhibition zone showed by all the bigger extracts compared to the positive control against *E. coli*. This suggests that *E. coli* is more susceptible to the extracts compared to commercial antibiotics (streptomycin).

TABLE 3: Antibacterial activity of different extracts of Manjakani galls at 24 hours

Types of Solvent	Diameter of inhibition zone (mm)± S.D			
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*
100% methanol (1)	17±0.00[a]	13±.0.14 [a]	16±0.07 [a]	16±0.21 [a]
70% methanol (2)	16±0.07 [a]	15±0.14 [a]	17±0.00 [a]	15±0.21 [a]
100% ethanol (3)	17±0.00 [a]	14±0.21 [a]	18±0.21 [a]	15±0.21 [a]
70% ethanol (4)	17±0.14 [a]	15±0.14 [a]	17±0.07 [a]	14±0.21 [a]
100% acetone (5)	18±0.14 [a]	12±0.21 [a]	19±0.14 [a]	14±0.14 [a]
100% aqueous (6)	15±0.00 [a]	13±0.14 [a]	17±0.14 [a]	14±0.21 [a]
Neg. control (7)	-	-	-	-
Streptomycin	15±0.07 [a]	15±0.14 [a]	19±0.07 [a]	17±0.21 [a]

Each bar represents means ± SD of three replicates. Different letters on the bars indicate that groups are significantly different from each other according to Tukey's test ($p<0.05$).

Meanwhile, the antibacterial activity of the extracts might be due to the presence of gallic acid and tannic acid, which are derivatives of tannins. Tannins are reactive with the secreted extracellular enzymes and bacteria cell wall. This bioactive compound will destroy the cell walls of the bacteria and alter the transportation of nutrients into the cell to hinder the growth of this microorganism. This fact can explain the significant inhibitory effect of the *Quercus infectoria* extracts and this current finding is in accordance with the previous study reported by Li *et al*. (2011) who claimed that tannins from *Terminalia chebula Fructus Retz.* contain antibacterial activity.

CONCLUSION

In this study, the HPLC analysis had identified and quantified the gallic acid and tannic acid in the *Quercus infectoria* (manjakani) gall extract. The results demonstrated that conventional soxhlet extraction is efficient to extract the bioactive compounds from the plants. The strong antioxidant and antibacterial activities of the *Quercus infectoria* extracts are probably due to the bioactive compounds that are present in the plants. However, further investigation of individual phenolic compounds, their in vivo antioxidant and antibacterial activity, is necessary before advocating the application of *Quercus infectoria* in pharmaceutical and food products.

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