CAFEi2012-138

BIOACTIVE COMPOUNDS FROM QUERCUS INFECTORIA (MANJAKANI) GALLS EXTRACT AND THEIR EFFECTS ON ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Gallic acid and tannic acid have been identified in *Quercus infectoria* (manjakani) galls extracts by using high performance liquid chromatography. The extraction process was conducted using conventional soxhlet extraction with six different types of solvents (100% methanol, ethanol, acetone, aqueous and 70% methanol and ethanol). The aqueous extracts contained the highest concentration of bioactive compounds compared to other types of solvent which are 51.14 mg/g sample and 1332.88 mg/g sample of gallic acid and tannic acid respectively. The antioxidant and antibacterial activity were tested using DPPH free radicals scavenging and disc diffusion assay. The result demonstrated that aqueous extracts gives the highest antioxidant activity approximately 94.55% while acetone extract gives the largest inhibition zone for disc diffusion assay which is 19.00mm respectively. The results 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 compounds, HPLC, Q .infectoria, antioxidant activity, antibacterial activity.*

INTRODUCTION

Bioactive compounds in plant are also referred to as nutraceuticals that reflects their existence in the human diet, their biological activity and present as natural constituents in food provide health benefits beyond the basic nutritional value of the product. The compounds studied most extensively are the antioxidants, an increased intake of which can alter the risk of chronic diseases including cancer and cardiovascular disease [1]. Extraction of bioactive compounds from medicals plants has been permitted the demonstration of their physiological activity by medical researchers. Therefore, there is a need to find the plants with the excellent medically valuable produced from varying substrates.

Quercus infectoria galls(QI) or widely known as manjakani is a small tree native to Greece, Asia Minor and Iran which also popular as oak tree. The galls arise on young branches of this tree as a result of attack by the gall-wasp *Adleria gallae-tinctoria*. It is locally known in Malaysia as a herbal drink to remedy the women after their childbirth to restore the elasticity of the uterine wall. While in India, it is well known as *Majuphal* and has been used as dental powder and in the treatment of toothache and gingivitis. In Asian, it has been used for centuries as traditional medicine for treating inflammatory disease [2-4]. Other than that, by using the hot water extract of manjakani as a mouth antiseptic, it can control the inflammation of tonsils, while the direct application of it onto the skin cures swelling or inflammation [5]. Hemorrhoids caused by inflammation of the skin can also be treated by applying the powdered manjakani in the form of ointment to the skin. Moreover, this plant also show promising result in cosmeticeutical where [6] reported that the galls possess high potential in skin whitening. The potential of manjakani in medical and cosmaticeucal areas have induced the researcher to study and investigate the further details about its usage and application.

Manjakani is greatly used as medicinal plant since ancient time because it was reported contains large amount of bioactive constituents such as tannins, gallic acid, syringic acid, ellagic acid, β-sitosterol, amentoflavone, hexamethyl ether, isocryptometrin, methyl betulate, methyl oleanate, hexagalloyglucose and

others [7-9]. 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, 10, 11]. Tannin which is derived from phenolic compounds has been reported to have antioxidant activity and has the ability to be antimicrobial [12], antibacterial [13] and the antifungal agent [14]. Therefore the bioactive compounds from the galls can be extracted by using several methods such as conventional soxhlet extraction (CSE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and others. However, CSE technique have been selected due to its simplicity, easy to handle and cheap. The extraction of the bioactive compounds were investigated by using six different types of solvents which are 100% methanol, 70% methanol, 100% ethanol, 70% ethanol, 100% acetone and aqueous and their effects on the antioxidant and antibacterial were assessed.

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.

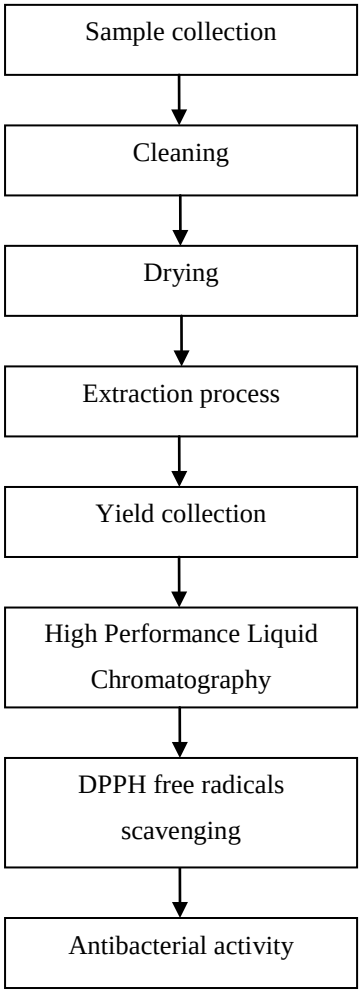

Fig. 1: Flowchart of antioxidant and antibacterial activity of manjakani galls.

Plant material

The galls of manjakani were purchased from the local market in Johor Bharu, Malaysia. Then, the galls were crushed to fine powder and washed under tap water in order to remove the undesired particles. After that, all of the samples were dried in the oven at 50ºC.

Extraction preparation

To prepare the extract,5 g of powdered galls were weight 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. Lastly,the extraction yield was put in the rotary evaporator at 40^0C to remove the solvent.All the steps were repeated using 70% methanol, 100% ethanol, 70% ethanol, acetone and aqueous. After that, the yield of the extracted samples were calculated using following equation:

$$\text{Percent of yield extraction} = \frac{\text{Final weight (g)}}{\text{Initial weight}} \times 100 \quad (1)$$

High Performance Liquid Chromatography (HPLC):

Determination of Gallic Acid

Determination of active constituents from extracted compounds was examined using high performance liquid chromatography as described by [15] with slight modification.

In order to evaluate the quality of extracted compounds, all of the samples will be analyzed by using high performance liquid chromatography using gallic acid as chemical marker. Waters 600E System Controller combined with Waters 996 Photodiode Array Detector will be used and C18 column was selected as stationary phase. Meanwhile, 0.1% orthophosphoric acid (H_3PO_4) will be consumed as solvent A and 100% and 100% acetonitrile (100%) as solvent B. Then, the flowrate of mobile phase will be adjusted at 1ml/min at 280nm and every injection was set until achieved 10 µL.

Determination of Tannic Acid

The identification of tannic acid from the extracts was determined as reported by [16] with slight modification. High performance liquid chromatography was performed by reversed-phase HPLC on a C18 column by using a binary gradient elution with consisting of an aqueous methanol eluents at low pH as mobile phase. The gradient system 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 were calculated using the appropriate calibration curves.

Antioxidant activity

This assay was carried out according to the method of [17] with a slight modification. DPPH or 2, 2-diphenyl-1-picrylhydrazyl is a stable free radical, which froms a purple-coloured solution when dissolved in methanol. Antioxidant components can scavenge this stable free radical and therefore the purple colour will be bleached. Extract solution was prepared by dissolving 0.025 g of dry extract in 10ml of methanol to give final concentration at 2.5mg/ml. After that, 77µL of the extract solution was mixed with 3ml of 6 x 10^{-5} M methanolic solution of DPPH. After that, the mixture was placed in the dark for 30 minutes at the room temperature and the decrease in the absorption was measure at 517nm by using spectrophotometer. Radical scavenging activity of the samples was calculated by using following formula:

$$\text{DPPH quenched}\,(\%) = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad (2)$$

Antibacterial Assay

The disc diffusion method was used to evaluate the antibacterial activity. Nutrient agar prepared earlier was used as the media for the test microorganism. The extracts from the galls of manjakani 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*). First, 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 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 negative control. All the plates were incubated for 24 hr at 37^{o}C. Then, the antibacterial activity was interpreted from the size of diameter of zone inhibition measured to the nearest millimeter (mm) as observed from the clear zone surrounding the disc [18]. The inhibition zone was measured after 24 hours.

RESULTS AND DISCUSSIONS

Yield of extracts

Figure 2 shows the result of yield on manjakani extract. There are six different solvent with different polarities were used to extract the manjakani which are 100% methanol, 70% methanol, 100% ethanol, 70% ethanol, 100% acetone and 100% aqueous (water).

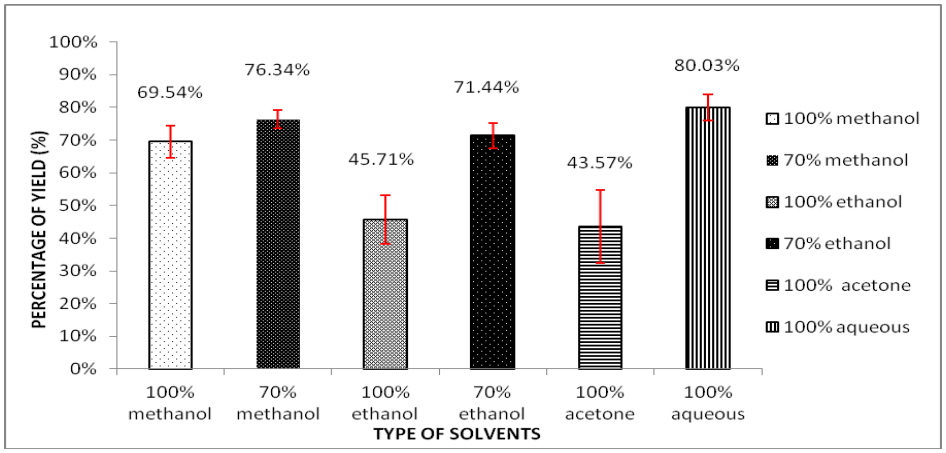

Fig.2 : Yield of manjakani extract based on different types of solvent.

Generally, all of the solvents gives the highest extraction yield varies from 45.71% until 80.03%. Based on the obtained results, the highest extraction yield was found with water extract (80.03%) and with slight difference followed by 70% methanol (76.34%) and 70% ethanol (71.44%). On the other hand, 100% acetone resulted on lowest extraction yield suggesting that polar compounds in biological plant is easier to extract with more polar solvents whereas the less polar solvent is enable to extract the polar bioactive compounds. The results above indicate that the mixture of the organic solvents give higher extraction yield compare to the pure solvent alone while the pure solvent of methanol gives higher yield compare to ethanol due to the higher polarity of the solvents. The preceding findings from [19] was contradictive with the result above where they found that pomegrated peel was extracted effectively using methanol followed water, ethanol and acetone. This inconsistent finding might be caused by the different extraction method and different raw material used.

The suitable solvent for extracting target compounds should be selected carefully because the extracted compound will be based on the type of solvents used [20]. A polar solvent will isolate polar compound and non-polar solvent will extract non-polar compound thus different solvents will yield different extracts and extract composition. The highest yield is commonly achieved by using methanol or ethanol and their mixture with water. However, ethanol and water are widely used solvents due to their low toxicity and high extraction yield and in advances their polarity can be modulated by mix them at selected ratio [21]. This finding was comparable with [22] where they found that 70% solvent-mixture isolated phenolics compound from cherry liqueur pomace more effectively compared to the pure solvents did.

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

High performance liquid chromatography coupled with UV detector was employed to separate, identify and quantify the gallic and tannin acid in *Quercus infectoria* galls extract. The concentrations of the bioactive compounds were determined by using the peak area from the calibration curves [23] and shown in table 1. Figure 2-5 show the chromatogram of standard and sample of gallic acid and tannic acid. These phenolic compounds have been identified in *Q.infectoria* according to their retention times against those of standards (Figs.2 and 4) as well as spiking the samples with standards.

Table 1: 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	51.14	1332.88
70% Methanol	71.13	1823.31
100% Ethanol	37.22	954.03
70% Ethanol	99.39	2512.22
100% Acetone	34.04	949.34
100% Aqueous	101.55	2975.11

As shown in table 1, aqueous extracts contain highest concentration of gallic acid (101.55mg/g sample) and tannic acid (2975.11mg/g sample) compared to the other solvents used for extraction process. This result indicates that aqueous solution is a better extraction solvent for the extraction of both gallic acid and tannic acid. This higher yield of these compounds might contribute to the pharmaceutical used such as anti-inflammantory, anticancer and antioxidant activity [24].

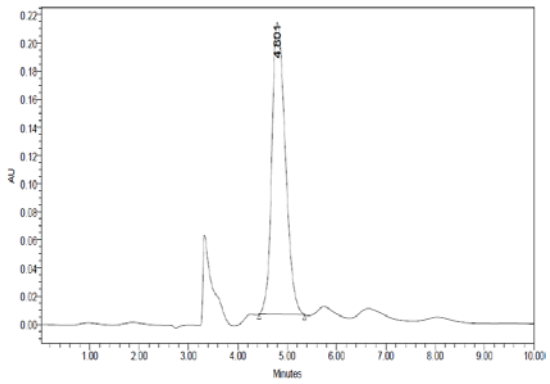

Fig. 3: HPLC chromatogram of standard Gallic acid.

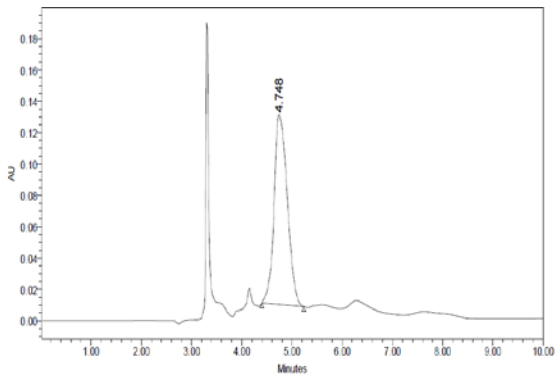

Fig. 4: HPLC chromatogram of Gallic acid from *Q. infectoria* galls extract.

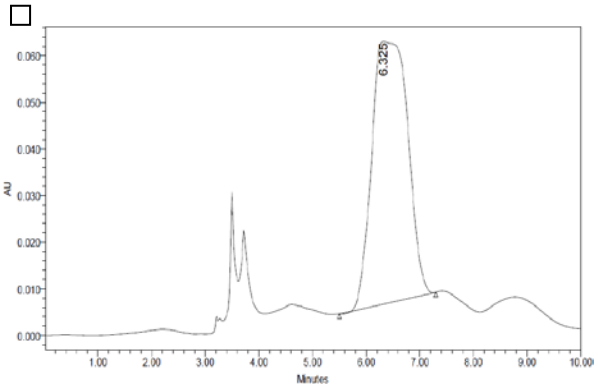

Fig. 5: HPLC chromatogram of standard Tannic acid.

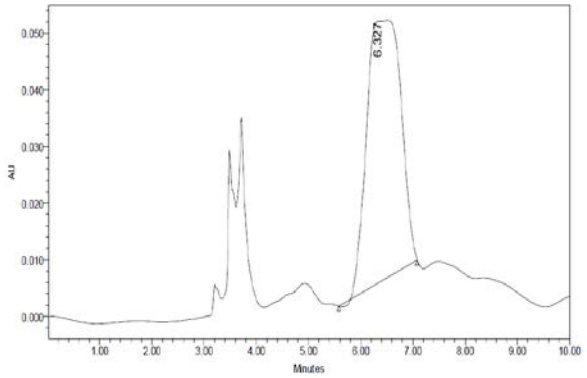

Fig. 6: HPLC chromatogram of Tannic acid from *Q.infectoria* galls extract .

Scavenging antioxidant activity

Extracts containing antioxidant are beneficial to health because it can regulates many degenerative processes and effectively lower the risk of having a cancer and cardiovascular. DPPH is a common abbreviation for an organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl. Other than that, it is a compound with stable free radical and has been widely used to screen phenolic compounds containing high free radical scavenging ability [14]. In addition, when a hydrogen atom or electron was transferred to the odd electron in DPPH, the absorbance at 515 until 517 nm decreased proportionally to the increases of non radicals forms of DPPH [17]. DPPH is a potent tool to ascertain antioxidant capacity of the extracted compound. Hence, figure 3 depicts the DPPH free radical scavenging by the manjakani extracts using different types of solvent.

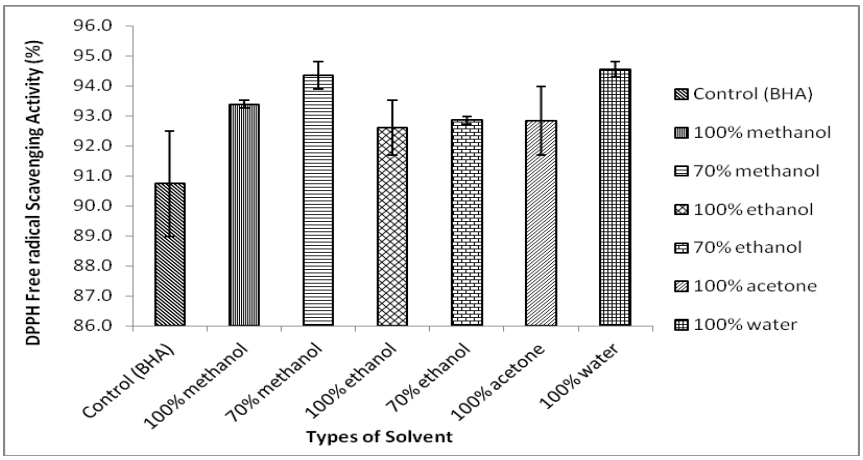

Fig. 7: DPPH scavenging activity of different types of solvent

The presented figure 7 shows that water extract (94.55%) gives the highest DPPH scavenging activity and with a little different by 70% methanol (94.35%) and 100% methanol (93.38%). However, other solvents also depicted high free radical scavenging varies from 92.60% (100% ethanol) until 92.83% (100% acetone). However, [24] documented on the contradictive result where they found that methanolic extracts gives higher reduction activity followed by ethanol, aqueous and acetone in the extraction of wild edible plant *Digera muricata* (L.) mart. The different finding is due to the ability of the solvent to extract the bioactive compounds is differ for different biological plant. To date, the antioxidant activity of manjakani galls extracts using different types of solvent has not been well documented but from the results obtain we can conclude that the types of solvents does not gives significant differences toward the scavenging of free radicals.

However, this findings was compatible with [25] where that found that galls extract of manjakani possess antioxidant activity by scavenge the free radicals about 71.5 % at 0.5µg/ml suggesting that the lower antioxidant activity might be due to the lower concentration used. From the observation, the extracted compound from the 70% methanol and ethanol give higher antioxidant activity compare to the absolute solvent and [26] reported the same findings where they found 50% and 80% of solvent mixture exhibited considerably higher DPPH radical scavenging activity compare to the pure solvent. On the basis of the result obtained, manjakani galls are found to be a potential source of natural antioxidant to replace the synthetic antioxidant which are proven can initiate the cancer. The high antioxidant activity might be due to the presence of gallic acid and tannic acid which are proven posses antioxidant activity.

Antibacterial activity

The antibacterial activity of manjakani galls extracts using different solvents was studied by using disc diffusion method and the result are tabulated in Table 2 and Figure 8-11.

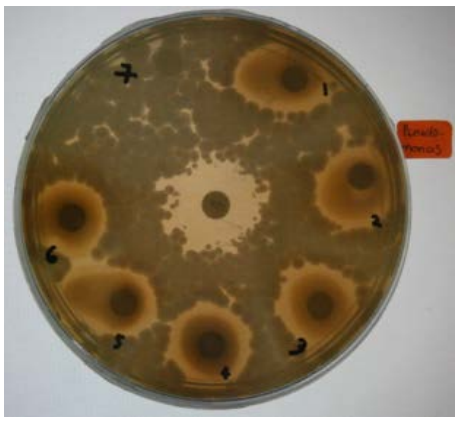

Fig. 8: Antimicrobial activity of the extracts on *E.coli* : Disc diffusion test for the effect of *Q.infectoria* against *E.coli* grown on nutrient agar medium. 1:100% methanol, 2:70% methanol, 3:100% ethanol, 4:70% ethanol, 5:100% acetone, 6:100% distilled water, 7: Negative control.

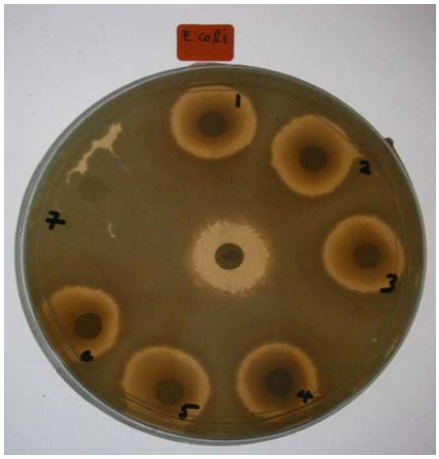

Fig. 9: Antimicrobial activity of the extracts on *P.aeruginosa*: Disc diffusion test for the effect of *Q.infectoria* against *P.aeruginosa* grown on nutrient agar medium. 1:100% methanol, 2:70% methanol, 3:100% ethanol, 4:70% ethanol, 5:100% acetone, 6:100% distilled water, 7:Negative control.

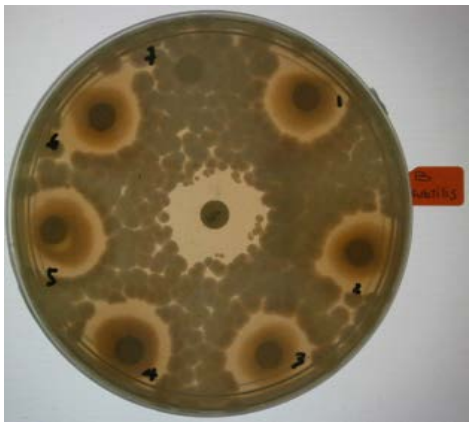

Fig. 10: Antimicrobial activity of the extracts on *B.subtilis*: Disc diffusion test for the effect of *Q.infectoria* against *B.subtilis* grown on nutrient agar medium. 1:100% methanol, 2:70% methanol, 3:100% ethanol, 4:70% ethanol, 5:100% acetone, 6:100% distilled water, 7: Negative control.

e-ISBN 978-967-960-322-4

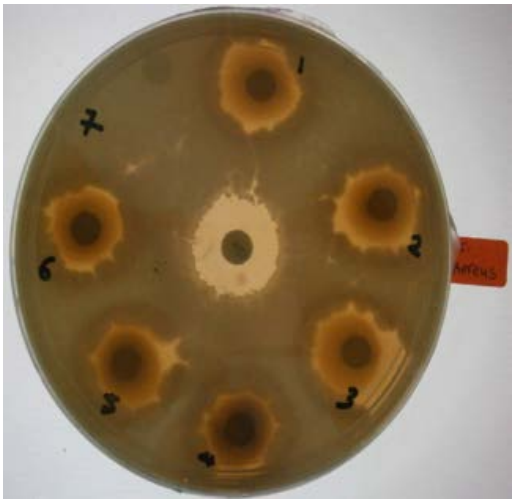

Fig. 11: Antimicrobial activity of the extracts on *S.Aereus*: Disc diffusion test for the effect of *Q.infectoria* against *S.aereus* grown on nutrient agar medium. 1:100% methanol, 2:70% methanol, 3:100% ethanol, 4:70% ethanol, 5:100% acetone, 6:100% distilled water, 7:Negative control.

All the samples were tested again two gram negative bacteria (*E.coli and P.aeruginosa*) and two gram positive bacteria (*B.subtilis and S.aereus*).The inhibition zone was measured after 24 hours.

Table 2: Antibacterial activity of different extracts of manjakani galls at 24 hours

Type of Solvents	Diameter of inhibition zone (mm)± S.D			
	E.coli	*P.aeruginosa*	*B.subtilis*	*S.aereus*
100% methanol (1)	17.0±0.00	13.0±.0.14	16.5±0.07	16.5±0.21
70% methanol (2)	16.5±0.07	15.0±0.14	17.0±0.00	15.5±0.21
100% ethanol (3)	17.0±0.00	14.5±0.21	18.0±0.21	15.5±0.21
70% ethanol (4)	17.0±0.14	15.0±0.14	17.5±0.07	14.5±0.21
100% acetone (5)	18.0±0.14	12.5±0.21	19.0±0.14	14.0±0.14
100% aqueous (6)	15.0±0.00	13.0±0.14	17.0±0.14	14.5±0.21
Neg. control (7)	-	-	-	-
Strepromycin	15.5±0.07	15.0±0.14	19.5±0.07	17.5±0.21

As can be obtained from table 2, all the solvent extracts showed significant inhibitory activity. After 24 hours, the largest inhibition zone was shown by 100% acetone (19.0mm) against *B.subtilisi* and it was comparable with the commercial antibiotics (19.5.mm) while other samples also showed strong inhibition zone varies 12.50mm until 18.0mm. From the findings, the antimicrobial properties of alcoholic and acetone extract were superior compare to aqueous extract for those selected bacteria. Our studies showed that the extracts from the galls inhibited the gram-positive bacteria are about equal with gram-negative. Generally, plant extracts are usually more active against gram-positive bacteria than gram-negative bacteria.

From the observation, the different types of solvent do not show any significant difference because all the sample extracts were observed to inhibit the growth of the bacterial effectively. Our findings were also supported by other researchers who reported that methanol, ethanol and aqueous extract showed strong inhibition toward pathogenic bacterial strains [26], while [18] also documented on the potential of aqueous and acetone extracts of galls as antibacterial agents.

Tannin is widely known as one of the phenolic compound that is easily dissolved in water, alcohol, and acetone and gives precipitate with protein [27]. The similarity of all solvents selected in the antimicrobial activity suggests that these extracts may have high total tannin content and can influence the antimicrobial activity of the biological plant [28].In addition, tannin might be the bioactive compound which may be

responsible for the antibacterial activity in this study due to the galls implied high amount of tannin [8, 10, 11]. Other than that, [28] also supported this finding where they claimed that tannin in plant extracts was found to possess antimicrobial activity.

CONCLUSIONS

HPLC analysis has been identified and quantified the gallic acid and tannic acid in *Q.infectoria* (manjakani) galls extract. The result demonstrated that conventional soxhlet extraction is efficient to extract the bioactive compounds from the plants. The strong antioxidant and antibacterial activities of manjakani extracts are probably due to the bioactive compounds presence in the plants. However, further investigation of individual phenolic compounds, their in vivo antioxidant and antibacterial activity is necessary before application of *Q.infectoria* in pharmaceutical and food product can be advocated.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial supports from Minister of Higher Education (MOHE) and acknowledgement is also extended to Universiti Teknologi Malaysia (UTM) for the use of laboratory instruments/ equipments and their kind supports and research grant GUP (Q.J130000.7125.02H01) during this study.

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