International Food Research Journal 21(3): 1067-1073 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of solvent extraction on antioxidant and antibacterial activities from *Quercus infectoria* (Manjakani)

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Article history

Received: 11 April 2013
Received in revised form: 19 January 2014
Accepted: 20 January 2014

Keywords

Quercus infectoria
Tannic acid
Gallic acid
Antioxidant activity
Antibacterial activity

Abstract

Bioactive compounds from *Quercus Infectoria* (manjakani) were extracted with six different types of solvents: 100% methanol, ethanol, acetone, aqueous and 70% methanol and ethanol. High Performance liquid chromatography (HPLC) was used to identify and quantify the active compounds, namely gallic acid and tannic acid. Total phenolics content were determined by Folin-Ciocalteu while antioxidant and antibacterial activity were tested using DPPH free radicals scavenging and disc diffusion assay. The result revealed that aqueous extract contained the highest concentration of bioactive compounds compared to other types of solvents which are 51.14 mg/g sample and 1332.88 mg/g sample of gallic acid and tannic acid respectively.. The highest level of phenolic compound was found in 100% acetone extract (121 mg GAE/g). The results demonstrated that aqueous extract gives the highest antioxidant activity approximately 94.55% while acetone extract gives the largest inhibition zone for disc diffusion assay which is 19.00 mm 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.

Introduction

Numerous studies have been conducted on the potential of traditional plants on their biologically activity for medicinal purpose. The biologically activity of these plants is related to their bioactive compounds which definitely produce physiological actions on human body (Mohemmadi *et al*., 2011). There are wide variety of bioactive compounds that gained interest among researchers such as tannins, saponins, alkaloids, flavonoids and phenolic compounds (Hill, 1952). The extraction of active compounds is highly depending on the polarity of the solvent because polar compound is easily extracted using polar solvent (Goli *et al*., 2004). Thus, the solvent used for the extraction of bioactive compounds must be critically chosen because it will influence the quantity and quality of the final extract (Sinero *et al*., 2008). *Q. infectoria* is one of the traditional plants that have great potential on the medicinal purpose and have been reported to possess antioxidant (Everest, 2005) and antibacterial (Hamid *et al*., 2005). In Asian, it has been used for centuries as traditional medicine for treating inflammatory disease (Galla, 1911; Kaur *et al*., 2004) while in Malaysia it has been used as a herbal drink to treat the women after their childbirth to restore the elasticity of the uterine wall. Other than that, by using the hot water extract of *Q. infectoria* as a mouth antiseptic, it can control the inflammation of tonsils, while the direct application of it onto the skin cures swelling or inflammation (Chopra *et al*., 1956). Moreover, this herb also show promising results in cosmeticeutical where it was (Rohana *et al*., 2004) reported that the galls possess high potential in skin whitening. The potential of *Q. infectoria* in medical and cosmaceutical areas have induced researchers to study and investigate further details about its usage and application.

The galls is greatly used as medicinal plant since ancient times because it was reported to contain large amount of bioactive constituents such as tannins, gallic acid, syringic acid and others (Dar *et al*., 1976: Ikram and Nowshad, 1977; Hwang *et al*., 2000). The main constituents found in the galls of Q.infectoria are tannin (50-70%) and small amount of free gallic acid and ellagic acid (Ikram and Nowshad, 1977; Evan, 1996; Wiart and Kumar, 2001). Most study on *Q. infectoria* was on identification and isolation, biological and pharmacological studies (Basri *et al*., 2005; Kaur *et al*., 2005; Asghari *et al*., 2011). However, none of the study reported on the effects of solvents toward antioxidant and antibacterial activity.

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Therefore, the objective of the present study was to identify and quantify gallic acid and tannic acid from the gall extracts using six different types of solvents by using HPLC, and to evaluate the antioxidant and antibacterial activity using DPPH free radicals scavenging and disc diffusion method.

Material and Method

Plant material

Q. infectoria galls were purchased from local herbal shop in Kota Tinggi, Johor Bharu, Malaysia.

Chemicals and reagents

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.

Extraction of plant material

To prepare the extract, 5 g of powdered galls were weighed and placed in a Whatman 25 mm x 100 mm cellulose thimble while 150 ml of methanol (100%) was placed at the bottom of the apparatus. The extraction process was done for 6 hours at the boiling point.. Then, the extraction yield was put in the rotary evaporator at 40°C 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 the following equation:

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

High Performance Liquid Chromatography (HPLC)

Determination of Gallic Acid

Determination of active constituents from extracted compounds were examined using high performance liquid chromatography as described by (Pin *et al*., 2006) with a slight modification.

In order to evaluate the quality of extracted compounds, all of the samples were analyzed by using high performance liquid chromatography using gallic acid as chemical marker. 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% acetonitrile (100%) as solvent B. Then, the flowrate of mobile phase was adjusted at 1 ml/min at 280 nm and every injection was set until achieved 10 µL.

Determination of tannic acid

The concentration of tannic acid from the extracts was determined as reported by Asghari *et al.* (2011) 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 consisted of solvent A (25 ml acetic acid and 975 ml distilled water) and solvent B (99.8% methanol) pumped at 1 mL/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 280 nm. The percentage of the tannin acid was calculated using the appropriate calibration curves.

DPPH radical scavenging assay

This assay was carried out according to the method of Miliauskas *et al.* (2004) with a slight modification. DPPH or 2,2-diphenyl-1-picrylhydrazyl is stable free radicals, which forms a purple-coloured solution when dissolved in methanol. Antioxidant components can scavenge these stable free radicals and therefore the purple colour will be bleached. Extract solution was prepared by dissolving 0.025 g of dry extract in 10 ml of methanol to give a final concentration of 2.5 mg/ml. After that, 77 µL of the extract solution was mixed with 3 ml of 6 x 10^{-5} M methanolic solution of DPPH. After that, the mixture was placed in the dark for 30 minutes at room temperature and the decrease in the absorption was measured at 517 nm by using spectrophotometer. Radical scavenging activity of the samples was calculated by using the following formula:

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

Determination of total phenolic content

The total phenolic content (TPC) of each sample was estimated using the Folin-Ciocalteu colorimetric method according to Slinkard and Singelton (1977) with minor modifications. Appropriately, 20 µL diluted test sample (1 mg/ml) was reacted with Folin-Ciocalteu reagent (100 µL) and 1.58 mL of water for 8 minutes at room temperature. Then, the mixture was neutralized with 300 µL of sodium carbonate (75 g/l) and the mixture was left for 2 hours in the dark at room temperature. The absorption was read at 765 nm against a reagent blank (containing all test reagents except for sample). All the determinations were performed in triplicate. Quantification was

done on the basis of a standard curve with gallic acid. Result was expressed as gram of gallic acid equivalent (GAE) per gram dry weight.

Antibacterial assay

The disc diffusion method was used to evaluate the antibacterial activity according to Basri *et al.* (2012) with a slight modification. Nutrient agar prepared earlier was used as the media for the test microorganism. The extracts from the galls of *Q. infectoria* at 50 mg/ml were screened against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). First, 50 µL of bacteria 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, 6 mm) was impregnated with 20 µl of each of the extracts (50 mg/ml). Then, streptomycin (10 µg/ disc) was used as a 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°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 (Basri and Fan, 2005). The inhibition zone was measured after 24 hours.

Statistical analysis

The data presented were analyzed by using SPSS 16.00 for windows (SPSS Inc., Chicago, IL). The significance difference between the data were analyzed by using one-way analysis of variance (ANOVA) and Tukey's test at 95% confidence level.

Results and Discussion

Solvent effects on extract yield

Based on Table 1, 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%). Tian *et al.* (2009) have reported similar findings, in which the highest yield of *Galla chinensis* was obtained from aqueous solvent. On the other hand, 100% acetone resulted on lowest extraction yield suggesting that bioactive compounds in *Q. infectoria* is easier to extract with solvents that are more polar. However, from ANOVA analysis, the extraction yield from all solvents did not show significant difference ($P > 0.05$). The results above also indicate that the mixture of organic solvents give higher extraction yield compared to the pure solvent alone. This finding was compatible with the previous findings as documented by Markom *et al.* (2007) where they found that the addition of water in ethanol significantly increased the extraction yield of *Phyllanthus niruri* Linn.

Table 1. Total extraction yield and concentration of gallic acid and tannic acid from *Q. infectoria* using different types of solvents

Types of solvents	Yield (%)	Gallic acid (mg/g sample)	Tannic acid (mg/g sample)
100% methanol	69.54±0.07[a]	51.14	1332.88
70% methanol	76.34±0.04[a]	71.13	1823.31
100% ethanol	45.71±0.13[a]	37.22	954.03
70% ethanol	71.44±0.06[a]	99.39	2512.22
100% acetone	43.57±0.19[a]	34.04	949.34
100% aqueous	80.03±0.07[a]	101.55	2975.11

Values are expressed as mean± SD of duplicate measurement in which different letters for each column (a-c) are significantly different at $p < 0.05$.

The suitable solvent for extracting target compounds should be selected carefully because the extracted compound will be based on the type of solvents used (Zarnowski and Suzuki, 2004). 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 (Franco *et al*., 2008).

High performance liquid chromatograph

In HPLC analysis (Table 1), the similar trend was found for both gallic and tannic acid in which 100% aqueous> 70% ethanol> 70% methanol> 100% methanol> 100% ethanol> 100% acetone. As shown in Table 1, aqueous extracts contain highest concentration of gallic acid (101.55 mg/g sample) and tannic acid (2975.11 mg/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. The analysis revealed that tannic acid constitute a major bioactive compound of the extract and due to its high concentration in gall extract, most properties of the extract can at least be attributed to tannic acid. In notion to this, gall extract was found to scavenge free radicals from DPPH (Yokozawa *et al.*, 1998; Lopes *et al.*, 1999; Gyamfi and Aniya, 2002) and inhibit the growth of gram positive and gram negative bacteria (Asghari *et al.*, 2011). In addition, higher yield of these compounds might contribute to other pharmaceutical use such as anti-inflammatory, anticancer, antimutagenetic and astringent activity (Gicher *et al.*, 1987). Previous study reported lower concentration of gallic acid and tannic acid from ethanolic extract of *Q. infectoria* which is 87.5

Table 2. DPPH free radicals scavenging activity and total phenolic content of *Q. infectoria* using different types of solvent

Types of solvents	DPPH free radicals scavenging (%)	Total phenolic content
100% methanol	93.38±0.18[a]	95.86 ±2.02[a]
70% methanol	94.36±0.64[a]	112.29±3.03[c]
100% ethanol	92.60±1.29[a]	109.79±6.57[b,c]
70% ethanol	92.86±0.18[a]	99.43±2.02[a,b]
100% acetone	92.83±1.61[a]	107.65±0.5[a,b,c]
100% aqueous	94.55±0.37[a]	95.86±1.01[a]

Values are expressed as mean ± SD of duplicate measurement in which different letters for each column (a-c) are significantly different at $p < 0.05$.

mg/g and 199.25 mg/g, respectively (Kaur *et al.*, 2008). However, this finding were also supported by literature as carried out from previous study which revealed the presence of tannic acid, gallic acid, syringic acid, ellagic acid, β-sitosterol, amentoflavone hexamethyl ether, isocryptomerin, starch, essential oils, anthocyanins, methyl-betulate, methyl- oleanate, hexagalloyl-glucose and polygalloyl-glucose (Dar *et al.*, 1976; Ikram and Nowshad, 1977; Hwang *et al.*, 2000) in gall extract of *Q. infectoria.*

DPPH free radical scavenging

Free radicals scavenging activity (Table 2) showed that water extract (94.55%) gives the highest DPPH scavenging activity and followed closely by 70% methanol (94.35%) and 100% methanol (93.38%). However, other solvents also depicted high free radicals scavenging varying from 92.60% (100% ethanol) to 92.83% (100% acetone). Tian *et al.* (2009) documented on the contradictive result where they found that ethanol extract of *Galla chinensis* gives higher reduction activity compared to the aqueous extract. The different finding is due to the ability of the solvent to extract the bioactive compounds is differ for different plant. To date, the antioxidant activity of *Q. infectoria* galls extracts using different types of solvents has not been well documented but from the results obtained, types of solvents does not give significant differences towards the scavenging of free radicals ($P > 0.05$).

However, this findings was compatible with Jain *et al.* (2011) where they found that *Q. infectoria* possess antioxidant activity by scavenge the free radicals about 90.02% while Kaur *et al.* (2008) reported lower antioxidant activity which is about 71.5%. This variation in finding might be due to the different concentration and solvent used. Moreover, the different origins of the raw material also influence the growth of plant itself because the different soil composition will yield different vegetative traits (Devkota and Jha, 2009).

From the observation, the extracted compound from the 70% methanol and ethanol gave higher antioxidant activity compared to the absolute solvent and Turkmen *et al.* (2006) reported the same findings

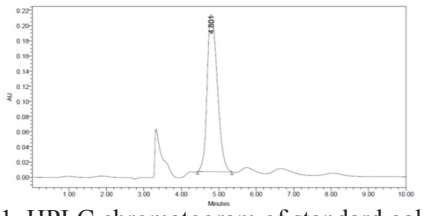

Figure 1. HPLC chromatogram of standard gallic acid

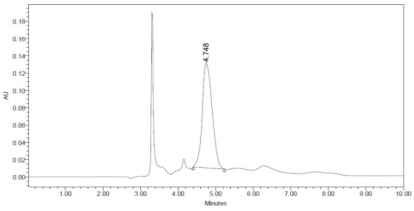

Figure 2. Gallic acid 100% methanol extract of *Q. infectoria*

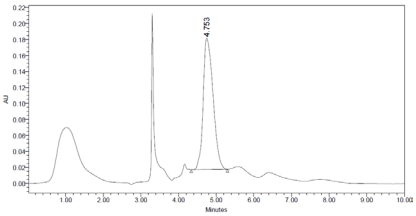

Figure 3. Gallic acid from 70% methanol extract of *Q. infectoria*

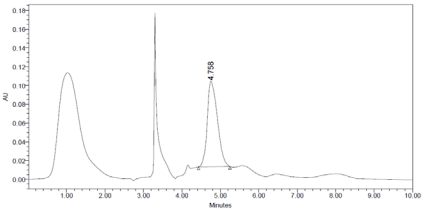

Figure 4. Gallic acid from 100% ethanol extract of *Q. infectoria*

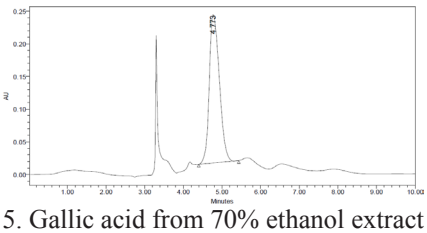

Figure 5. Gallic acid from 70% ethanol extract of *Q. infectoria*

where they found 50% and 80% of solvent mixture exhibited considerably higher DPPH radicals scavenging activity compared to the pure solvent. On the basis of the result obtained, *Q. infectoria* 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 of *Q. infectoria* might be due to the presence of gallic acid and tannic acid which are proven to possess antioxidant activity (Robert *et al.*, 1999; Govindarajan *et al.*, 2005).

Total phenolic content

As shown in Table 2, the total phenolic content of *Q. infectoria* was ranged from 95.86 to 112.29

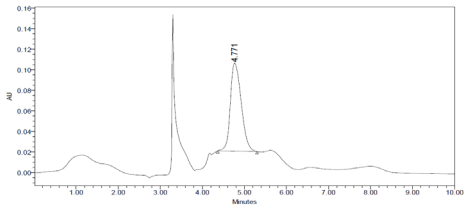

Figure 6. Gallic acid from 100% acetone extract of *Q. infectoria*

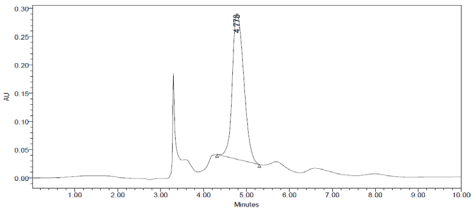

Figure 7. Gallic acid from 70% ethanol extract of *Q. infectoria*

mg GAE/ respectively. Total phenolic content was estimated by using Folin-Ciocalteu colorimetric method, based on the procedure of Slinkard and singleton (1977) and using gallic acid as a standard phenolic compound. From the finding, extract using 70% methanol shows the highest total phenolic content while 100% methanol and aqueous extracts show the lowest phenolic content. The results obtained in the present study are lower than reported by Kaur *et al.* (2008) where they reported the ethanolic extract from *Q. infectoria* was found to contain 416 ± 10.6 mg GAE/g. The differences could be attributed to the different age of plant, climate and growth location. This obtained amount of phenolic content might be the reason for the high scavenging activity of *Q. infectoria* since antioxidant activity may be directly correlated to the polyphenolic content (Kaur *et al.*, 2008). In addition, there are wide ranges of research found the positive linear correlation between antioxidant activity and total phenolic content (Velioglu *et al.*, 1998; Miliauskas *et al*., 2004; Dorman *et al*., 2004; Surweswaran *et al*., 2007; Tawaha *et al.*, 2007).

The Folin-Ciocalteu assay gives a crude estimate of total phenolic content in the plant extracts. Moreover, sugars, ascorbic acids and some simple phenols also react with the Folin-Ciocalteu reagents although they are not effective radical scavenging antioxidants which in turn giving elevated apparent phenolic (Huang *et al*., 2005; Prior *et al*., 2005). Generally, each phenolic compounds of extracts respond in different way in this assay, depending on the number of phenolic groups they have (Singelton and Rossi, 1965). Thus, this might explain the equivocal correlation between total phenolic content and antioxidant of several extracts. For example, although the total phenolic content of aqueous extracts (95.85 mg GAE/g) is lower than extracts from 100% ethanol (109.79 mg GAE/g), the corresponding scavenging activity of aqueous extracts (94.55%) is higher than that of 100% ethanol extracts (92.60%). However, from the high performance liquid chromatography, *Q. infectoria* is proven to contain gallic acid and tannic acid which might be the reason to the high scavenging activity of stable free radicals.

Antibacterial activity

As can be seen from Table 3, all the extracts showed inhibitory effects which were not significantly different ($P > 0.05$) against each bacterial species tested. In other words, the sizes of inhibitory zones showed by all of the organic and aqueous solvent did not differ significantly against all of the tested bacteria.

After 24 hours, the largest inhibition zone was shown by the extracts of 100% acetone (19.0 mm) against *B. subtilis* and it was comparable with the commercial antibiotics (19.5 mm) but other samples also showed inhibition zone varying from 12.50 mm to 18.0 mm. However, *B. subtilis* was found to be most susceptible towards all of the extracts. The smallest inhibition zone was exhibited by 100% acetone extract against *P. aeruginosa*.

From table 3, the antimicrobial properties of alcoholic and acetone extract were superior compared to aqueous extract for those selected bacteria. This finding was in accordance with previous research which had reported that the plant extracts using organic solvent exhibited more antibacterial activity compared to the extract using aqueous as a solvent (Parekh *et al.*, 2005; Turkmen *et al*., 2006) while Basri *et al.* (2005) also documented on the potential of aqueous and acetone extracts of galls as antibacterial agents. Furthermore, this present study also showed that the extracts from the galls were active in both gram positive and gram negative bacteria which were contradictive with the previous study where they found that extracts from the galls were more active against gram positive bacteria rather than gram negative bacteria (Basri *et al*., 2005). This difference might be due to the differences bacteria used which are *Streptococcus mutans* and *Streptococcus salivirus* for gram positive bacteria and *Porphyromonas gingivalis* and *Fusobacterium nucleatum* for gram negative bacteria. In addition, the most interesting finding was that inhibition zone showed by all of the extracts was bigger compared to the positive control against *E. coli*. This suggests that *E. coli* was more susceptible to the extracts compared to the commercial antibiotics (streptomycin).

The antibacterial activity of the extracts might be due to the presence of gallic acid and tannic acid which are derivatives of tannin. Tannin is widely known as

one of the phenolic compound that is easily dissolved in water, alcohol, and acetone and gives precipitate with protein (Leela and Satirapipathkul, 2011). The similarity of all solvents selected in the antimicrobial activity might be due to the high tannic acid and gallic acid concentration found in the extracts. Moreover, these bioactive compounds have reported to possess and can influence the antimicrobial activity of the biological plant (Ikram and Nowshad, 1977; Evans, 1996; Wiart and Kumar, 2001, Leach, 1986; Moller *et al*., 2009). Other than that, Leach (1986) also supported this finding where they claimed that tannin in plant extracts was found to possess antimicrobial activity.

Conclusion

HPLC analysis has been identified and quantified the gallic acid and tannic acid in *Q. infectoria* (manjakani) galls extract. The strong antioxidant and antibacterial activities of *Q. infectoria* extracts are probably due to the bioactive compounds presence in the plants. However, different types of solvents do not give significant effect toward the free radicals scavenging and antibacterial activity.

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.

References

Asghari, J., Ondruschka, B. and Mazaheritehrani, M. 2011. Extraction of Bioactive Chemical Compounds from the Medicinal Asian Plants by Microwave Irradiation. Journal of Medicinal Plants Research 5(4): 495-506.

Basri, D. F. and Fan, S. H. 2005. The Potential of Aqueous and Acetone Extracts of Galls of Quercus Infectoria as Antibacterial Agents. Indian Journal of Pharmacology 37: 26-69.

Basri, D. F., Tan, L. S., Shafiei, Z. and Zin, N. M. 2012. In vitro Antibacterial Activity of Galls of Quercus infectoria Oliver against Oral Pathogens. Evidence-Based Complementary and Alternative Medicine 632796.

Chopra, R.N., Nayar, S. I. and Chopra, I.C. 1956. Glossary of Indian Medicinal Plant. Council of Scientific and Industrial Research India 208.

Dar, M. S., Ikram, M. and Fakouhi, T. 1976. Pharmacology of Quercus Infectoria. Journal of Pharmaceutical Sciences 65: 1791-4.

Devkota, A. and Jha, P. K. 2009. Variation in growth of centella asiatica along different soil composition. Botany Research International 2(1): 55-60.

Dorman, H. J. D., Bachmayer, O., Kosar, M. and Hiltunen, R. 2004. Antioxidant properties of aqueous extracts from selected *Lamiaceae* species grown in Turkey. Journal of agricultural and Food Chemistry 52: 762-770.

Evans, W. C. 1996. Pharmacopoeial and Related Drugs of Biological Origin. London.: WB saunders Co. Ltd, 1996.

Everest, A. and Ozturk, E. 2005. Focusing On the Ethnobotanical Uses of Plants in Mersin and Adana Provinces (Turkey). Journal of Ethnobiology and Ethnomedicine 1: 1-6.

Franco, D., Sineiroz, J., Rubilar, M., Sanchezz, M., Jerezz, M., Pinelo, M., Costoya, N. and Nunez, M. J.2008. Polyphenols from Plant Materials: Extraction and Antioxidant Power. Electronic Journal of Environmental, Agricultural and Food Chemistry 7(8): 3210-3216.

Galla, B. P. 1911. Council of the Pharmaceutical Society of Great Britain.

Gichner, T., Pospisil, F., Veleminsky, J., Volkeova, V. and Volke, L. 1987. Two types of antimutagenic effects of gallic acid and tanni acids N- nitroso-compounds-induced mutagenicity in the Ames *Salmonella* assay. Folia Microbiologia.

Goli, A. H., Barzegar, M., Sahari, M. A. 2004. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. Food Chem 92: 521–525

Govindarajan, R., Vijayakumar, M. and Pushpangadan, P. 2005. Antioxidant approach to disease management and the role of Rasayana herbs of Ayurveda. Journal of Ethnopharmacology 99: 165-178.

Gyamfi, M. A. and Aniya, Y. 2002. Antioxidant properties of Thonninggianin A, isolated from the African medical herbs, Thonningia sanguine, Biochem. Pharmacol 63: 1725-1737.

Hamid, H., Kaur, G., Abdullah, S.T., Ali, M., Athar, M. and Alam, M.S. 2005. Two New Compounds From The Galls Of Quercus Infectoria With Nitric Oxide And Superoxide Inhibiting Ability. Pharmaceutical Biology 43: 317-323.

Hill, A.F. 1952. Economic Botany A Textbook of useful Plant Products. 2nd Edn, McGraw - Hill Book Company p. 430- 432. New York:McGraw - Hill.

Huang, D., Ou, B. and Prior, R.L. 2005. The chemistry behind antioxidantcapacity assays. J Agric Food Chem 53: 1841–1856.

Hwang, J. K., Kong, T. W., Baek, N. I. and Pyun, Y.R. 2000. α-Glycosidase Inhibitory Activity Of Hexagalloylglucose From The Galls Of Quercus Infectoria. Plant Medicine 66: 273-4.

Ikram, M. and Nowshad, F. 1977. Constituent of Quercus Infectoria. Plant Medicine 31: 286-7.

Jain, N., Goyal, S. and Ramawat, K. G.2011. Evaluation of antioxidant properties and total phenolic content of medicinal plants used in diet therapy during

postpartum healthcare in Rajasthan. International Journal of Pharmacy and Pharmaceutical Sciences Vol 3, Issue 3.

Kaur, G., Athar, M. and Alam, M.S. 2008. Quercus Infectoria Galls Possess Antioxidant Activity and Abrogates Oxidative Stress-Induced Functional Alterations In Murine Macrophages. Chemico-Biologocal Interaction 171(3): 272-282.

Kaur, G., Hamid, H., Ali, A., Alam, M.S. and Athar, M. 2004. Antiinflammatory Evaluation of Alcoholic Extract of Galls of Quercus Infectoria. Journal of Ethnopharmacology 90(2-3): 285- 292.

Leach, C.K. 1986. The Phenolic Contents of Some British Cynipid Galls. Cecidology, 1: 10-2.

Leela, T. and Satirapipathkul, C.2004. Studies on the Antibacterial Activity of Quercus Infectoria Galls. Proceedings of the International Conference on Bioscience, Biochemistry and Bioinformatics, March 10-11, Singapore: IPCBEE, 5.

Lopes, G. K., Schulman, H.M. and Hermes-Lima, M.1999. Polyphenol tannins acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions, Biochim.Biophys. Acta 1472; 142-152.

Markom, M., Hasan, M., Wan Daud, W., Sigh, H. and Jahim, J. M. 2007. Extraction of hydrolysable tannins from *Phyllanthus niruri* Linn.: Effects of solvents and extraction methods. Separation and Purification technology 52: 487-496.

Miliauskas, G., Venskutonis, P. R. and van Beek, T, A. 2004. Screening of Radical Scavenging Activity of Some Medicinal and Aromatic Plant Extracts. Food Chemistry 85: 231-237.

Mohemmadi, Z. 2011. Impact of Solvent Extraction Type on Total Polyphenols Content and Biological Activity from *Tamarix aphylla* (L.) Karst. International Journal of Pharma and Bio Sciences 2(1): 609-615.

Moller, C., Hansen, S. H. and Cornett, C. 2009. Characterisation of Tannin-Containing Herbal drugs by HPLC.Phytochemical Analysis 20: 231-239.

Parekh, J., Jadeja, D. and Hanlin, R.L. 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turkish Journal of Biology 29, 203-210.

Pin, K.Y., Chuah, T.G., Abdull Rashih, A., Rasadah, M. N., Choong, T.S.Y. and Law, C.L. 2006. Effects of the Concentration of Quercus Infectoria Galls (Manjakani) Extract on Moisture Content and Quality of Its Freeze-Dried Product. International Journal of Engineering and Technology 3(2): 167-174.

Prior, R.L., Xianli, W.U. and Schaich, K.2005. Standardized methods for the determination of antioxidants capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53: 4290-4302.

Robert, R. E., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical Biology and Medicine 26: 1231-1237.

Rohana, S., Vimala, S., Abdull Rashih, A. and Mohd Ilham, A. 2004. Skin Whitening and Antioxidant Properties of Quercus Infectoria Galls. Proceedings, of the Seminar on Medicinal Plants edited by Y.S.

Sineiro, J., Franco, D., Rubilar, M., Sanchez, M., Jerz, M., Pinelo, M., Costoya, N. and Jose Nunez, M. 2008. Polyphenols from Plant Materials: Extraction and Antioxidant Power. EJEAFChe 7(8): 3210-3216.

Singleton, V. L. and Rossi, V. L. 1965. Colorimetry of total phenolics with phospo-molybdic-phosphotungustic acid reagents. American Journal of enolofy and Viticulture 16; 144-158.

Slinkard K. and Singleton V. L. 1977. Total phenol analyses: Automation and Comparison with Manual Methods. Am. J. Enol. Vitic 28: 49-55.

Surweswaran, S., Cai, Y. Z., Rorke, H. and Sun, M. 2007. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plant. Food Chemistry 102; 938-953.

Tawaha, K., Ali, F. Q., Ghairabeh, M., Mohammad, M. and El-Elimat, T. 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food chemistry 104: 1372-1378.

Tian, F., Li, B., Ji, B., Yang, J., Zhang, G., Chen, Y. and L, Y. 2009. Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*: The polarity affects the bioactivities. Food Chemistry 113; 173-179.

Turkmen, N., Sari, F. and Velioglu, Y. S. 2006. Effects Of Extraction Solvents on Concentration and Antioxidant Activity of Black and Black Mate Tea Polyphenols Determined by Ferrous Tartrate and Folin-Ciocalteu Methods. Food Chemistry 99: 835-841.

Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry 46, 4113-4117.

Wiart, C. and Kumar, A. 2001. Practical Handbook of Pharmacognosy Malaysia. Malaysia. Pearson Education Malaysia Sdn Bhd.

Yokozawa, T., Chen, C.P., Dong, E., Tanaka, T., Nonaka, G. I. and Nishioka, I. 1998. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical,Biochem. Pharmacol 56: 213-222.

Zarnowski, R. and Suzuki, Y. 2004. Expedient Soxhlet Extraction of Resorcinolic Lipids from Wheat Grains. Journal of Food Composition and Analysis 17: 649-656.