IJPSR (2016), Vol. 7, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 May, 2016; received in revised form, 25 June, 2016; accepted, 29 June, 2016; published 01 November, 2016

PROXIMATE ANALYSIS AND QUANTITATIVE ESTIMATION OF GALLIC ACID IN *QUERCUS INFECTORIA* OLIV. GALLS BY HPTLC

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Keywords:

Quercus infectoria; proximate analysis; HPTLC; gallic acid

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ABSTRACT: The galls of *Quercus infectoria* Oliv. (Family: Fagaceae) are commonly known as "Manjakani" by Malaysian community. It is one of the most popular traditional medicines used by the females to help regression of prolapsed uterus. The aim of this study was to perform the proximate analysis and quantitative estimation of gallic acid in the methanol extract of locally available galls of *Q. infectoria* using HPTLC. The gall powder was subjected to the quantitative determination of moisture content, ash and extractive values according British Pharmacopoeia. Preliminary phytochemical studies were performed on different extracts to find out the nature of phytoconstituents they contain. Quantitative estimation of gallic acid in the methanol extract was assessed through denstiometric scanning using a TLC Scanner 3 (Camag, Switzerland) with win CATS software. The results of the preliminary phytochemical screening of various extracts revealed presence of steroids, triterpenoids, saponins, flavonoids, tannins and phenolic compounds, carbohydrates, gums and mucilages respectively in the galls. Amount of of gallic acid in the sample was found to be 0.218 ± 0.0011% w/w. The present method was validated for linearity, accuracy, precision, and specificity with reference to ICH guidelines. The developed method is capable of quantifying gallic acid in locally available samples of *Q. infectoria.*

INTRODUCTION: The use of herbal medicines represents a substantial portion of global market in recent years. Considering the growing importance of herbal medicines, concerns about their safety and claimed efficacy and lack of proper scientific evaluation has also been discussed in several forums. The quality control of herbal medicines therefore, still remains a challenge and requires special approaches.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(11).4400-06

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (11).4400-06

The WHO emphasizes the importance of the qualitative and quantitative methods for standardization, chemical fingerprint profiles and quantification of the markers [1]. Markers serve to determine the quality of herbal medicinal product if determined quantitatively in the sample.

The galls of *Quercus infectoria* Oliv. (Family: Fagaceae) are commonly known as 'Manjakani' by Malaysian community. It is one of the most popular traditional medicines used by the females to help regression of prolapsed uterus. The powdered gall mixed with alum and tied in a muslin gauge is inserted in to the vagina to get the effect. Alternatively, it has been used as herbal drink to treat the women after their childbirth to restore the elasticity of the uterine wall. As an astringent, the

decoction is used orally to treat diarrhoea and dysentery, and applied locally as washings to treat all types of haemorrhoids, anal fissures and prolapsed rectum [2]. There are also ointments with powders of galls incorporated in to it as a local application for these anal diseases [3]. Sometimes, the galls are recommended as an antidote for plant poisons due to opium, nux vomica and aconite [4]. The galls are reported to contain tannins, gallic acid, syringic acid and ellagic acid [5]. Most of the earlier studies on *Q. infectoria* were on the pharmacognostic, pharmacological and biological studies including anti-inflammatory, antidiabetic, larvicidal, antiulcerogenic, gastroprotective, antibacterial and antiviral activities [5, 6]. However, there are no reports on the quantitative estimation of gallic acid from the galls. In the present study, we report the proximate analysis of locally available galls of *Q. infectoria* along with quantitative estimation of gallic acid using HPTLC.

MATERIALS AND METHODS:

Plant material:

The dried galls of *Q. infectoria* were procured from the local market in Ipoh and identified based on their physical characteristics described earlier[7,8]. The collected galls were pulverised to powder. The dried gall powder was preserved in a desiccator and used for further study.

Proximate analysis:

The gall powder was subjected to the quantitative determination of moisture content, ash and extractive values according to the procedure described in British Pharmacopoeia [9].

Preliminary phytochemical studies:

A known quantity of dried powder (50 g) was extracted successively with petroleum ether (40-60°C), chloroform, methanol and water. Following extraction, the liquid extracts were concentrated under vacuum and further subjected to preliminary phytochemical studies to find out the nature of phytoconstituents they contain [8, 10].

HPTLC analysis:

Sample preparation: Dried gall powder (2.5 g) was extracted exhaustively in 60 ml methanol (20 ml methanol each time) by ultrasonication for 30 min, until the extract was free from gallic acid which was confirmed from ferric chloride test and TLC studies. Following extraction, the liquid extract was filtered through a Whatman filter paper and the filtrate was then subjected to centrifugation at 3000 rpm for 10 min. The supernatant liquid was used for the analysis. Solution of standard gallic acid (0.1 mg/ml) was prepared by dissolving 10 mg gallic acid in 100 ml methanol.

Chromatographic condition:

HPTLC studies were performed on aluminium plates of silica gel 60 F254 (20 × 10 cm with 0.2 mm thickness; E. Merck, Darmstadt, Germany) [11]. Sample application on the plates was done through automatic TLC applicator under a flow of nitrogen gas as 6 mm band with a Linomat V automatic sample spotter (Camag, Switzerland), positioned at 10 mm from the bottom of the plate. The mobile phase used was Toluene: Ethyl acetate: Formic acid: Methanol with a ratio of 12: 9: 4: 0.5. Sample solution (5 µl/spot) was applied (n = 4). Standard solution of gallic acid was applied (4 µl) on the plate. After application of the test samples, the plates were dried in hot air oven at 105˚C for 5 min. The plate was developed to a height of 8 cm in a Camag twin trough chamber at 25± 2°C and 40% relative humidity (RH). Denstiometric scanning was performed by using a TLC Scanner 3 (Camag, Switzerland) with win CATS software. The wavelength of the detector was set at 272 nm.

Preparation of calibration curve of gallic acid and quantification of gallic acid in the sample:

From a working standard solution of gallic acid (0.1 mg/ml), 1, 2, 3, 4, 5, 6 and 7 µl of the solution was applied on the plate, corresponding to concentrations of 98.4, 196.8, 295.2, 393.6, 492.0, 590.4 and 688.8 ng/spot respectively. The plates were then developed in a twin trough chamber (20 x 10 cm) up to a distance of 8 cm using the same mobile phase at 25± 2°C and 40% RH. Following development, the plates were dried and scanned densitometrically at 272 nm. Calibration curve of gallic acid was obtained by plotting peak areas against concentrations of the standard solutions. The quantification of gallic acid (in quadruplet) in the sample was measured from the above calibration curve of standard gallic acid and the results were expressed as Mean ± SD.

Validation of the method:
The proposed method was validated according to ICH guidelines for linearity, precision, accuracy, limit of detection and limit of quantification [12, 13].

Linearity:
The linearity was confirmed from the calibration curve obtained by plotting peak areas against concentrations of the standard gallic acid solution.

Precision:
The precision was determined by analyzing 98.4, 393.6 and 688.8 ng/spot of gallic acid standard solution after applying on a TLC plate (n = 3) on the same day for intraday precision and on 3 different days for interday precision. The precision was expressed as percent relative standard deviation (%RSD).

Accuracy:
Accuracy of the method was confirmed by determination of recovery. The recovery of gallic acid from the extract was calculated on sample spiked with three concentration levels of standard gallic acid (50, 100, and 150% of the determined content of the *Q. infectoria* extract solution) (n = 3).

LOD and LOQ:
Limit of detection (LOD) and Limit of quantification (LOQ) were determined by scanning the blank (methanol) spot and noise. Signal-to-noise ratios of 3:1 and 10:1 were considered as LOD and LOQ, respectively.

RESULTS:
Proximate analysis:
The results of the proximate analysis are presented in **Table 1**. The moisture content of the powdered galls was 7.06% w/w. The sulphated ash value registered a higher value than the total ash value. The water soluble extractive value was higher than the ethanol soluble extractive indicating presence of more water soluble components in the drug.

TABLE 1: PROXIMATE ANALYSIS OF POWDERED SAMPLE OF *Q. INFECTORIA*

Parameters	Obtained value (% w/w)
Total ash	6.04 ± 0.303
Acid insoluble ash	2.08 ± 0.166
Water soluble ash	4.31 ± 0.184
Sulphated ash	6.71 ± 0.148
Moisture content	7.06 ± 0.243
Ethanol soluble extractive	9.12 ± 0.252
Water soluble extractive	14.33 ± 0.211

Results expressed as Mean ± SD from three observations

Preliminary phytochemical studies:
The results of the preliminary phytochemical screening of various extracts revealed presence of steroids, triterpenoids, saponins, flavonoids, tannins and phenolic compounds, carbohydrates, gums and mucilages respectively **(Table 2**).

TABLE 2: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *Q. INFECTORIA* GALL EXTRACTS

Test	Pet. Ether	Chloroform	Methanol	Aqueous
Steroids	+	+	-	-
Triterpenoids	+	+	-	-
Alkaloids	-	-	-	-
Saponins	-	-	+	-
Tannins and phenolic compounds	-	-	+	+
Flavonoids	-	-	+	+
Carbohydrates	-	-	-	+
Gums and mucilages	-	-	-	+

+; Present, -; Absent

HPTLC analysis:
HPTLC studies carried out using the mobile phase of Toluene: Ethyl acetate: Formic acid: Methanol (12: 9: 4: 0.5) yielded acceptable resolution and separation of the components in the extract (**Fig. 1**). The specificity of the bands of gallic acid (Rf = 0.45) in the *Q. infectoria* extracts was confirmed by overlaying the absorption spectra of the extract

with gallic acid reference standard **(Fig. 2**). The maximum absorption of gallic acid was at 272 nm; and this wavelength was chosen for the analysis. Densitogram of standard gallic acid is shown in **Fig. 3**. The proposed TLC-densitometric method showed acceptable validation parameters (**Table 3**). The calibration curve of gallic acid was linear over the range of 98.4 to 688.8 ng/spot. The correlation coefficient value was 0.99956, confirming the linearity of the method (**Fig. 4**). The percentage of relative standard deviation value of intraday and interday precisions was lower than 2% and the average recovery of gallic acid was 99.96 ± 1.56 %, indicating the high precision and accuracy of the method. LOD and LOQ were found to be 19 and 60 ng/spot, respectively.

The quantification of gallic acid (in quadruplet) in the sample was measured from the calibration curve of standard gallic acid and found that the galls contain 0.218 ± 0.0011% w/w of gallic acid (**Fig. 4).**

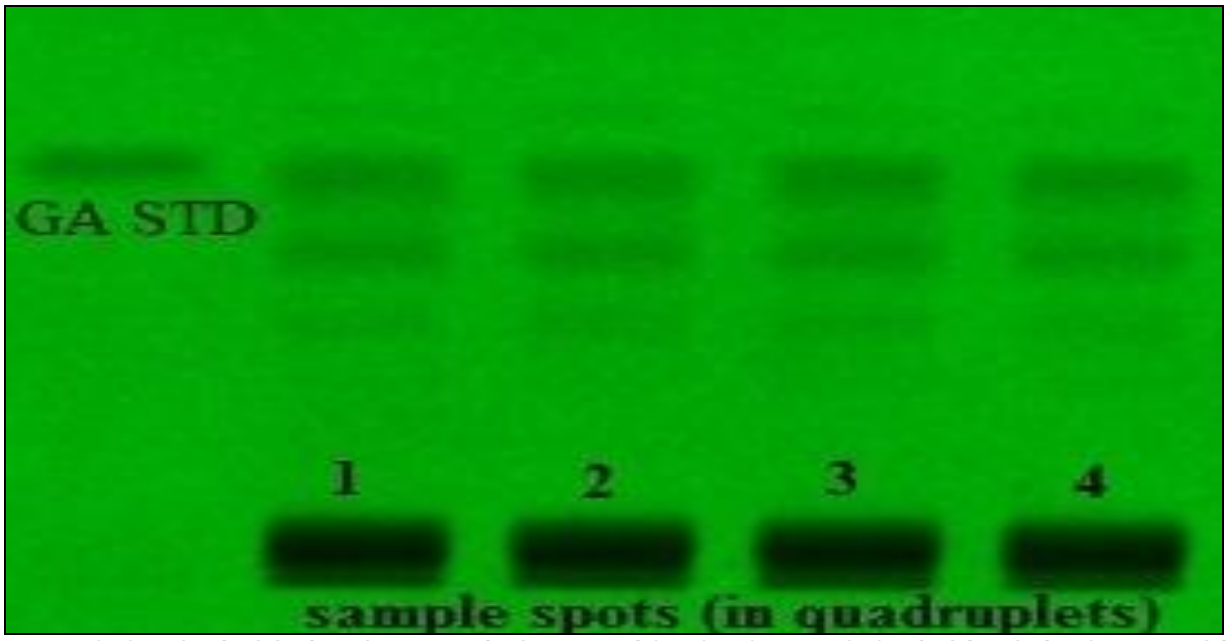

FIG. 1: HPTLC CHROMATOGRAM OF METHANOL EXTRACT OF *Q. INFECTORIA* GALLS AT 254 NM, GA STD: STANDARD GALLIC ACID

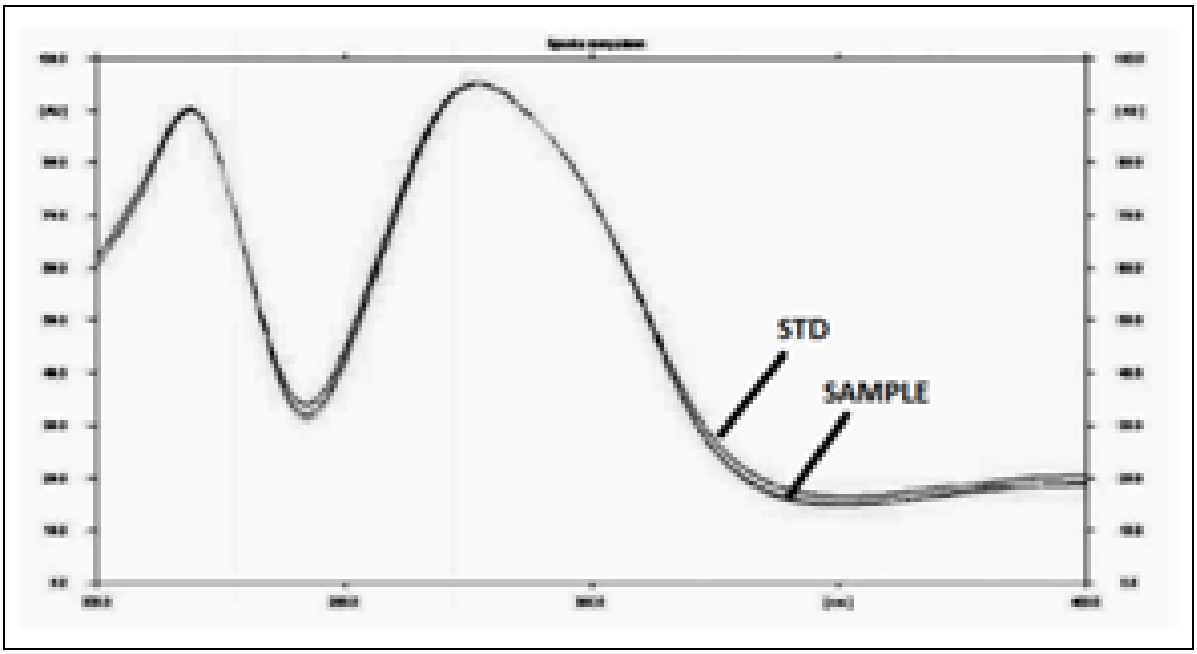

FIG. 2: PEAK MATCHING OF UV ABSORPTION SPECTRA OF GALLIC ACID REFERENCE STANDARD AND *Q. INFECTORIA* EXTRACT

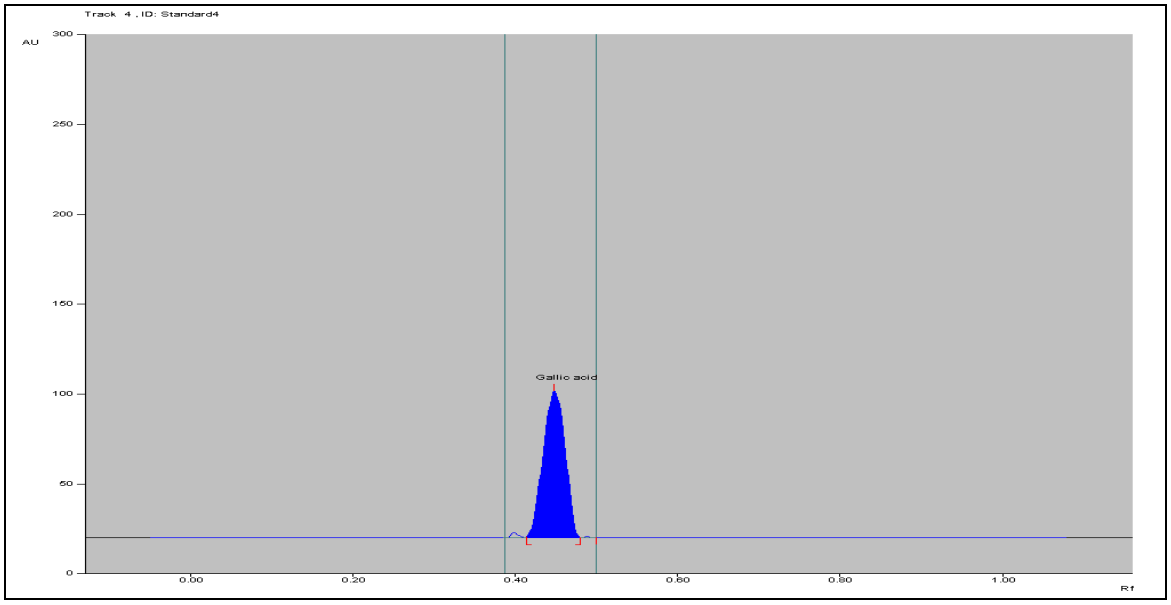

FIG. 3: DENSITOGRAM OF GALLIC ACID REFERENCE STANDARD

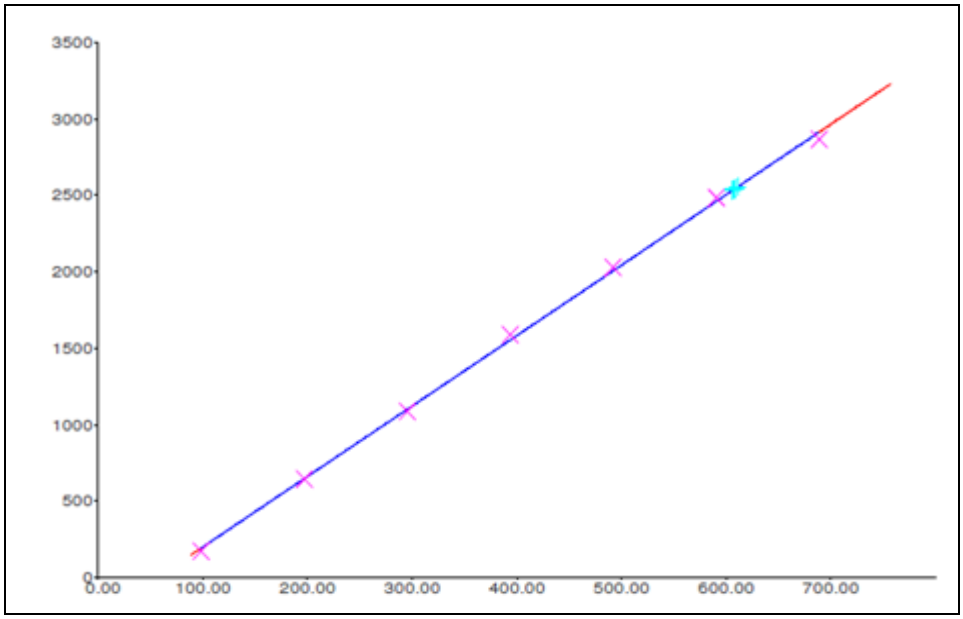

FIG. 4: CALIBRATION CURVE OF GALLIC ACID STANDARD (λ_{max} 272 nm)

TABLE 3: METHOD VALIDATION PARAMETERS FOR THE PROPOSED HPTLC METHOD

Sl. no	Parameter	Gallic acid
1	Range of linearity	98.4 – 688.8 ng/spot
2	Regression equation	Y = 265.9 ± 4.612 X
3	Correlation coefficient	0.99956
4	Limit of detection (ng)	19
5	Limit of quantification (ng)	60
6	% RSD intraday precision (n = 3)	0.59%
7	% RSD intraday precision (n = 3)	1.87%
8	Accuracy (average % recovery)	99.96 ± 1.56

DISCUSSION:

Proximate analysis:

Proximate analysis of crude drugs includes determination of total ash, acid-insoluble ash, water soluble ash, sulphated ash, water soluble extractive, ethanol soluble extractive and moisture content. Ash is the inorganic residue resulted after incineration and usually consists of carbonates, phosphates, oxalates and silicates of sodium, potassium, calcium and magnesium. Ash values are

particularly helpful in determining the quality and purity of powdered crude drugs. The water soluble ash indicated presence of inorganic compounds in crude drugs. The acid insoluble ash consist mainly of silica and represent presence of earthy material in the sample. Moisture content is an important parameter since excessive moisture in herbal samples encourages microbial growth and enzymatic degradation of crude drugs during storage. Estimation of extractive values indicates the amount of phytochemicals present when extracted with a particular solvent. The compositions of phytoconstituents vary with the type of solvent used[14].

In the present study, the sulphated ash value was higher than the total ash value. The sulphated ash is a measure of the content of inorganic impurities in the sample. The sulphated ash gives more reliable ash content for a sample. The water soluble extractive value was higher than the ethanol soluble extractive indicating presence of more water soluble components in the drug.

Preliminary phytochemical studies:

Preliminary screening of phytochemicals is a valuable step in the detection of the classes of phytochemicals present in crude drugs that may subsequently help in drug discovery process.

HPTLC analysis:

Recently, HPTLC has been recommended for routine analysis of herbal drugs due to its advantages of reliability in quantitation of analytes even at nanogram levels with low operating cost and high sample throughput. The developed method was simple, rapid and precise for routine analysis of gallic acid content in *Q. infectoria* galls and the products containing them. The method was validated in terms of linearity, accuracy, precision, and specificity according to ICH guidelines. The developed method is capable of quantifying amounts of gallic acid in locally available samples of *Q. infectoria*.

CONCLUSION: The results of this work will support in the standardization process of *Q. infectoria.* Further, the developed method for the quantitative estimation of gallic acid as the biomarker for *Q. infectoria* will help in the determination of quality of the crude drug.

ACKNOWLEDGEMENTS: The authors are thankful to Universiti Kuala Lumpur Royal College of Medicine Perak for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Dash GK, Md. Ansari T, Sami F and Majeed S: Proximate Analysis and Quantitative Estimation of Gallic Acid in *Quercus infectoria* Oliv. Galls by HPTLC. Int J Pharm Sci Res 2016; 7(11): 4400-06.doi: 10.13040/IJPSR.0975-8232.7(11).4400-06.