Research article

High-performance liquid chromatography method development and validation for the quantification of mangiferin in *Coffea arabica* leaves

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ABSTRACT

Introduction and Aim: Mangiferin is accompanied by the therapeutic potential for various human ailments. The present study is aimed to develop a validated HPLC method for the quantification of mangiferin in coffee (*Coffea arabica*) leaves.

Materials and Methods: The high-performance liquid chromatography (HPLC) was carried out on Agilent Technologies 1260 LC Infinity Series liquid chromatography system (Agilent, USA) using an isocratic mobile phase of a mixture of methanol and 1% orthophosphoric acid v/v (70:30) at a flow rate 1mL min^{-1} for 14 min with the controlled temperature at 25°C. The mangiferin content was detected by a UV diode array detector at 258 nm.

Results: The linearity of the HPLC system was established in the range of 10-100 ppm mL^{-1} with a regression coefficient (R^2) of 0.99670. The accuracy of the method was assessed by a study performed using 3 different levels, with a 99% average recovery. The limit of detection(LOD) and limit of quantification (LOQ) were 1.3 and 3.9 ppm mL^{-1}. Intraday and interday precision analysis showed the relative standard deviation ≤0.012342 separately. The content of mangiferin in the coffee leaves was 94 mg/gram (w/w).

Conclusion: All necessary factors, such as linearity, accuracy, LOD, LOQ, precision, and % relative standard deviation(RSD), were inside the appropriate limits. The HPLC was validated using all necessary variables with a successful estimation of mangiferin.

Keywords: HPLC; Mangiferin; coffee leaves; method validation; LOD; LOQ.

INTRODUCTION

Coffee (*Coffea Arabica*) is a fragment beverage, consumed during the early morning to begin the day and several times throughout the day by a significant portion of the population of the world. After water and tea, coffee is the third most well-known drink polished off overall (1). Coffee is cultivated mostly in developing countries employing over 26 million people and traded worldwide as raw or processed beans (2). Coffee is currently second just to oil in significance among rural merchandise exchanged universally. The two principal species exchanged are Arabica and Robusta. Global coffee production in 2017 was reported to be huge with an export value of 32.7 billion USD (3). No less than 748 thousand tons of coffee, or 6.6% of the worldwide result, were created in Ethiopia in 2012. Coffee beans are for the most part filled in the Gimbi, West Welega Zone of the Oromia District, Ethiopia.

Nearly all coffee taxa include caffeic, p-coumaric, vanillic, ferulic, and protocatechuic acids. 5-caffeoylquinic acid (5-CQA) is the most prevalent soluble ester in the Coffea species. Caffeine, sucrose, chlorogenic acids, and trigonelline are major constituents of coffee and are attributed to flavor and the characteristic bitter taste of the coffee beverage (4,5). Besides, several studies reported the presence of iridoid glycosides, anthraquinones, and tannins (6). Caffeine (2,4,8-tri methylxanthine) is synthesized in the immature leaves and fruits of *C. arabica*. Caffeine diminishes drowsiness, improves transient memory, influences human circadian timing, and builds the adequacy of specific meds (1).

The coffee bean is considered a chief wellspring of caffeine on the planet (7). Polyphenol accumulates more in seeds and leaves when compared with other parts of plants (7,8). Several biological activities including antioxidant, anti-cancer, chronotropic, antiarrhythmic, and anti-atherosclerosis reported from coffee earlier (9-11).

Noteworthy research is undergoing to comprehend the chemistry of coffee leaves. The countries Ethiopia, South Sudan, and Indonesia are considered as the major coffee producers. In these countries, coffee leaves are usually used to create infusions. The coffee leaves infusion is supposed to aid in the management of numerous ailments, for instance, fever, anemia, and intestinal discomfort (12). Several investigations were carried out to decipher the chemistry of coffee leaves and identify bioactive principles (4, 13). The outcomes of all these investigations were an exploration of chlorogenic acids, alkaloids, and xanthones.

Xanthones are popularly known as mangostin and mangiferin and are attributed to various biological and pharmacological activities (14-16). The presence of mangiferin is reported in African coffee leaves (12). Indeed, comparable to *M. indica* leaves, mangiferin is found in coffee leaves (17). Several coffee species are known to accumulate mangiferin in the leaves (13). Mangiferin works as an anti-inflammatory agent, antioxidant agent, anti-diabetic drug, immuno-modulatory agent, and anticancer drug. It is also found to lower cholesterol and fatty acids. Additionally, it supports and strengthens cardiac tissue while assisting the body in controlling metabolism. Mangiferin has also been shown to have neuroprotective advantages that help against neuroinflammation and neuropathic pain.

The development and validation of an effective analytical method is a vital part of the quality control of the source material, to guarantee the safety and efficacy of the subsequent compound (18). Consequently, the pharmacological potential of mangiferin and the lack of interventions for quality control from coffee leaves led us to investigate to develop a method and validate the method for the estimation of mangiferin in coffee leaves by using HPLC and following parameters such as linearity, range, detection limit, quantitation limit, accuracy, and precision.

MATERIALS AND METHODS

Plant collection

The Coffee leaves were collected from Gimbi, West Welega Zone of the Oromia District, Ethiopia. Gimbi has a latitude and longitude of 9°10′N35°50′E with an elevation range of 1845 and 1930 meters above sea level.

Stock and working solutions of standard compounds

To prepare the standard (calibration) curve, a stock solution of 1mg/ml of mangiferin was made in methanol. The resulting stock solution was further diluted to 10, 20, 50, and 100 ppm for the development of the standard curve of mangiferin.

Preparation of the coffee leaf powder sample solutions

About 25 mg of coffee leaf powder was precisely weighed and transferred in 5 mL of methanol: water (75:25) v/v. Preceding the infusion and investigation by HPLC, the concentrate was sifted through a 0.22 µm nylon layer channel.

Mangiferin estimation by high-performance liquid chromatography (HPLC)

HPLC Framework: The Agilent Advances 1260 LC Endlessness Series fluid chromatography framework, (Agilent, USA) was utilized for the recognizable proof and assurance of mangiferin content in the coffee leaf material. The chromatographic framework is furnished with a twofold siphon, a degasser, an Agilent Innovations G7129A Autosampler and diode cluster finder (Father), and a segment indoor regulator. The Supelco ® C18 section (5 µm molecule size, 250 x 4.6 mm) was utilized. The instrument control, information securing, and examination was performed utilizing Chemstation programming (Agilent Advances).

Chromatographic condition: The elution was performed on an isocratic dissolvable framework utilizing a combination of methanol and 1% ortho-phosphoric corrosive v/v (70:30) at a stream pace of 1 mL/min for 14 min with a controlled temperature at 25°C. The infusion volume was 20 µL for standard and tests. The diode cluster locator was set at a frequency of 258 nm to recognize the eluent.

Validation of the analytical method

Linearity of the method

To assess the linearity parameter, four solutions (10, 20, 50, and 100 ppm) of reference compound mangiferin were prepared. The HPLC was then filled with 20 µl of each arrangement using an auto-sampler, and the tests were repeated many times while being examined at 258 nm. Plotted against the foci were the traditional pinnacle zones. Using alignment bend to calculate the coefficient of relationship, slant, and capture values, the suggested technique's linearity was estimated.

LOD and limit of LOQ

By comparing the signals from tests and known low groups of the experts with those of clear instances and putting out the base focus at which the scientific might be consistently identified under the suggested chromatographic situation, the assurance of the sign-to-commotion proportion was pear-shaped. The limit of detection (LOD) and limit of quantification (LOQ) were determined by centralization of the logic that established a sign-to-commotion ratio of 3:1 and 10:1, separately.

Accuracy/ recovery

The accuracy of a scientific strategy communicates the closeness between the normal worth and the worth found. It is communicated by ascertaining the percent recovery (% R) of insightful recuperation. For this situation, to assess the exactness of the suggested strategy, progressive examination (n = 3) for three unique fixations (10, 20, 50, and 100 ppm) of standard mangiferin arrangement was done utilizing the proposed technique. The information of the analysis was genuinely examined utilizing the recipe [% Recovery = (Recovered conc. /Injected conc.) x 100] to concentrate on the recovery and legitimacy of the suggested strategy.

Precision

Regarding repeatability and reproducibility, the accuracy of the examination was evaluated. The degree of arrangement in a scientific technique's results if the method is repeatedly used on various samples is known as precision. After triplicate infusions, it was examined for intra- and interday repeatability of responses and reported as percentage relative standard deviation (% RSD) among reactions using the formula [RSD (%) = (Standard deviation/Mean) x 100%]. Three duplicate tests using the suggested method were conducted at the convergence of 15 ppm of the standard mangiferin setups in the continuing technique advancement and validation convention, and the results were not completely resolved.

Statistical analysis

Experimental values were recorded twice or three times. Using Microsoft Excel 2010, the mean values and standard deviations (S.D.) were computed. The results are shown as a bar diagram with the mean value and S.D.

RESULTS

The HPLC was carried out on Agilent Technologies 1260 LC Infinity Series liquid chromatography system (Agilent, USA) using an isocratic mobile phase of a mix of methanol and 1% orthophosphoric acid v/v (70:30) at a flow rate of 1mL min^{-1} for 14 min with the controlled temperature at 25°C. The mangiferin content was detected by a UV diode array detector at 258 nm. The mangiferin from coffee leaves powder was extracted in methanol: water (75:25) v/v solvent. The chromatograms obtained are shown in Fig. 1.

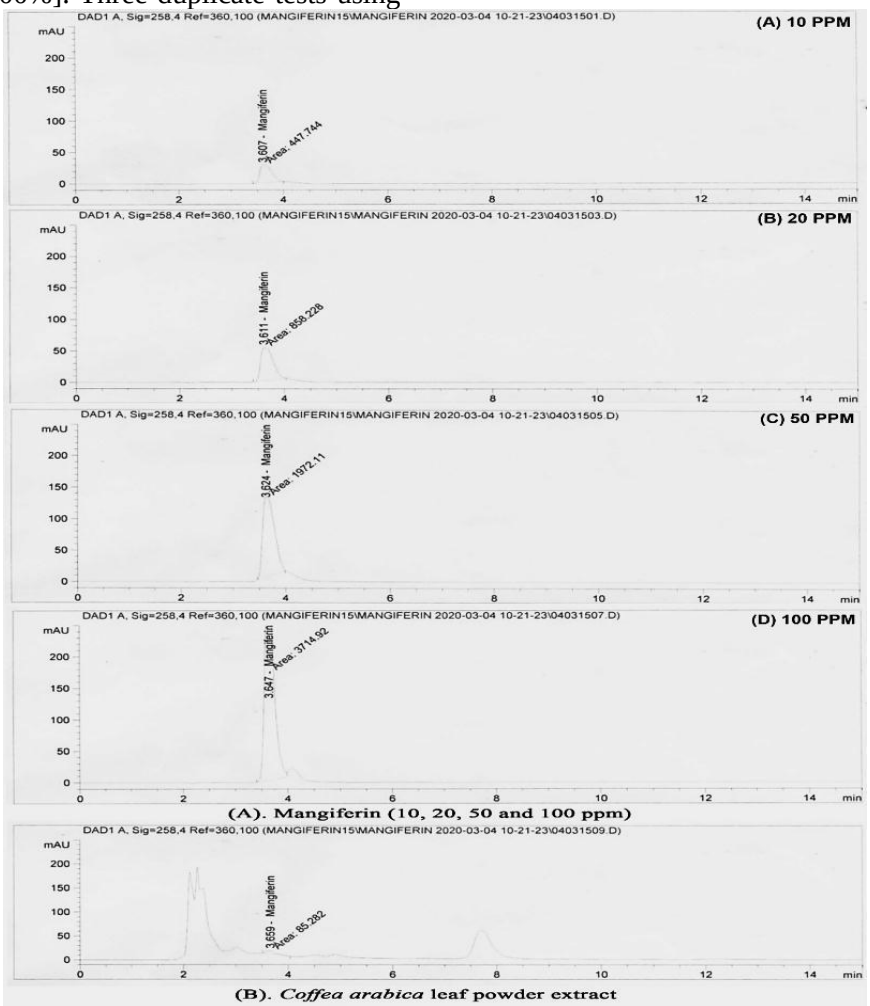

Fig. 1: Chromatograms of (A). Reference mangiferin (10 ppm, 20 ppm,100 ppm) and (B). Methanol/water (75:25) extract of *Coffea arabica* leaves.

Table 1: Linear regression data obtained from the standard curve of mangiferin

Concentration (ppm)	Mean area (y) (n=3)	Width (mins)	Intercept (c)	Slope (m)	Correlation coefficient (r^2)
10	448.87±1.30	0.2599	144.98691	36.24093	0.99670
20	859.30±1.24	0.2683			
50	2079.19±12.37	0.2577			
100	3715.96±1.20	0.2222			

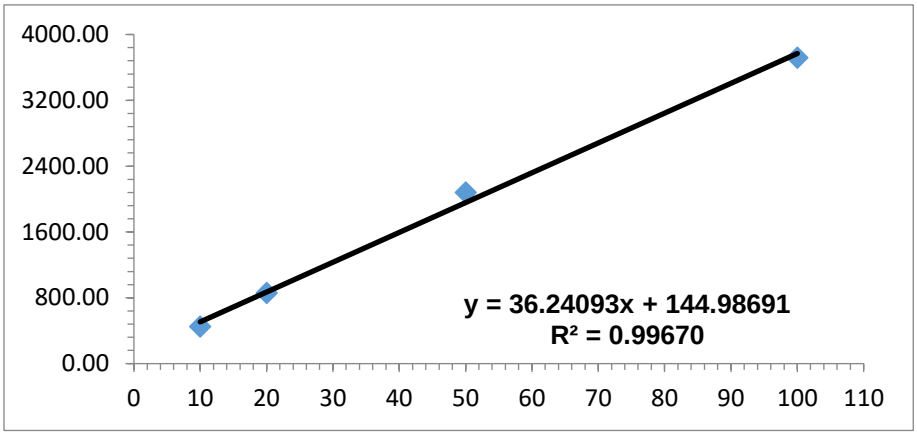

Fig. 2: Calibration curve of mangiferin by HPLC method.

Linearity study by calibration curve

The results of the linearity study are presented in Table 1 and the calibration curve of the mangiferin is presented in Fig. 2. The calibration curve for mangiferin was linear in the 10 ppm to 100 ppm range, and the correlation coefficient (r), which was 0.99670, indicated that the peak area's dependence on concentration was good (Figure 2). For mangiferin, the standard curve was given by the equation y = 36.24093x + 144.98691. (Where y represents peak area and x represents concentration) (Table 1). Mangiferin's estimated concentration in coffee leaf extract was determined to be 94 mg/g.

LOD and LOQ

The LOD and LOQ for mangiferin were shown to be 1.3 ppm and 3.9 ppm, respectively. Because mangiferin has a low LOD and LOQ, as indicated in Table 2, it can be measured in *C. arabica* at low quantities. Hence the proposed method is effective to detect mangiferin at less concentration range.

Accuracy/ recovery

Accuracy was studied by investigating three replicates (n=3) of mangiferin (standard) solution earlier analyzed (10 ppm, 20 ppm, 50 ppm, and 100 ppm). The percentage recoveries of the three concentrations were established to be 99.10 % to 99.89 %, which is suggestive of high accuracy (Table 3).

Precision

The repetitiveness of the suggested HPLC method, by intraday assay and inter-day assay, is stated in the expressions of % RSD, and the recovery esteems that were attained in the current investigation are listed in Table 4. Intra-day precision based on the content of mangiferin with a concentration of 15 ppm was found to be 0.039, 0.082, and 0.058, whereas inter-day precision based on the content of mangiferin with a concentration of 15 ppm was found to be 0.039, 0.082, and 0.058 respectively (Table 5).

Table 2: LOD) and LOQ of the suggested HPLC method

Sample	Range	α	S	LOD	LOQ	R^2
Mangiferin	10-100	23.25	59.96	1.3 ppm	3.9 ppm	0.9967

Table 3: Repeatability and recovery study for the mangiferin

Injected conc. (ppm)	Mean peak area (n=3)	Slope (m)	Intercept (c)	Mean recovery (ppm)	% Recovery
10	448.8695	**36.24093**	**144.98691**	444.8721	99.10873
20	859.3000			855.3026	99.53443
50	2079.1900			2075.193	99.80759
100	3715.9550			3711.958	99.89234

Table 4: Intra-day HPLC precision measurement data for mangiferin

Day	Injected conc. (ppm)	Area	Slope (m)	Intercept (c)	Recovered (ppm)	Mean recovered (µg/mL)	SD	%RSD
1	15	601.394	36.24093	144.98691	14.90022	14.90344	0.005837	0.039166
		663.552			14.90956			
		645.117			14.90698			
		571.382			14.89497			
		634.972			14.90549			
2	15	543.793	36.24093	144.98691	14.88965	14.89981	0.012342	0.082836
		634.558			14.90543			
		515.912			14.88368			
		673.455			14.91089			
		662.183			14.90938			
3	15	571.128	36.24093	144.98691	14.89493	14.89373	0.008698	0.0584
		537.415			14.88834			
		592.539			14.89872			
		627.96			14.90444			
		509.502			14.88222			

Table 5: Intra-day mangiferin recovery

Day	Injected concentration (ppm)	Area	SD	Slope (m)	Intercept (c)	Mean recovered (ppm)	SD	%RSD
1	15	623.2834	36.76884	36.24093	144.9869	14.90344	0.005837	0.039166
2	15	605.9802	71.60276	36.24093	144.9869	14.89981	0.012342	0.082836
3	15	567.7088	46.27143	36.24093	144.9869	14.89373	0.008698	0.0584

DISCUSSION

This study aimed to develop and validate the HPLC technique to quantify mangiferin in Coffee (C*offea arabica*) leaves. Mangiferin possesses biological activities that include antioxidant, anti-cancer, chronotropic, antiarrhythmic, and anti-atherosclerosis activities (19-20). The mangiferin from coffee leaves may be an active ingredient in therapeutic formulations prepared to treat human ailments. The correlation coefficient is a statistical technique used to quantify the degree or intensity. A high correlation coefficient value (a number extremely close to 1.0) implies a high level of linear association between the concentration of mangiferin and the peak area. According to the requirements for acknowledging linearity, the correlation coefficient (R2) should be about 0.990 (21-22). This demonstrates that the linearity of the mangiferin calibration curve is appropriate.

LOD and LOQ were assessed as per the ICH Guidelines Q2(R1) (21). Both parameters limit the amount of analyte that may be evaluated (23). The ratio of the detected amount to added amount was calculated to assess the average percentage of recoveries (18). The mean percentage recovery values, near to 100%, specified the high accuracy of the analytical method. The degree of agreement between a set of measurements made using several samples of the same homogenous sample under the specified conditions is expressed as the analytical procedure's precision (24). Precision is the degree of coordination between the acquired attributes (25). The HPLC technique was precise with % RSD for intra-day and inter-day measurements. These precision introduced % RSD values are under 1.0%, so the technique was viewed as exceptionally exact and reproducible.

In the current study for the determination of mangiferin, methanol/water (75:25) extract of *Coffea arabica* leaves was prepared. The devised HPLC method was precise and accurate for the determination of mangiferin from this extract. Statistical analysis ascertained that the method is inevitable for the determination of mangiferin. The approach can be employed for repetitive analysis of mangiferin from *Coffea arabica* leaves because the suggested mobile phase efficiently extracts mangiferin.

CONCLUSION

A simple HPLC technique has been developed for the analysis of mangiferin from *Coffea arabica* leaves. The proposed HPLC method is precise, specific, and accurate. The devised HPLC approach has undergone thorough validation with positive outcomes. The method's ease of use and consistency allowed it to be effectively applied to routine analysis of mangiferin from *Coffea arabica* leaves.

CONFLICT OF INTEREST

The authors declare no conflict of interest for this research work.

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