ORIGINAL ARTICLE

RP-HPLC Method for Rhein Quantification in *Cassia fistula* L. (Fabaceae) Leaves

Jade P. Rodriguez, RPh, MS

Institute of Herbal Medicine, National Institutes of Health, University of the Philippines Manila

ABSTRACT

Objectives. The aim of this study is to establish a Reversed Phase – High Performance Liquid Chromatographic (RP-HPLC) method for the quantification of Rhein from *Cassia fistula* L. leaves.

Methods. A Shimadzu system equipped with a C18 Column (150 x 4.6 mm, 5 µm) with an isocratic elution of Acetonitrile (solvent A) and 0.1% trifluoroacetic acid aqueous solution (solvent B) (Merck, 1.08178.0050) with a 55:45 ratio, respectively and a flow rate of 1.0 mL/min and sample injection of 10 µL detection was done at 230 nm. Standard solution of Rhein (Chengdu Biopurify) was prepared for method development. This study was validated using the guidelines set under "ICH Topic Q2 R2 or the Validation of Analytical Procedures". Procedures for linearity, precision, accuracy, limit of detection, and limit of quantitation were performed.

Results. The retention time of Rhein standard was determined at 5.10 minutes. LOD and LOQ were determined to be 1.278 mcg/mL and 3.872 mcg/mL, respectively with good linearity (R2 ≥0.996) with a linear range of 2.5-20 ug/mL of the Rhein standard. The accuracy of the method was determined based on % recovery method and ranged from 94.75%-100.32% (intraday, n=3) with %RSD of 0.71. The intraday precision %RSD was 2.92 (n=6) while interday precision %RSD was 3.75 (n=3). The method was able to check the Rhein quantity among 10 samples of *Cassia fistula* L. leaves from different locations in the Philippines.

Conclusion. The method was found to be sensitive and accurate for the quantification of Rhein. The method was found to be useful for the quantification of the amount of Rhein and can be used as a Quality Control tool for the assessment of *Cassia fistula*.

Keywords: Cassia fistula, Rhein, HPLC, method validation, Kanya pistula

INTRODUCTION

Cassia fistula L. belongs to the family Fabaceae and is locally known in the Philippines as Bitsula (C. Bis), caña fistula (Sp.), kaña pestula (Ibn.), kaña-pistola or kanya pistula (Tag), fistula (C.Bis), ibabau (Bis), lombayong (Bis), and lapad-lapad (Tagb).[1,2] A search from the World Flora Online showed that there are no other varieties of *C. fistula L.* but only a synonym namely *Cassia fistula var. ovata* DC.[3] Kanya pistula or KP is a moderate-sized erect deciduous tree popularly remembered as "golden shower" due to its fragrant flower with a bright yellow color. An illustration of KP is shown in Figure 1 where its leaves are pinnate, smooth, around 30 to 40 centimeters long, with leaflets having an ovate shape around 8 to 16 centimeters long. Calyx is 6 to 8 millimeters long, smooth, and deciduous. Petals are veined, obovate, 18 to 25 millimeters long, bright yellow, and short-clawed at the base. Stamens are all furnished with anthers, the 2 or 3 lower ones being longer. The pod is cylindric, 30 to 60 centimeters long about 2.5 centimeters thick, dark brown,

eISSN 2094-9278 (Online)
Published: December 18, 2024
https://doi.org/10.47895/amp.vi0.8803

Corresponding author: Jade P. Rodriguez, RPh, MS
Institute of Herbal Medicine
National Institutes of Health
University of the Philippines Manila
PM 104 Paz Mendoza Hall
Pedro Gil Street, Ermita, Manila 1000, Philippines
Email: jprodriguez2@up.edu.ph
ORCiD: https://orcid.org/0009-0002-9600-9087

pendulous, smooth, and shiny. Seeds are numerous, embedded in black, sweet pulp, completely separated by thin, transverse dissepiments, small, ovoid, slightly compressed, smooth shining, and yellowish brown.[1,3,4] The University of Florida in the United States listed *C. fistula* or Golden Shower as one of the ornamental trees planted in streets, providing shade, and as a tree in parking lots.[5] In the Philippines, *Cassia fistula*, or golden shower tree, is found throughout the country, from Luzon to Mindanao. It is commonly used as an ornamental tree and can be seen planted along main roads and highways, such as Roxas Boulevard in Manila and the South Luzon Expressway. Local government units often include them in the landscaping of their main plazas, like in Silay, Negros Occidental, or in Rizal Park, Manila. Additionally, schools and universities often incorporate *Cassia fistula*, along with other ornamental plants like fire trees or caballero, within their properties. *C. fistula* is also found in India, Thailand, Malaysia, and other Southeast Asian countries.

Multiple studies listed down the phytochemical constituents of the bark, leaves, and pods showing the presence of different anthraquinones, lupeol chrysophanol, and physcion, butyric and formic acids, pectins, tannin, B-sitosterol sennosides A and B, oxalic acid, lignans, and flavonoids.[6-9] The phytochemical screening of the leaves showed the presence of free rhein and its glycosides (sennosides A and B).[9] It also has anthraquinone, tannin, oxyanthraquinone, and volatile oils. The cuticular wax of leaves contain hentriacontanoic, triacontanoic, nonacosanoic, and heptacosanoic acids.[9,10] ASEAN Monograph for *Cassia fistula* listed down the contents of the leaves to be anthraquinones, carbohydrates, carotene, cellulose, chitorin, chlorophyll A, chlorophyll B, chrysophanic acid, hemicelluloses, inorganic elements kampferol-3-O-b-D-glucoside, kaempferol- 3-O-b-D-neohesperidoside, kaempferlo-3-glucoside, kaempferol-3-rhamnosied, kaempferol-3-robinobioside-7-rhamnoside, lignins, myricetin, 3-neohesperidoside, phenolic ester and ethers, alcohol, pigments, protein, quercetin, quercetin-3-rutinoside, quercetin-3-xyloside, rhein, rhein glucoside, sennoside, sennoside A, sennoside B, steroids, tannins, vecein (6,8-di-C-glucosylapigenin, xylose D).[11]

Quisumbing records its folkloric use as purgative for both the leaves and fruit pulp. Fruit pulp was also used as a cathartic while its extract is used for habitual constipation. Leaves are usually grounded to become a paste and are rubbed on ringworm and other fungal skin infections. The pleasant taste of the fruit pulp makes it ideal as a purgative for children who would need to take about 4-10 segments. External pods are used for the removal of the placenta. Roots are given as a tonic and febrifuge but also for their purgative action. It is also used in heart disease. Flowers are known to be demulcent, laxative, and purgative.[2]

In Ayurvedic medicine, *Cassia fistula* root is used as a tonic, astringent, febrifuge, and strong purgative. The leaves possess anti-periodic and laxative properties, also used for jaundice, piles, rheumatism ulcers as well as skin diseases like boils, insect bites, ringworms, and eczema. Leaves and bark are mixed with oil and applied to pustules. Roots are used for chest pain, joint pain, and headaches.[10] The extract of the leaves reduced the mutagenicity of *E. coli*. The leaves are also used as a laxative and used externally as an emollient. Both the leaves and flowers are purgative like the pulp.[9,10]

In the Thailand Materia Medica, the meat on the pod of *C. fistula* or Khun-fak in their local language has been traditionally used to relieve constipation in both children and women during pregnancy. This property was tested in-vivo in rats and dogs.[12] The ripe pod pulp of *C. fistula* has been traditionally used among Southeast Asian countries as a laxative owing to its anthraquinone glycoside constituents.[11]

The major anthraquinone found in the leaves and pulp of *C. fistula* was Rhein.[13,14] Rhein and rhein anthrone (Figure 2) are the active metabolites of sennosides which stimulates peristalsis and increases fecal water content to increase the movement of feces through the large intestine.[6,15]

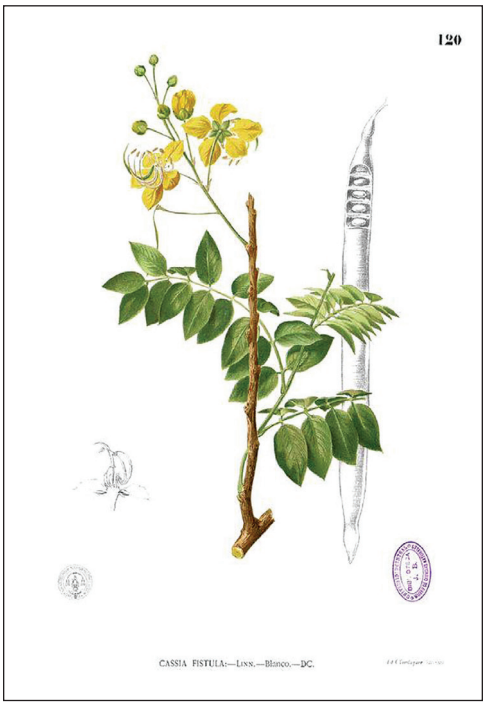

Figure 1. Illustration of *Cassia fistula* L.[4]

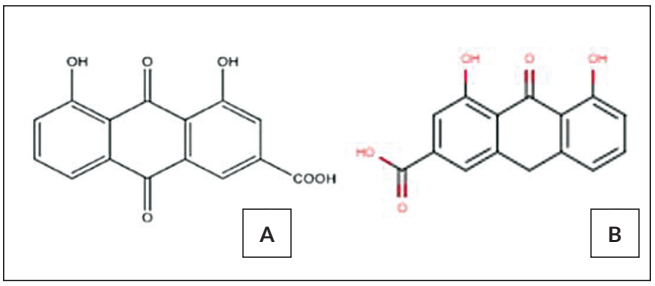

Figure 2. Rhein **(A)** and Rhein anthrone **(B)**.[6]

Accordingly, the amount of Rhein was found to be greatest in the young maturing green pods and in mature leaves. It is recommended, for medicinal use, to extract anthraquinone glycosides from young maturing green pods instead of extracting from fully-grown pods as well as from fully-grown leaves rather than from young developing leaves.

Rhein, being the major component, had been regarded as the marker compound for quality assessment of *C. fistula* pod pulp extract in Thailand.[13] Studies determining the amount of Rhein among the pods using HPLC method were previously developed.[15,16] However, the seasonal availability of the pods makes continuous production impossible. The choice of leaves allows a sustainable harvest all year long. An HPLC Method for detection of Rhein using leaves could be validated using the guidelines for Method validation set by "ICH Topic Q2 R2 or the Validation of Analytical Procedures".[17]

The aim of this study is to establish a Reversed Phase – High Performance Liquid Chromatographic (RP-HPLC) method for the quantification of Rhein from *Cassia fistula* L. leaves.

MATERIALS AND METHODS

Chemicals, Reagents, and Materials

Methanol (Labscan®), Trifluoroacetic acid (Merck®) and Acetonitrile (JT Baker®) solvents used were of HPLC Grade. Methanol (Labscan®) used for extraction was of Analytical Reagent grade. Ultra Pure water was used in the mobile phase. The Rhein standard (98.9% Purity) was acquired from Biopurify® Chengdu, China. Solvents used as Mobile phase were filtered using a 0.45-µm 47-mm (diameter) nylon membrane filter discs (Whatman®) while solutions injected to the HPLC System were filtered using a 0.45-µm 25-mm Syringe filter.

Rhein Standard Solution Preparation

An accurately weighed quantity of Rhein reference standard (70mg, 98.9% Purity) was dissolved with 100 mL methanol HPLC to prepare the stock solution. Standard working solutions of Rhein were prepared by diluting the stock solution with methanol to obtain five solutions of varying concentrations ranging from 2.5 mcg/mL to 20 mcg/mL. A standard curve was then prepared using the Rhein standard solutions.

Instrument

A Shimadzu system was used consisting of a pump (Shimadzu Nexera X2, LC-30AD), a pump controller (CBM-20A), a degassing unit (DGU-20A5R), an autosampler (Nexera X2, SIL-30AC), and a photodiode array detector (Nexera X2, SPD-M30A). Shimadzu LabSolutions ver. 5.81 software was used for the data processing and acquisition of the HPLC profile. C18 Reverse Phased Column Purospher® STAR RP-18 endcapped (5 µm) LiChroCART® 150-4.6 (Merck) was used as the column for the HPLC Analysis.

Optimization of Chromatographic Conditions

The High-Performance Liquid Chromatography (HPLC) of the standard Rhein solution and the crude extracts were run on the Shimadzu system. The total running time was 15 minutes, and a flow rate of 1.0 mL/min was set. The UV detector was set at 230 nm, while the sample injection volume was 10 µL.

Mobile Phase

The mobile phase was filtered and degassed before incorporation into the HPLC system. The mobile phase was then allowed to run for one hour to equilibrate the column. The final mobile phase used was a mixture of Acetonitrile and 0.1% Trifluoroacetic Acid (TFA) at a ratio of 55:45, respectively.

Method Validation

Linearity, LOD, and LOQ

The validation of the method was performed following the "ICH Topic Q2 R2 or the Validation of Analytical Procedures".[17] Five concentrations of Rhein reference standard ranging from 2.5 mcg/mL to 20 mcg/mL were prepared with 100% methanol HPLC Grade. Each concentration was run through the HPLC in triplicate. The resulting calibration curve was obtained by plotting the peak areas from each concentration versus the concentrations of the standard. The regression equation and correlation coefficient (r2) were then calculated. Limit of Detection is the smallest amount that can be measured and distinguished from the zero point. Limit of Quantification on the other hand is the smallest concentration of an analyte in a test sample that we can determine with an acceptable reproducibility and accuracy. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated using the standard deviation (SD) of the response and slope of the calibration curve.

Accuracy

The accuracy of the HPLC Method was determined based on its %recovery. The %recovery was performed on samples spiked with three concentration levels of Rhein reference standard solutions (50%, 100%, and 150%) of the determined content of the Rhein on the *Cassia fistula* L. leaves (n=3 per concentration level). An acceptable %RSD is <5%.

Precision

Intraday precision was performed by analyzing 20 mcg/mL of Rhein reference standard solution (n=6) within one day. Interday precision was done by analyzing 5, 15, and 20 mcg/mL Rhein reference standard solution (n=3 per concentration) on three different days. The precision is

Table 1. Collection of *Cassia fistula* L. from Different Locations

Sample Batch Name	Location	Date Received
201019-TQ-KP	Tiaong, Quezon	October 19, 2020
201019-LB-KP	Los Banos, Laguna	October 19, 2020
201125-PNG-KP	Pangasinan	November 25, 2020
210507-DAV-KP	Davao	May 7, 2021
210607-PNG-KP	Pangasinan	June 7, 2021
210607-TAR-KP	Tarlac	June 7, 2021
210614-TAR-KP	Tarlac	June 14, 2021
210614-LB-KP	Los Banos, Laguna	June 14, 2021
210712-LAC-KP	LAC, Nueva Ecija	July 12, 2021
210906-DAV-KP	Davao	September 6, 2021

Table 2. Intraday Precision

20 mcg/mL	Area	Concentration (mcg/mL)
1	1434202	20.38
2	1347934	19.573
3	1346864	19.551
4	1343621	19.487
5	1353377	18.951
6	1342715	18.763
Average	1361452.167	19.451
%RSD	2.6324	2.9197

reported as the relative standard deviation. An acceptable %RSD is <5%.

Quantification of Rhein in Different Samples

Samples of *Cassia fistula* L. leaves were collected from different locations in the Philippines (Table 1). These were washed, garbled, and dried prior to milling producing the final powdered form. Powdered leaves were macerated using methanol, AR for 24 hours. The extracts were then evaporated to dryness in a water bath. These are then transferred to suitable containers and stored in a refrigerator with a temperature range of 2 to 8 degrees Celsius.

About 1.0 g of dried methanolic extract was then dissolved with an initial amount of methanol HPLC grade. These were then mixed and sonicated for 15 minutes at 32 degrees Celsius. It was then filled to volume in a 100-mL volumetric flask. These were then filtered using 0.45-µm 25-mm syringe filter. Filtered sample solutions were run through the HPLC method that passed the Method Validation.

RESULTS

Retention Time

This study used materials available in the laboratory for the mobile phase. The optimum mobile phase that was tested for this study was found to be a combination of Acetonitrile (ACN) and 0.1% TFA. Different concentrations were tested before arriving at the final mobile phase. The final 3 combinations were 60:40, 55:45, and 50:50 (Acetonitrile and 0.1% TFA) (Figure 3). The combination of 55:45 Acetonitrile and 0.1% TFA gave the best retention time at 5.101 minutes.

Linearity, Limit of Detection and Limit of Quantification, Precision

Five concentrations of the Rhein reference standard solution (2.5 ug/mL-20 ug/mL) were run in triplicates under the HPLC Method developed. The resulting equation of the line was y= 50561.6x + 358310 with an R-value of 0.998 and an r2 value of 0.996 (Figures 4 and 5). The Limit of Detection and Limit of Quantification was calculated with the results being 1.278 mcg/mL and 3.872 mcg/mL, respectively.

Precision

For Intraday precision, the Average %Recovered for 20 mcg/ml was calculated at 97.25% and the %RSD at 2.92 (Table 2).

For Interday precision, the Average %Recovered from the 20 mcg/mL reference solution was calculated at 97.79% and %RSD of 2.39. For the 15 mcg/mL, the Average %Recovered was calculated at 99.93% and %RSD of 4.96. Lastly, for the 5 mcg/mL, the Average %Recovered was calculated at 101.64% and %RSD of 3.91 (Table 3).

Accuracy

Accuracy was done by the standard addition method. A known concentration of Rhein from the LAC Nueva Ecija sample (19.4 mcg/mL) was spiked with 0%, 50%, 100%, and 150% of the Rhein reference standard. The experiment

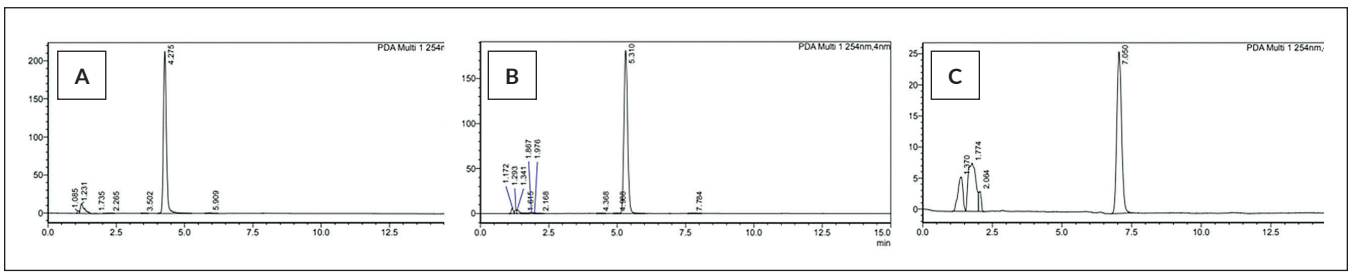

Figure 3. **(A)** Chromatogram of 60:40 ACN-TFA, **(B)** Chromatogram of 55:45 ACN-TFA, **(C)** Chromatogram of 50:50 ACN-TFA.

was done in triplicates and the results are shown in Table 4. The %recovery was computed by using the formula:

$$\%\text{Recovery} = \frac{\text{Derived Concentration} - \text{Original Concentration x } 100\%}{\text{Original Concentration}}$$

Original Concentration

After tabulating all the %Recovery of the samples and spiked samples, %RSD was computed and checked based on the acceptable limit of 5%RSD.

Quantification of Rhein

A total of 10 Samples collected from different areas in the countries were tested using the validate method. The amount of Rhein quantified are shown in Table 5. The highest concentration was from 210614-LB-KP while the lowest was from the 210507-DAV-KP.

DISCUSSION

The method in this study was validated using the guidelines set under "ICH Topic Q2 R2 or the Validation of Analytical Procedures".[17] Procedures for Linearity, Precision, Accuracy, Limit of Detection, and Limit of Quantitation were performed.

The standard used for the HPLC method developed was Rhein with a Retention time or RT of 5.101 minutes and maximum absorbance at 230 nm. The method developed

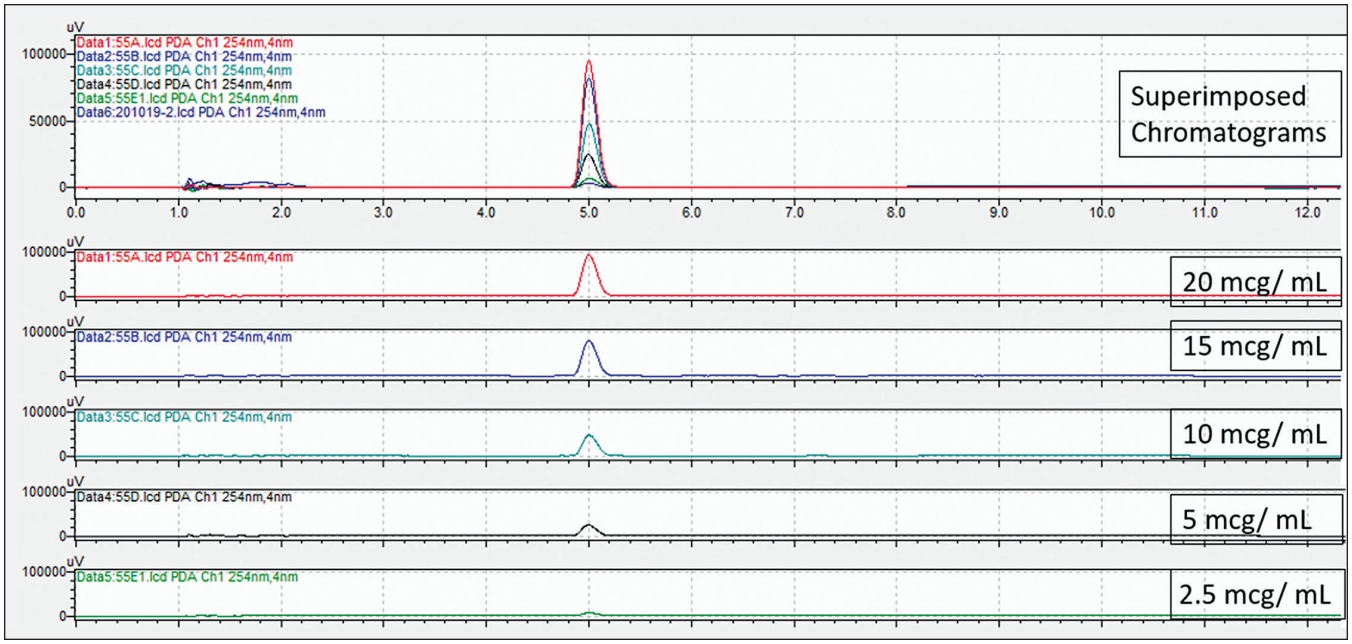

Figure 4. Chromatograms of Rhein Standards.

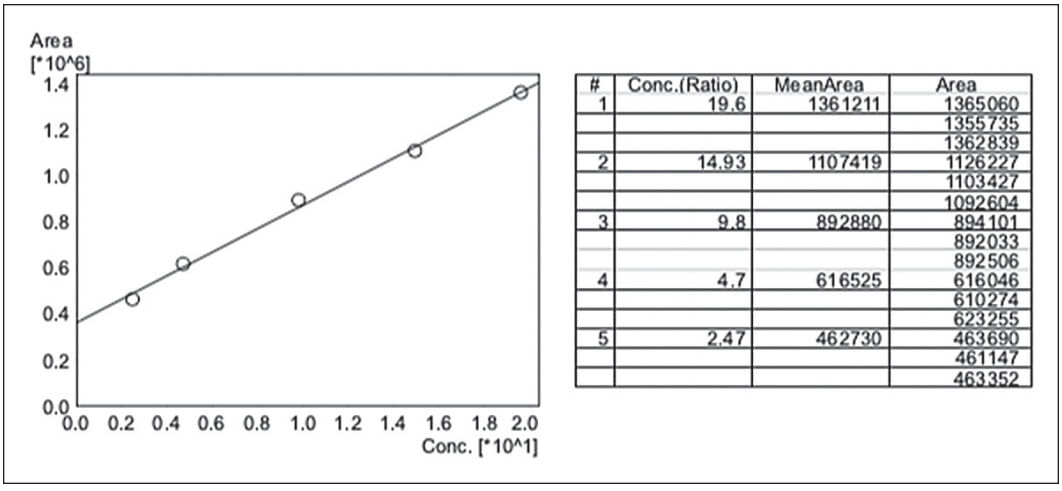

Figure 5. Calibration curve of Rhein Standard.

was linear with an R-value and r2 value of 0.998 and 0.996, respectively. Additional results from the tests above showed that the %RSD for Intraday Precision or within-the-day repeatability studies was 2.63% while for Interday Precision was 3.91%. The %RSD for both Interday and Intraday Precision where within the ≤5% acceptable range for RSD of analytical procedures.

As for accuracy, spiking of a sample with the known concentration of Rhein was performed. The %Recovery for the spiked samples of 0%, 50%, 100%, and 150% were 0.13%, 48.92%, 94.68%, and 150.48%, respectively. An average %RSD of 0.7155 indicates the reliability of the test. These values were close to their target concentrations and indicate the accuracy of the method. The Limit of Detection and Limit of Quantification were calculated by using the standard deviations calculated from the calibration curve of the line and the results being 1.278 mcg/mL and 3.872 mcg/mL, respectively. These values were able to detect the amount of Rhein present among the samples of *Cassia fistula* L. leaves.

Samples of the methanolic crude extract of *Cassia fistula* L. leaves were run through the validated HPLC Method. Among all 10 samples, the highest concentration of Rhein for 1 g crude extract was that of 210614-LB at 14.14mg or about 14.14%. Out of the 10 samples, the 210507-DAV-KP gave the lowest value of 0.22mg or 0.22%.

Table 3. Interday Precision

20 mcg/mL	Area	Concentration (mcg/mL)
Day 1 A.1	1407418	19.907
Day 1 A.2	1347934	19.573
Day 1 A.3	1346864	19.551
Day 2 A.1	1343621	19.487
Day 2 A.2	1353377	18.951
Day 2 A.3	1342715	18.763
Day 3 A.1	1293870	20.263
Day 3 A.2	1285765	19.893
Day 3 A.3	1279055	19.640
Average	1333402.111	19.55866667
%RSD		2.39

15 mcg/mL	Area	Concentration (mcg/mL)
Day 1 A.1	1098057	14.631
Day 1 A.2	1097488	14.619
Day 1 A.3	1092604	14.523
Day 2 A.1	1057973	13.838
Day 2 A.2	1046891	15.292
Day 2 A.3	1043765	15.101
Day 3 A.1	1043887	15.116
Day 3 A.2	1055718	15.231
Day 3 A.3	1412307	16.551
Average	1105410	14.98911111
%RSD		4.96

5 mcg/mL	Area	Concentration (mcg/mL)
Day 1 A.1	612018	5.018
Day 1 A.2	605188	5.281
Day 1 A.3	610923	5.061
Day 2 A.1	524226	4.647
Day 2 A.2	577047	5.148
Day 2 A.3	626808	5.121
Day 3 A.1	527186	5.176
Day 3 A.2	59400	5.318
Day 3 A.3	604151	4.970
Average	527438.5556	5.082222222
%RSD		3.91

Table 4. Standard Addition Method

Concentration (%) added	Theoretical content (mcg/mL)	Concentration	%RSD	Recovery (%)
0%	19.4	19.426±0.176	0.905	100.13±0.9
50%	29.1	28.891±0.038	0.131	97.84±0.13
100%	38.8	37.767±0.653	1.729	94.68±1.68
150%	48.5	48.593±0.205	0.421	100.32±0.42
			Average	98.24%

Table 5. Quantification of Rhein in Different Samples

Sample Batch Name	Concentration (mg/g)
201019-TQ-KP	1.32±0.058
201019-LB-KP	3.08±0.008
201125-PNG-KP	3.66±0.048
210507-DAV-KP	0.22±0.021
210607-PNG-KP	2.465±0.353
210607-TAR-KP	2.461±0.154
210614-TAR-KP	4.045±0.010
210614-LB-KP	14.14±0.08
210712-LAC-KP	1.96±0.012
210906-DAV-KP	2.38±0.035

Table 6. Method Validation Parameters for the Proposed HPLC Method

Parameter	Results
Regression Equation	y = 50561.6x + 358310
Correlation coefficient (r2)	0.996
LOD (µg mL^{-1})	1.278
LOQ (µg mL^{-1})	3.872
Range (µg mL^{-1})	2.5-20 µg mL^{-1}
%Recovery	98.24%
%RSD Accuracy	0.72
%RSD Intraday Precision	2.63
%RSD Interday precision	3.91

CONCLUSION

The method developed in this study (Table 4) underwent the Method Validation of the International Council for Harmonisation (ICH). The summary of the method parameters is tabulated in Table 6. It was found to be accurate having a %RSD of 0.7155 and precise with a %RSD of 2.63% (Intraday Precision) and 3.91% (Interday Precision) (Good %RSD is <5%). The method was found to be useful for the quantification of the amount of Rhein among 10 different samples sourced around the country. This method could be used as a Quality Control tool for the assessment of *Cassia fistula* L. leaves and help future studies as well as future regulations for the proper identification of the plant material and quantification of its Rhein content.

Statement of Authorship

The author certified fulfillment of ICMJE authorship criteria.

Author Disclosure

The author declared no conflicts of interest.

Funding Source

This study was completely funded by the author. No external funding was provided.

REFERENCES

1. Merrill ED. An Enumeration of Philippine Flowering Plants. Vol. 2. Manila, Philippines: Bureau of Printing [Internet]. 1923 [cited 2023 Sep]. Available from: http://www.forgottenbooks.org/books/An_Enumeration_of_Philippine_Flowering_Plants_v2_1000888542.
2. WFO. Cassia fistula var. ovata DC. [Internet]. 2023 [cited 2023 Jun]. Available from: http://www.worldfloraonline.org/taxon/wfo-0001445103.
3. Quisumbing E. Medicinal Plants of the Philippines. Quezon City, Philippines: Katha Publishing House Co. Inc., 1978. p. 977.
4. Gilman EF, and Watson DG. Cassia fistula: Golden Shower 1 [Internet]. 2023 [cited 2023 Jun]. Available from: http://plants.ifas.ul.edu.
5. Blanco FM. Flora de Filipinas, ed. 3 Manila 1845 [Internet]. 2019 [cited 2023 Jun]. Available from: https://commons.wikimedia.org/wiki/File:Cassia_fistula_Blanco1.120.png
6. Mwangi RW, Macharia JM, Wagara IN, Bence RL. The medicinal properties of Cassia fistula L.: A review. Biomed Pharmacother. 2021 Dec;144: 112240. doi: 10.1016/j.biopha.2021.112240. PMID:34601194.
7. Mehta JP. Separation and characterization of Anthraquinone derivatives from Cassia fistula using chromatographic and spectral techniques. Int J Chem Sci. 2012;10(1):306–16.
8. Sharma DK. Enumerations on phytochemical, pharmacological and ethnobotanical properties of Cassia fistula Linn: yellow shower. J Phytopharmacol. 2017; 6(5):300–6. doi: 10.31254/phyto.2017.6509.
9. Saeed,M, Naseer S, Hussain S, Iqbal,M. Phytochemical composition and pharmacological effects of Cassia fistula. Sci Inquiry Rev. 2020 Jan-Mar;4(1):59-69. doi:10.32350/sir.41.05.
10. Mohd D, Singh P, Mishra G, Srivastava S Jha KK, Khosa RL. Cassia fistula L. (Amulthus) – an important medicinal plant: a review of its traditional uses, phytochemistry and pharmacological properties. J Nat Prod Plant Resour. 2011; 1(1):101-18.
11. ASEAN. ASEAN Herbal and Medicinal Plants. ASEAN Secretariat. 2010; pp. 244,274.
12. Subcommittee on the Preparation of Monographs of Selected Thai Materia Medica. Khun-Fak. Journal of Thai Traditional & Alternative Medicine. 2018; 16(3):474-8.
13. Grtisanapan W, Sakulpanich A. Determination of anthraquinone glycoside content in Cassia fistula leaf extracts for alternative source of laxative drug. Int J Biomed Pharm Sci. 2009;3(1):42-5.
14. van Gorkom, BAP Timmer-Bosscha H, de Jong S, van der Kolk DM, Kleibeuker JH, de Vries EGE. Cytotoxicity of rhein, the active metabolite of sennoside laxatives, is reduced by multidrug resistance-associated protein 1. Br J Cancer. 2002 May 6;86(9): 1494-500. doi: 10.1038/sj.bjc.6600255. PMID: 11986786; PMCID: PMC2375364.
15. Sakulpanich A, Chewchinda S, Sithisarn P, Gritsanapan W. Standardization and toxicity evaluation of Cassia fistula pod pulp extract for alternative source of herbal laxative drug. Pharmacogn J. 2012 Mar-Apr;4(28):6-12.doi: 10.5530/pj.2012.28.2.
16. Chewchinda S, Wuthi-udomlert M, Gritsanapan W. HPLC quantitative analysis of Rhein and antidermatophytic activity of cassia fistula pod pulp extracts of various storage conditions. Biomed Res Int. 2013:2013:821295. doi: 10.1155/2013/821295. PMID: 24171175; PMCID: PMC3792547.
17. International Conference on Harmonization (ICH). Q2(R1) – Validation of Analytical Procedures: Text and Methodology [Internet].1996 [cited 2023 Apr]. Available from: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf