European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Extraction of Medicinal Important Glycyrrhizin from Achras zapota L

Article History:

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Received: 28-12-2025
Revised: *18*-01-2026
Accepted: 05-02-2026
Published: 20-02-2026

Abstract: Glycyrrhizic acid (glycyrrhizin, GA) is a biologically active triterpenoid saponin widely recognized for its sweetening potential and pharmacological activities including anti-inflammatory, antiviral, anti-ulcer, and hepatoprotective effects. Although GA has been reported in Achras zapota L., a simple and reproducible extraction and quantitative method has not been well established. In the present study, extraction variables such as solvent polarity, ethanol–water composition, extraction method, time, and temperature were systematically optimized to maximize GA recovery from A. zapota fruit powder. Preliminary solvent screening demonstrated that GA is preferentially extracted using polar solvents, with water showing the highest extraction efficiency (2.40 mg/g), while non-polar solvents such as chloroform showed no detectable GA. Further optimization using ethanol–water mixtures identified ethanol/water (10:90, v/v) as the most effective extraction solvent. Dipping (maceration) extraction was found superior to ultrasonic extraction in terms of yield and practicality. Extraction kinetics revealed increasing GA concentration up to 90 min, after which no significant improvement was observed. Temperature optimization indicated that extraction efficiency increased markedly up to 60°C and remained constant thereafter, enabling rapid extraction within 60 min. Quantification was performed using validated reversed-phase high-performance liquid chromatography (RP-HPLC) with a C18 column and methanol/water (70:30, v/v) containing 1% acetic acid as mobile phase, with UV detection at 252 nm. The method exhibited excellent linearity ($r^2 > 0.9996$), acceptable precision (intra- and inter-day RSD < 0.6%), and a low limit of detection (464 ng/mL). Under optimized conditions, GA content reached 2.50 mg/g with recovery between 88.7–90.3%, confirming accuracy and suitability for routine analysis. This optimized extraction–RP-HPLC approach provides a convenient platform for GA standardization and phytochemical quality assessment of A. zapota.

Keywords: Achras zapota, glycyrrhizic acid, glycyrrhizin, extraction optimization, RP-HPLC, method validation.

INTRODUCTION

Natural products remain a major source of therapeutically important biomolecules, and their continued exploration is essential for the development of safe, effective, and affordable health-promoting agents. Among the most widely recognized phytoconstituents, glycyrrhizic acid (glycyrrhizin, GA) is a triterpenoid saponin traditionally associated with licorice (*Glycyrrhiza* spp.), a medicinal plant that has been used for more than 4000 years in ethnomedicine and classical systems of healing [1]. The genus *Glycyrrhiza* includes nearly 30 species, and at least six species are well-known producers of GA, a compound valued not only for its medicinal importance but also for its intense sweetness and commercial utility [2]. The compound is reported to be nearly 50 times sweeter than sucrose, which has led to its wide use as a natural sweetener and flavoring ingredient in the food and pharmaceutical industries [4]. Due to its dual functional role as both a bioactive and a taste-masking agent, GA continues to attract major interest in nutraceutical formulation and herbal standardization. glycyrrhizic acid has been extensively investigated for its broad-spectrum biological activities. Several studies have described its anti-inflammatory effects, potentially mediated

through modulation of immune and inflammatory responses, including inhibition of complement activation pathways [6]. GA has also demonstrated anti-ulcer activity, supporting its traditional relevance in gastrointestinal disorders [7]. Additionally, it has been used clinically in various countries as a supportive therapeutic agent for chronic viral hepatitis, and its protective role against hepatic damage and toxicity has been supported by clinical and mechanistic research [5,8]. Beyond hepatoprotection, GA has also been linked with antiviral activity, including effects on viral replication and infection progression in specific viral disease contexts [9,10]. Moreover, neuroprotective potential has been reported through suppression of inflammatory transcription regulators such as nuclear factor-κB (NF-κB), particularly in excitotoxic neuronal injury models [3]. Collectively, these findings position GA as a high-value compound with relevance across pharmaceutical, biomedical, and functional food research domains.

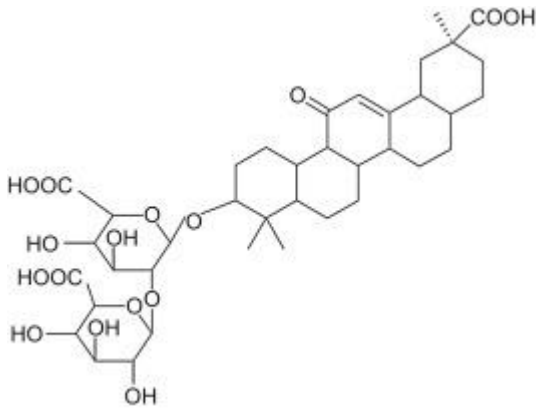

Figure 1. Molecular structures of glycyrrhizic acid

Although licorice remains the primary and commercially dominant natural source of GA, the identification of alternative plant matrices containing glycyrrhizin is valuable for expanding supply sources, improving availability, and enabling comparative phytochemical evaluations. In this context, *Achras zapota* L. (commonly known as sapodilla) has gained attention due to reports indicating the presence of glycyrrhizin-like constituents in its fruit matrix [2,9]. However, despite preliminary evidence of GA occurrence, the extraction process for glycyrrhizic acid from *A. zapota* has not been clearly standardized. For any bioactive marker compound, lack of an optimized extraction and validated quantification method becomes a major limitation in quality evaluation, reproducibility of experimental findings, and industrial feasibility. Therefore, establishing a scientifically optimized, scalable extraction procedure supported by robust analytical separation is essential for advancing *A. zapota* as a potential plant source for GA. Extraction of triterpenoid saponins such as glycyrrhizin is strongly influenced by several key variables including solvent polarity, solvent composition, extraction time, temperature, and technique (maceration, sonication, reflux, etc.). Since GA contains both hydrophilic sugar moieties and a hydrophobic aglycone segment, it typically demonstrates strong affinity for polar or hydroalcoholic extraction systems. However, extraction efficiency is not governed by solvent polarity alone; it also depends on mass-transfer kinetics, matrix swelling, diffusion behavior, and thermal enhancement of solubility. While ultrasonic extraction has become popular due to shorter processing time, it may not always provide higher yields for all compound classes and may demand additional energy input and equipment dependence. In contrast, dipping/maceration extraction is often preferred when the goal is to develop a method that is simple, reproducible, low-cost, and suitable for routine laboratory adoption, especially in phytochemical standardization workflows.Reliable quantification of GA requires a selective and sensitive analytical platform. Reversed-phase high-performance liquid chromatography (RP-HPLC) remains one of the most widely accepted techniques for phytochemical analysis due to its robustness, reproducibility, and compatibility with complex herbal matrices. Several studies have successfully employed chromatographic approaches and LC-based detection strategies for licorice-derived constituents and related compounds [1,10]. Optimizing the mobile phase composition, acidity modifier, detection wavelength, and stationary phase selection is essential to achieve good resolution, stable peak shapes, and accurate quantification. Importantly, method validation parameters such as linearity, precision (intra-day and inter-day), recovery, and limit of detection (LOD) provide scientific assurance of method reliability, making such analytical approaches applicable to quality control and research settings.

The present study was designed to develop an optimized and validated approach for extracting glycyrrhizic acid from *Achras zapota* L. fruits. The work systematically evaluated the influence of different extraction solvents, ethanol–water ratios, extraction method (dipping vs ultrasonic extraction), extraction time and temperature conditions to establish a practical optimum procedure for maximum GA yield. Subsequently, GA was separated and quantified using a validated RP-HPLC method employing an acidic methanol–water mobile phase and UV detection. The outcome of this work provides a reproducible and convenient platform for GA extraction and determination from *A. zapota*, supporting its potential application in phytochemical standardization, quality assessment, and further exploration of medicinally relevant bioactive constituents.

MATERIALS AND METHODS

2.1. Chemicals and Reagents

Glycyrrhizic acid (GA) standard (mono-ammonium salt hydrate) was procured from Raj Scientific, Nagpur (India). Analytical grade solvents including methanol, ethanol, acetonitrile, chloroform and glacial acetic acid were used for extraction and chromatographic analysis. Ultrapure water was used throughout the study. All mobile phase solvents and sample solutions were filtered through 0.2 µm disposable syringe filter units prior to HPLC injection to remove particulate matter and ensure column protection.

2.2. Plant Material and Sample Preparation

Fresh fruits of *Achras zapota* L. were collected and processed for extraction experiments. The fruits were oven-dried sliced into small segments, and then pulverized into a fine powder to improve surface area and enhance extraction efficiency. The powdered material was stored in airtight containers under dry conditions until further use. For each experimental run 1.0 g of fruit powder was accurately weighed for extraction studies.

2.3. Optimization of Extraction Conditions

To establish an optimized extraction protocol, the effects of solvent type ethanol–water composition, extraction method, extraction time and extraction temperature were systematically evaluated.

2.3.1. Screening of Extraction Solvents

To determine solvent suitability 1.0 g of *A. zapota* powder was mixed with 50 mL of different solvents: water, methanol, ethanol, acetonitrile and chloroform. Each extraction was conducted for 240 minutes at room temperature followed by separation of the extract for chromatographic analysis. The extracted GA concentration (mg/g) was calculated and compared across solvents.

2.3.2. Effect of Ethanol–Water Composition

Based on the polarity preference observed in solvent screening, hydroalcoholic mixtures were evaluated. Different ratios of ethanol/water (v/v) were prepared as: 90:10, 70:30, 50:50, 30:70, and 10:90. In each case 50 mL of solvent mixture was used to extract 1.0 g of powdered sample for 240 minutes and GA yield was quantified using RP-HPLC.

2..3.3. Comparison of Extraction Methods (Dipping vs Ultrasonication)

Two extraction techniques were compared:

- Dipping (Maceration) Extraction: Powdered sample (1.0 g) was mixed with 50 mL ethanol/water solvent and stirred continuously. Extraction was performed at different time intervals to determine saturation kinetics.
- Ultrasonic Extraction: Equivalent sample and solvent volumes were subjected to ultrasonication for varying time periods, and the extracted GA levels were compared against dipping extraction. The method was assessed based on yield, practicality, and energy requirement.

2.3.4. Effect of Extraction Time

To determine the time required for maximum recovery, dipping extraction was conducted at various time points ranging from 10 to 150 minutes under fixed solvent composition conditions. Extracts were analyzed, and GA yield versus time was plotted to identify the extraction plateau.

2.3.5. Effect of Extraction Temperature

The influence of temperature was studied by performing dipping extraction at temperatures ranging from 20°C to 70°C while maintaining a fixed extraction time (60 minutes). The extracts were quantified and compared to identify the optimal temperature required for maximum GA extraction efficiency.

2.4. Preparation of Standard and Sample Solutions

A stock solution of GA was prepared by dissolving the standard compound in methanol followed by serial dilution to prepare working standards. For calibration, GA standard solutions of 0.1, 0.2, 0.4, 0.8, and 1.0 mg/mL were prepared. Each concentration was injected three times to generate calibration data. Extracts obtained from *A. zapota* were filtered (0.2 µm) and directly injected into the HPLC system for quantification. Extraction steps followed the optimized procedure established during experimental optimization.

2.5. RP-HPLC Instrumentation and Chromatographic Conditions

Quantitative estimation of GA was performed using a reversed-phase HPLC (RP-HPLC) system consisting of:

M930 solvent delivery pump
UV detector (M720 Absorbance Detector)
Integrated data processing system (Autochrowin Ver. 1.42)

Rheodyne injector with 25 µL sample loop

Chromatographic separation was carried out on a C18 column (150 × 4.6 mm, 5 µm) under the following conditions:

- Mobile Phase: Methanol: Water (70:30 v/v) containing 1% acetic acid
- Flow Rate: 1.0 mL/min
- Detection Wavelength: 252 nm
- Injection Volume: 25 µL

The mobile phase was filtered through 0.2 µm filters before use.

2.6. Method Validation

Method validation was performed to ensure reliability and reproducibility of GA quantification.

2.6.1. Linearity

Calibration curves were constructed by plotting GA peak area versus concentration using the least-square regression method. The regression equation and correlation coefficient (r^2) were calculated to confirm linearity.

2.6.2. Precision

Precision was evaluated by repeated injections of 0.5 mg/mL GA solution:

- Intra-day precision: multiple injections in a single day
- Inter-day precision: repeated measurements over a 5-day period

Precision was expressed as relative standard deviation (RSD, %).

2.6.3. Recovery Studies

Recovery was assessed using a standard addition method by spiking sample extracts with known GA concentrations (0.5, 0.6, and 0.8 mg/mL). Each level was analyzed in triplicate. Recovery (%) was calculated.

2.6.4. Limit of Detection (LOD)

The LOD (ng/mL) for GA was determined as part of method sensitivity evaluation and reported along with validation parameters.

RESULTS AND DISCUSSION

The present investigation focused on developing an optimized and reproducible method for the extraction of glycyrrhizic acid (GA) from *Achras zapota* L. fruits, followed by its quantification using RP-HPLC. Extraction performance was evaluated by systematically varying solvent polarity, ethanol–water ratio, extraction method, extraction time, and temperature as these variables strongly influence solubility, diffusion, and mass transfer of saponin-type phytoconstituents.

3.1. Effect of Different Extraction Solvents

Solvent polarity is a primary determinant of extraction efficiency for GA, a triterpenoid saponin containing hydrophilic glycosidic groups. In this experiment, water, methanol, ethanol, acetonitrile and chloroform were screened under identical extraction conditions (50 mL solvent, 1.0 g powder, 240 min, room temperature). GA was predominantly extracted using polar solvents, confirming its high affinity toward aqueous systems.

Water produced the highest GA yield (2.40 mg/g) whereas moderately polar alcohols (methanol and ethanol) extracted only ~0.86–0.88 mg/g. No detectable GA was found in acetonitrile or chloroform, demonstrating that GA is poorly soluble in less polar/organic non-aqueous media.

Table 1. Extracted Amounts of GA Using Different Solvents

Solvent	GA (mg/g)
Water	2.40
Methanol	0.86
Ethanol	0.88
Acetonitrile	*Not detected*
Chloroform	*Not detected*

Discussion: These results confirm that GA extraction from *A. zapota* requires a highly polar solvent system, with water showing maximum recovery due to better dissolution of glycosidic fractions and effective swelling of plant matrix.

3.2. Effect of Ethanol–Water Composition

Although water provided the highest yield, hydroalcoholic systems were studied to determine whether ethanol addition improves matrix penetration and overall recovery. Various ethanol/water ratios (90:10 to 10:90 v/v) were evaluated (50 mL solvent, 1.0 g sample, 240 min).

The results showed a strong dependence of GA recovery on ethanol proportion. The most efficient extraction occurred at high water content.

Table 2. Extracted Amounts of GA with Different Ethanol–Water Ratios

Ethanol:Water (v/v)	GA (mg/g)

10:90	2.40
30:70	1.86
50:50	1.32
70:30	1.13
90:10	1.09

Discussion: The maximum extraction yield at 10:90 ethanol/water indicates that GA behaves as a highly water-favoring polar phytoconstituent. Increasing ethanol content reduced extraction efficiency, likely due to reduced solubility of GA in alcohol-rich media and reduced hydration-driven matrix swelling.

3.3. Effect of Extraction Method (Dipping vs Ultrasonication)

Two extraction approaches were compared under optimized solvent composition (ethanol/water 10:90):

- Dipping (maceration) extraction
- Ultrasonic extraction

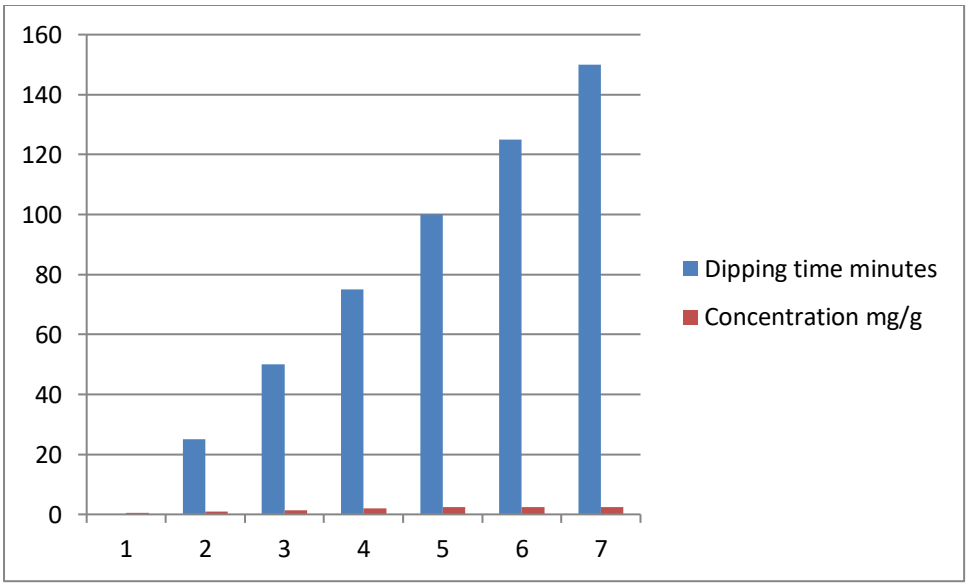

Figure 2. Effect of Dipping Time on GA Extraction

(Trend: GA increases with time and reaches plateau after ~90 min)

Table 3 -Effect of Dipping Time on GA Extraction

Dipping Time (min)	GA (mg/g)
0	0.6
25	0.9
50	1.4
75	2.1
100	2.5
125	2.5
150	2.5

GA increased steadily from 10–90 min and then became nearly constant, indicating extraction equilibrium.

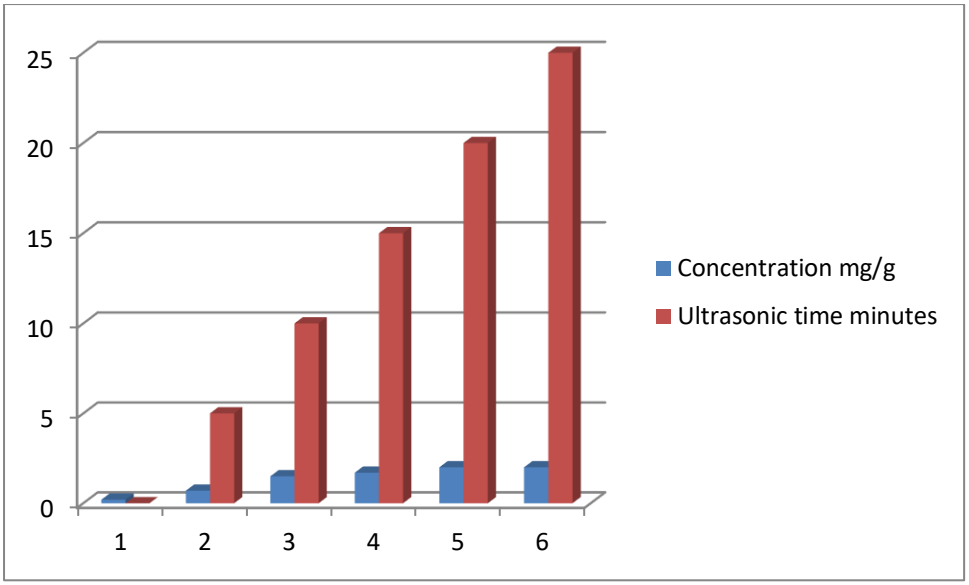

Figure 3. Effect of Ultrasonic Time on GA Extraction

Table .4 - Effect of Ultrasonic Time on GA Extraction

Ultrasonic Time (min)	GA (mg/g)
0	0.2
5	0.7
10	1.5
15	1.7
20	2.0
25	2.0

Discussion: Although ultrasonic extraction enhanced yield with time, its maximum recovery (~2.0 mg/g) was lower than dipping extraction (2.5 mg/g). Additionally, ultrasonication requires higher energy input and equipment dependence. Therefore, dipping extraction was selected as the most suitable method, offering higher yield and operational simplicity.

3.4. Optimization of Extraction Temperature

Extraction temperature impacts solubility and diffusion of GA within the plant matrix. Dipping extraction was performed across 20–70°C for 60 min.

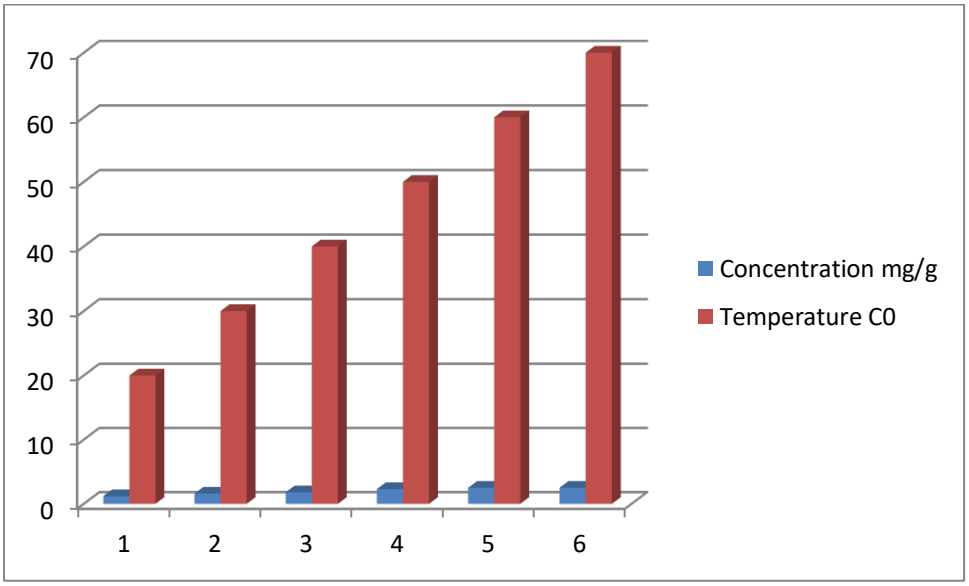

Figure 4. Effect of Temperature on GA Extraction

Table 5. ***Effect of Temperature on GA Extraction***

Temperature (°C)	GA (mg/g)
20	1.2
30	1.6
40	1.8
50	2.3
60	2.5
70	2.5

GA recovery increased sharply up to 60°C**, after which it plateaued.**
Discussion: The optimized temperature was 60°C, because it achieved maximum extraction rapidly without further gain at higher temperature. Practically, this means 60 min at 60°C provides a yield comparable to significantly longer extraction at room temperature.

3.5. RP-HPLC Method Validation

Method validation confirmed that the analytical procedure is reliable for routine quantification. Calibration standards (0.1–1.0 mg/mL) showed excellent linearity.

- Regression equation: $Y = 6623.7x + 18.794$
- Linearity: $r^2 > 0.9996$
- Precision: Intra-day and inter-day RSD values were both < 0.6%
- LOD: 464 ng/mL

Table 6. Precision, Recovery, and LOD of GA

Parameter	GA
Intra-day RSD (%)	0.54
Inter-day RSD (%)	0.59
Added (mg/mL)	0.5 / 0.6 / 0.8
Recovery (%)	88.7 / 90.1 / 90.3
Recovery RSD (%)	0.66
LOD (ng/mL)	464

Discussion: High linearity and low RSD values confirm excellent precision and repeatability of the RP-HPLC method. Recovery values (~89–90%) demonstrate acceptable analytical accuracy, supporting the suitability of the method for quantitative estimation of GA from complex fruit matrix.

CONCLUSION

The present study successfully developed and validated a simple, efficient, and reproducible extraction–quantification strategy for the determination of glycyrrhizic acid (GA) from *Achras zapota* L. fruits. Since glycyrrhizic acid is an important bioactive saponin with well-established medicinal value, the availability of a reliable extraction protocol is essential for phytochemical standardization, quality evaluation, and future pharmacological exploration of *A. zapota* as a non-conventional plant source of GA. A systematic optimization approach was adopted in which key extraction variables such as solvent type, ethanol–water composition, extraction method, extraction time, and temperature were critically assessed. Solvent screening demonstrated that GA is highly extractable in polar media, with water and water-rich solvent systems producing markedly higher yields than organic solvents. This confirms that GA extraction from the fruit matrix is largely dependent on solvent polarity and the ability of aqueous phase to enhance matrix swelling and solubilization of glycosidic constituents. Among ethanol–water combinations, the study confirmed that a water-dominant composition was optimal, supporting improved dissolution and mass transfer of GA from powdered plant material into the extraction medium. With respect to extraction techniques dipping (maceration) extraction was found to be more suitable than ultrasonic extraction. Although ultrasonication enhanced extraction up to a certain duration, it produced comparatively lower GA yields and required additional energy input and equipment dependence. The dipping method, on the other hand, provided higher extraction efficiency, better practicality, and easier scalability for routine laboratory and industrial applications. Extraction kinetics suggested that GA recovery increased progressively with time and achieved equilibrium after an optimal duration, indicating saturation of the extraction process beyond that point. Temperature optimization further revealed that elevated temperature significantly improves extraction efficiency up to an optimal level. The extraction yield increased rapidly as temperature increased and reached a plateau beyond the optimized temperature, confirming that higher temperature accelerates solute diffusion and solubility without offering additional

benefit beyond the established limit. Under optimized conditions, the extraction process produced a maximum GA yield of approximately 2.50 mg/g from *A. zapota* fruit powder, demonstrating that *A. zapota* is a measurable and promising matrix for GA recovery. For quantitative assessment, the extracted GA was effectively separated and measured using a validated RP-HPLC method employing a C18 column and an acidic methanol–water mobile phase. The analytical method demonstrated excellent linearity high precision (low intra-day and inter-day variability), and acceptable sensitivity. Accuracy was further supported through recovery experiments, which yielded recoveries close to ~89–90%, confirming that the method is robust for routine quantification in complex plant extracts.

The developed methodology provides a cost-effective, accurate, and practical platform for GA extraction and determination from *Achras zapota* L. fruits. The optimized extraction conditions and validated RP-HPLC protocol can be utilized for phytochemical profiling, raw material standardization, and further medicinal/industrial applications and also serve as a foundation for future studies involving scale-up, formulation development, and bioactivity correlation of GA-rich extracts.

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