

Phytochemical Analysis and Comparative HPTLC Profiling of *Cassia siamea* Leaf Extracts Using Different Solvents

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Abstract:

This study presents the results of phytochemical analysis and comparative High-Performance Thin-Layer Chromatography (HPTLC) profiling of *Cassia siamea* leaf extracts obtained using three different solvents. *Cassia siamea*, a plant with diverse traditional uses and potential pharmacological properties, was subjected to Soxhlet extraction with methanol, ethanol, and water. Phytochemical analysis revealed the presence or absence of various compounds in each solvent extract. Subsequently, HPTLC analysis was conducted to profile the constituents of the extracts, both before and after derivatization. The HPTLC profiles were compared in terms of colors and R_f values to assess the variation in chemical composition among the extracts. The findings provide insights into the phytochemical composition of *Cassia siamea* leaves and the influence of solvent polarity on extract constituents. This research contributes to the understanding of the medicinal and pharmacological potential of *Cassia siamea* and highlights the importance of solvent selection in plant extraction procedures.

Keywords: *Phytochemical Analysis, High-Performance Thin-Layer Chromatography (HPTLC), Cassia siamea, Soxhlet Extraction, Solvent Polarity*

Introduction:

Cassia siamea, commonly known as Siamese *Cassia* or Siamese senna, is a plant species belonging to the Fabaceae family. Native to Southeast Asia, particularly Thailand and Myanmar, *Cassia siamea* is widely distributed in tropical and subtropical regions [1]. This evergreen tree is valued for its ornamental beauty, fast growth, and various traditional uses in folk medicine.

Several pharmacological studies have investigated the bioactive constituents and potential medicinal properties of *Cassia siamea*. Phytochemical analysis of *Cassia siamea* extracts has revealed the presence of various secondary metabolites, including flavonoids, alkaloids, tannins, saponins, and phenolic compounds. These bioactive constituents are attributed to the plant's diverse pharmacological activities, such as antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, antidiabetic, and analgesic effects [2].

Despite the traditional uses and pharmacological potential of *Cassia siamea*, there is still a need for comprehensive scientific investigations to elucidate its phytochemical composition and pharmacological properties. Moreover, understanding the influence of extraction solvents on the chemical profile of *Cassia siamea* extracts is crucial for optimizing extraction protocols and maximizing the yield of bioactive compounds.

Phytochemical analysis involves the qualitative and quantitative determination of bioactive compounds present in plant materials. This includes the identification of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, terpenoids, and saponins, which contribute to the therapeutic effects of medicinal plants [2]. Phytochemical analysis helps researchers understand the chemical profile of plant extracts, which is essential for assessing their medicinal potential and establishing correlations between chemical composition and biological activities.

Phytochemical analysis and High-Performance Thin-Layer Chromatography (HPTLC) play significant roles in plant research by providing valuable insights into the chemical composition and quality of plant extracts. These analytical techniques are instrumental in identifying and quantifying various phytoconstituents, elucidating their pharmacological properties, and ensuring the standardization and quality control of herbal medicines.

HPTLC is a powerful analytical technique used for the separation, identification, and quantification of complex mixtures of phytochemicals in plant extracts. HPTLC offers several advantages, including high resolution, reproducibility, sensitivity, and cost-effectiveness [3]. By employing stationary phases of different polarities and mobile phases of varying compositions, HPTLC allows researchers to separate and visualize individual components within a plant extract. Moreover, HPTLC can be coupled with densitometric detection, enabling quantitative analysis of phytoconstituents based on their chromatographic signals.

The significance of phytochemical analysis and HPTLC in plant research lies in their contributions to the discovery and characterization of bioactive compounds with potential pharmacological activities. These analytical techniques facilitate the screening of plant extracts for lead compounds, the optimization of extraction and purification processes, and the development of standardized herbal formulations for therapeutic use. Additionally, phytochemical analysis and HPTLC play crucial roles in quality control and authentication, ensuring the consistency and safety of herbal products in the pharmaceutical and nutraceutical industries.

Phytochemical analysis and HPTLC are indispensable tools in plant research, offering valuable insights into the chemical composition, pharmacological properties, and quality of medicinal plants and their derivatives.

The objectives of this study are as follows:

1. To conduct phytochemical analysis of *Cassia siamea* leaf extracts prepared using different solvents (acetone, hexane, and methanol) to determine the presence or absence of various phytochemical constituents.
2. To perform High-Performance Thin-Layer Chromatography (HPTLC) profiling of the *Cassia siamea* leaf extracts before and after derivatization, utilizing acetone, hexane, and methanol as solvents, and to characterize the chromatographic profiles based on colors and R_f values.
3. To compare the phytochemical composition and HPTLC profiles of the *Cassia siamea* leaf extracts obtained with acetone, hexane, and methanol, thereby elucidating the influence of solvent polarity on the chemical constituents extracted from the plant material.

Materials and methods

1. Collection of *Cassia siamea* Leaves:

Cassia siamea leaves were collected from healthy, mature trees in a local botanical garden. Leaves were carefully harvested to avoid damage and contamination. The collection was carried out during the appropriate season to ensure optimal phytochemical content.

2. Preparation of *Cassia siamea* Leaf Extracts:

- The collected leaves were thoroughly washed with distilled water to remove any dirt or debris.
- The washed leaves were air-dried in a well-ventilated area at room temperature to avoid degradation of phytochemical constituents.
- Once dried, the leaves were finely powdered using a mechanical grinder to increase the surface area for extraction.
- Three solvent systems, namely acetone, hexane, and methanol, were selected for extraction based on their varying polarities and extraction efficiencies [4].

3. Extraction Procedure:

- Soxhlation extraction was employed to extract the phytochemical constituents from the powdered *Cassia siamea* leaves.
- Approximately 75 grams of the powdered leaves were placed in the Soxhlet extractor.
- Each solvent (acetone, hexane, and methanol) was added to the Soxhlet extractor in separate extraction cycles.
- The extraction process was carried out for 8 hours per cycle under reflux conditions to ensure efficient extraction of the plant constituents.
- After completion of the extraction process, the solvent was evaporated under reduced pressure using a rotary evaporator to obtain concentrated leaf extracts.

4. Storage of Leaf Extracts:

- The obtained *Cassia siamea* leaf extracts were transferred to clean, airtight containers and stored in a cool, dark place to prevent degradation of phytochemicals.

5. Phytochemical Analysis:

- Standard qualitative tests were performed according to established protocols to detect the specific classes of phytochemicals like protein, sterols, alkaloids, Tannins, amino acids, carbohydrates, monosachharides, saponin, flavons, glycosides along with HPTLC present in each extract as described in pharmacopeia, book by Harborne [5] and other authors.

Procedure for each compound is briefly mentioned as:

1. Biuret Test (For detection of Protein) [6]:

- Take a small amount of the sample extract in a test tube.
- Add a few drops of Biuret reagent (typically a solution of copper sulfate and potassium hydroxide).
- Mix the contents gently and observe the color change.
- A pink or violet color indicates the presence of proteins.

2. Salkowski Test (For detection of Sterols) [7]:

- Take a small amount of the sample extract in a test tube.
- Add a few drops of Salkowski reagent (consisting of concentrated sulfuric acid and glacial acetic acid).
- Observe the formation of a reddish-brown color at the interface.
- The appearance of the color indicates the presence of sterols.

3. Wagner's Test (For detection of Alkaloids) [7].

- Take a small amount of the sample extract in a test tube.
- Add Wagner's reagent (iodine in potassium iodide solution) drop by drop.
- Observe the formation of a reddish-brown precipitate.
- The appearance of the precipitate indicates the presence of alkaloids.

4. Ferric Chloride Test (For detection of Tannins) [8] :

- Take a small amount of the sample extract in a test tube.
- Add a few drops of ferric chloride solution.
- Observe the color change.
- A blue-black or greenish-black color indicates the presence of tannins.

5. Ninhydrin Test (For detection of Amino Acids) [8]:

- Take a small amount of the sample extract in a test tube.
- Add a few drops of ninhydrin reagent (typically a solution of ninhydrin in ethanol).
- Heat the mixture gently.
- Observe the appearance of a purple color.
- The appearance of the color indicates the presence of amino acids.

6. Molisch Test (For detection of Sugars) [8].

- Take a small amount of the sample extract in a test tube.
- Add a few drops of Molisch reagent (a solution of alpha-naphthol in ethanol).
- Carefully layer concentrated sulfuric acid down the side of the test tube.
- Observe the formation of a purple ring at the interface.
- The appearance of the ring indicates the presence of sugars.

7. Barford Test (For detection of Monosaccharides) [9].

- Take a small amount of the sample extract in a test tube.
- Add a few drops of Barford reagent (solution of copper sulfate and potassium sodium tartrate).
- Heat the mixture gently.
- Observe the appearance of a red or orange precipitate.
- The appearance of the precipitate indicates the presence of monosaccharides.

8. Foam Test (For detection of Saponins):

- Take a small amount of the sample extract in a test tube.
- Shake the test tube vigorously with water.
- Observe the formation of stable foam or froth.
- The formation of foam indicates the presence of saponins.

9. Shimoda Test (For detection of Flavones) [7].

- Take a small amount of the sample extract in a test tube.
- Mix with concentrated hydrochloric acid and magnesium metal.
- Observe the appearance of pink, red, or purple colorations.
- The appearance of the color indicates the presence of flavones.

10. Borntrager's Test (For detection of Glycosides) [7]:

- Take a small amount of the sample extract in a test tube.
- Add equal volume of dilute hydrochloric acid.
- Shake and filter.
- Add an equal volume of chloroform to the filtrate and shake.
- Observe the interface.
- The appearance of a pink or red color in the chloroform layer indicates the presence of glycosides.

High-Performance Thin-Layer Chromatography (HPTLC) method is used for analyzing the *Cassia siamea* leaf extracts:

1. Instrumentation:

- HPTLC plates: Silica Gel precoated plates 60 F254 with a thickness of 0.2 mm were used (from Merck India Limited, Mumbai) (Anno, 2008).
- TLC Applicator: Camag Linomat-5 (from Camag Switzerland, Model Number: 140443) was utilized for band application.
- Photo Documentation Unit: Camag Reprostar-3 (from Camag Switzerland, Model Number: 140604) was employed for the documentation of chromatographic fingerprints [8]

2. Preparation of Test Solutions:

- Prepare separate test solutions of *Cassia siamea* leaf extracts in methanol, acetone, and hexane.
- Ensure that the test solutions are properly labeled and stored in clean, tightly-sealed containers.

3. HPTLC Procedure:

- Apply 6 µl of each extract test solution as 6 mm bands onto the HPTLC plates using the TLC applicator.
- Develop the plates separately in the following solvent systems to a distance of 9.5 cm:
 - Toluene:ethyl acetate (7:3) for methanolic extract.
 - Toluene:ethyl acetate:formic acid (7:2.5:0.5) for acetone extract.
 - Toluene:ethyl acetate:formic acid (7:2.8:0.2) for hexane extract [7].
- Allow the developed plates to dry at room temperature.

4. Visualization Before Derivatization:

- Examine the dried plates under UV light at wavelengths of 254 nm and 366 nm [10].
- Record any visible bands or spots observed on the plates and note their colors.

5. Derivatization Procedure:

- Derivatize the plates using 5% methanolic-sulfuric acid reagent.
- Heat the plates at 105°C until the bands become clearly visible [6].
- Ensure that the plates are adequately cooled before further examination.

6. Visualization After Derivatization:

- Examine the derivatized plates under UV light at a wavelength of 366 nm.
- Record the R_f values and colors of the bands observed on the plates after derivatization.

7. Data Recording and Analysis:

- Record all observations, including Rf values and colors, in a systematic manner.
- Compare the chromatographic fingerprints of the different extracts to identify similarities and differences [7]
- Analyze the obtained data to draw conclusions regarding the phytochemical composition of the *Cassia siamea* leaf extracts.

Observation and results:

Primary chemical tests for the qualitative detection of various compounds in three different extracts were recorded. Result summarized in **Table1**.

Test	For detection of	Acetone	Hexane	Methanol
Biuret test	Protein	-	+	+
Salkowski	Sterols	-	+	+
Wagner's	Alkaloids	-	+	+
Ferric chloride	Tanins	-	-	+
Ninhydrin	Amino acid	-	-	+
Molisch	Sugar	+	+	+
Barford	Monosachharides	+	+	+
Foam test	Sapnins	+	+	+
Shimoda	Flavones	-	-	+
Borntrager's	Glycosides	-	-	+

Table 1: Qualitative result of phytochemical test, Here + = present, - = absent

- The presence of proteins was observed in the hexane and methanol extracts but not in the acetone extract. This suggests that hexane and methanol are more effective solvents for extracting proteins from *Cassia siamea* leaves compared to acetone.

- Sterols were detected in the hexane and methanol extracts but not in the acetone extract. This indicates that hexane and methanol are better solvents for extracting sterols from *Cassia siamea* leaves.

- Alkaloids were present in the hexane and methanol extracts but not in the acetone extract. This suggests that hexane and methanol are more efficient solvents for extracting alkaloids from *Cassia siamea* leaves.

- Tannins were only detected in the methanol extract, indicating that methanol is the most effective solvent for extracting tannins from *Cassia siamea* leaves.

- Amino acids were detected only in the methanol extract, suggesting that methanol is the preferred solvent for extracting amino acids from *Cassia siamea* leaves.

- Sugars were present in all three extracts, indicating that *Cassia siamea* leaves contain sugars that are extractable by acetone, hexane, and methanol.

- Monosaccharides were present in all three extracts, indicating that *Cassia siamea* leaves contain monosaccharides that are extractable by acetone, hexane, and methanol.

- Saponins were present in all three extracts, indicating that *Cassia siamea* leaves contain saponins that are extractable by acetone, hexane, and methanol.

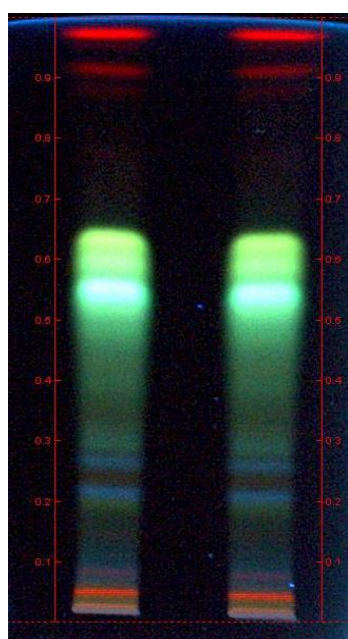
- Flavones were not detected in any of the extracts, suggesting that *Cassia siamea* leaves may not contain significant amounts of flavones.

- Glycosides were not detected in any of the extracts, indicating that *Cassia siamea* leaves may not contain significant amounts of glycosides.

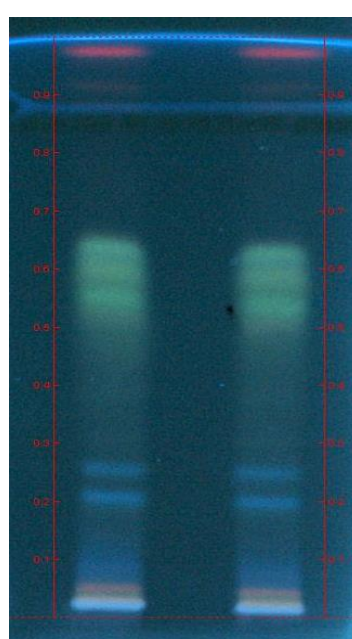
The phytochemical analysis revealed that the choice of solvent significantly influences the types of compounds extracted from *Cassia siamea* leaves. Methanol appeared to be the most effective solvent for extracting a wide range of compounds, followed by hexane, while acetone showed limited extraction efficiency for certain compounds.

Based on the provided HPTLC profiling results for the methanol, acetone, and hexane extracts of *Cassia siamea* leaves before and after derivatization at 366nm, following R_f values and band colour were obtained, summarized in **Table2**, **Table3** and **Table4**.

1. Methanolic Extract HPTLC result



A B
Fig. I, 366nm before derivatization



A B
Fig. II, 366nm after derivatization

R _f values	366nm before derivatization	366nm after derivatization
R _{f1}	0.06 (red)	0.04 (pink)
R _{f2}	0.08 (pink)	0.08 (sky blue)
R _{f3}	0.10 (sky blue)	0.22 (sky blue)
R _{f4}	0.22 (sky blue)	0.26 (sky blue)
R _{f5}	0.26 (sky blue)	0.60 (brownish yellow)
R _{f6}	0.54 (white)	0.96 (pink)
R _{f7}	0.60 (yellowish white)	-
R _{f8}	0.92 (red)	-
R _{f9}	0.96 (red)	-

Table2: HPTLC profiling of methanolic extract of *Cassia siamea* leaf.

2. Acetone Extract HPTLC result



A
Fig. III, 366nm before derivatization



A B
Fig. IV, 366nm after derivatization

R _f values	366nm before derivatization	366nm after derivatization
R _{f1}	0.06 (pink)	0.06 (pink)
R _{f2}	0.60 (sky blue)	0.60 (sky blue)
R _{f3}	0.94 (pink)	0.94 (pink)

Table4: HPTLC profiling of acetone extract of *Cassia siamea* leaf.

3. Hexane Extract HPTLC result



A B
Fig. V, 366nm before derivatization



A B
Fig. VI, 366nm after derivatization

R_f values	366nm before derivatization	366nm after derivatization
R_{f1}	0.04 (sky blue)	0.08 (sky blue)
R_{f2}	0.56 (sky blue)	0.56 (sky blue)
R_{f3}	0.90 (sky blue)	-
R_{f4}	0.96 (light pink)	-

Table5: HPTLC profiling of acetone extract of *Cassia siamea* leaf.

Methanol Extract:

- R_f 1: The band shifted from 0.06 (red) to 0.04 (pink) after derivatization, indicating a change in polarity or chemical structure.
- R_f 2: The band remained at 0.08 (pink) before and after derivatization, suggesting stability or minimal chemical change.
- R_f 3 to R_f 6: The bands shifted to higher R_f values and displayed various color changes after derivatization, indicating alterations in polarity or chemical structure.
- R_f 7 to R_f 9: Some bands were not detected after derivatization, suggesting possible decomposition or transformation of compounds.

Acetone Extract:

- R_f 1 to R_f 3: The bands remained at similar R_f values and colors before and after derivatization, indicating stability or minimal chemical change.
- Overall, the profile of the acetone extract showed minimal alterations after derivatization compared to the methanol extract.

Hexane Extract:

- R_f 1 to R_f 2: The bands remained at similar R_f values and colors before and after derivatization, suggesting stability or minimal chemical change.
- R_f 3 to R_f 4: Some bands were not detected after derivatization, similar to the methanol extract, indicating potential decomposition or transformation of compounds.

Discussion:

- The HPTLC profiling results show variations in band positions and colors before and after derivatization, indicating changes in chemical properties or structures of the compounds present in the extracts.
- Derivatization at 366nm resulted in shifts in R_f values and alterations in band colors, suggesting modifications in the polarity or chemical composition of the compounds.
- The methanol extract exhibited more pronounced changes in band positions and colors after derivatization compared to the acetone and hexane extracts, indicating potentially more diverse or reactive compounds present.
- Some bands were not detected after derivatization in all three extracts, suggesting possible degradation or transformation of compounds under the derivatization conditions.
- Further analysis and identification of the specific compounds corresponding to the bands observed before and after derivatization would provide insights into the phytochemical composition and potential bioactive components of the *Cassia siamea* leaf extracts.

Conclusion:

Our study investigated the phytochemical composition and comparative HPTLC profiling of *Cassia siamea* leaf extracts using three different solvents: methanol, acetone, and hexane. Through qualitative phytochemical tests and HPTLC analysis, we observed varying profiles of phytoconstituents extracted by each solvent. Methanol extraction yielded a diverse range of compounds, including proteins, sterols, alkaloids, sugars, and saponins, as evidenced by the HPTLC bands' shifts and color changes before and after derivatization. Acetone and hexane extracts displayed fewer detected compounds, indicating differences in the efficiency of solvent extraction. Our findings highlight the importance of solvent selection in phytochemical analysis and demonstrate the potential pharmacological diversity of *Cassia siamea* leaf constituents. Further studies employing advanced analytical techniques are warranted to elucidate the specific bioactive compounds responsible for the observed phytochemical profiles, thereby contributing to the understanding of *Cassia siamea*'s therapeutic potential.

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