Jurnal Farmasi Teknologi Sediaan Dan Kosmetika
Volume 2, Nomor 3, Tahun 2025
E-ISSN 3063-2226
DOI : 10.70075/jftsk.v2i3.143

Characterization of Methanol Fraction Compounds from Formulation Chinese Senna Leaves (Cassia Alata L.) Using UV-Vis Spectrophotometry Method

A. Mu'thi Andy Suryadi[1],Wicawati Van Solang[2*],Mohamad Aprianto Paneo[3]

[1,2,3]*Department of Pharmacy, Faculty of Sport and Health, Universitas Negeri Gorontalo, Jenderal Sudirman Street No. 06 Gorontalo City 96128, Indonesia*

** Penulis Korespondensi. Email: wicawativansolang@gmail.com*

ABSTRAK

Daun Ketepeng Cina (Cassia Alata L.) bagian penting dari kehidupan manusia sehari-hari yang digunakan sebagai obat-obatan, kurap, cacingan, sembelit, dan sariawan. Ketepeng cina (Cassia Alata L.) atau lebih dikenal dengan nama gelinggang memiliki rasa pedas dan bersifat hangat. Penelitian ini bertujuan untuk mengetahui identifikasi senyawa metabolit sekunder dari formulasi daun ketepeng cina. Metode yang digunakan pada penelitian ini yaitu maserasi dengan menggunakan pelarut metanol. Analisis kualitatif pada penelitian ini menggunakan metode uji warna, kromatografi lapis tipis (KLT) dengan eluen n-heksan : etil asetat (4:1) dan analisis kuantitatif menggunakan spektrofotometri UV-Vis pada panjang gelombang 260,00 nm, 213,18 nm, 345,24 nm, 202,56 nm, dan 363,63 nm. hasil menunjukan bahwa pada daun ketepeng cina (Cassia Alata L.) positif mengandung senyawa flavonoid, alkaloid, steroid, saponin, dan tanin. Disimpulkan bahwa identifikasi senyawa metabolit sekunder daun ketepeng cina (Cassia Alata L.) dengan menggunakan spektrofotometri UV-Vis menggunakan pelarut metanol memberikan spectrum yang mempunyai senyawa metabolit sekunder

Kata Kunci: Cassia Alata L.; Metabolit sekunder; Spektrofotometri UV-Vis; Maserasi; Kromatografi lapis tipis (KLT)

Diterima:	*Disetujui:*	*Online:*
15-12-2024	*12-10-2025*	*12-10-2025*

ABSTRACT

The leaves of Cassia Alata L. (Chinese Senna) are an important part of daily human life and are used as medicine for treating ringworm, worms, constipation, and canker sores. Cassia Alata L., also known as Gelinggang, has a spicy taste and is warm in nature. This study aims to identify the secondary metabolite compounds present in Formula Cassia Alata L. leaves. The method used in this study is maceration with methanol as the solvent. Qualitative analysis in this study utilized color tests, thin-layer chromatography (TLC) with a hexane:ethyl acetate (4:1) solvent system, and quantitative analysis using UV-Vis spectrophotometry at wavelengths of 260.00 nm, 213.18 nm, 345.24 nm, 202.56 nm, and 363.63 nm. The results show that Cassia Alata L. leaves positively contain flavonoids, alkaloids, steroids, saponins, and tannins. It is concluded that the identification of secondary metabolite compounds from Cassia Alata L. leaves using UV-Vis spectrophotometry with methanol as a solvent provides spectra that correspond to secondary metabolite compounds

Keywords: *Cassia alata L.; Secondary metabolites; UV–Vis spectrophotometry; Maceration ; Thin layer chromatography (TLC)*

Received:	*Accepted:*	*Online:*
2024-12-15	*2025-10-12*	*2025-10-12*

1. Introduction

Indonesia harbors extraordinary biological diversity and a long tradition of employing plants as primary health care. Among the numerous species used in folk medicine, Cassia alata L. (locally known as ketepeng cina or gelinggang) is notable for its year-round availability, ability to thrive in humid tropical and subtropical environments, and breadth of empirical indications. The leaves are commonly applied to manage dermatophytosis, helminthiasis, constipation, and oral lesions, pointing to a rich repertoire of bioactive constituents [1,2].

Secondary-metabolite classes frequently associated with Cassia species include tannins, anthraquinones such as aloe-emodin and chrysophanic acid, alkaloids, saponins, glycosides, flavonoids, steroids, and terpenoids. Many members of these classes display astringent, antidiarrheal, antimicrobial, and antioxidant actions, properties that align with traditional claims and support further investigation. Their broad bioactivity has also stimulated interest in C. alata as a candidate source of botanical pesticides for sustainable plant protection. The pharmacological breadth implied by long-standing use, together with the chemical plausibility of these metabolite classes, underscores the need for rigorous chemical characterization [3,4].

Extraction solvent and polarity profoundly influence the metabolite profile recovered from plant matrices. Polar solvents such as aqueous ethanol or methanol tend to enrich phenolics and flavonoids, whereas less polar solvents recover terpenoids and certain alkaloids. Fresh and dried leaves may also differ qualitatively because enzymatic activity, moisture content, and matrix microflora affect the stability and accessibility of labile constituents. These considerations highlight the importance of standardized sample handling, optimized extraction conditions, and transparent reporting to support reproducibility and future pharmacopoeial standardization [5,6].

Phytochemical screening offers a pragmatic first step to map major classes before targeted isolation. Thin-layer chromatography enables rapid separation and visualization of constituent groups, while UV–Vis spectrophotometry provides class-informative absorption signatures across the 200–700 nm range that can support preliminary identification. Together, these techniques yield reproducible, cost-effective fingerprints that can be compared across batches and correlated with downstream bioactivity assays, paving the way for mechanism-directed research [7,8].

This work aims to identify and characterize the principal classes of secondary metabolites present in C. alata leaves using maceration extraction followed by qualitative color tests, thin-layer chromatography, and UV–Vis spectrophotometry. By establishing a coherent analytical workflow and describing spectral features consistent with key metabolite classes, the study seeks to provide a chemical rationale for the plant's traditional applications, inform the initial standardization of botanical raw materials, and prioritize fractions for subsequent purification and mechanism-directed evaluation. Clarifying the chemistry of this widely used species is a necessary step toward the rational development of safe, effective phytotherapeutics and the responsible integration of Indonesia's botanical resources into evidence-based health strategies [9,10].

2. Methods

Research design

This laboratory study investigated leaves of Cassia alata L. using methanolic maceration. Dried leaf simplicia were pulverized and macerated (1:10 w/v) for 72 h at room temperature, protected from light, with periodic agitation. The filtrate was filtered and concentrated under reduced pressure using a rotary evaporator (≤40 °C); extract yield was calculated. The extract underwent phytochemical screening (color tests) and TLC with n-hexane:ethyl acetate (4:1) as the mobile phase; spots were visualized at 254/366 nm and Rf values determined. UV–Vis spectrophotometry (200–700 nm) profiled absorption bands. Measurements were performed in triplicate with solvent controls. Data were presented descriptively and compared across secondary-metabolite classes, with replication ensuring accuracy and precision.

Material and tools

This study explicitly distinguishes Equipment from Materials. Equipment comprised a glass stirring rod; a Philips blender; filter cloth; a Herma® graduated cylinder; an Osuka® analytical balance; a ruler; a spatula; capillary tubes; a UV–Vis spectrophotometer; a vacuum rotary evaporator; and sealable storage containers. Materials consisted of aluminum foil; distilled water; leaves of ketepeng kecil (Cassia alata L.); methanol; ethyl acetate; concentrated HCl; n-hexane; parchment paper; filter paper; quercetin reference standard; and silica gel TLC plates. Equipment was used to grind the dried leaves, measure volumes and masses with precision, develop chromatograms, acquire UV–Vis spectra, evaporate solvents under reduced pressure, and protect samples from light and contamination.

Materials provided the chemical media and substrates: methanol and n-hexane served as extraction and partitioning solvents; ethyl acetate formed the mobile phase; quercetin acted as a flavonoid comparator; concentrated HCl supported colorimetric tests; distilled water rinsed glassware and prepared solutions; aluminum foil and parchment paper limited light and moisture exposure; and TLC plates and filter paper enabled separation and clarification. Stating the categories explicitly ensures reproducibility and minimizes ambiguity regarding roles in the experimental workflow. This emphasis also clarifies procurement lists, instrument calibration needs, and safety considerations for laboratory personnelPengumpulan Sampel *(Cassia Alata L.)*.

Processed of sample (Cassia Alata L.)

Leaves of Cassia alata L. were processed under standardized conditions. Freshly collected material underwent wet sorting to remove soil, insects, and damaged tissues, then was rinsed thoroughly under running water and drained to constant surface dryness. Clean leaves were cut into small pieces to increase surface area, air-dried at ambient temperature in the shade with adequate ventilation until brittle, and subjected to dry sorting to discard remaining impurities. Dried material was milled with a grinder and sieved to obtain a uniform fine powder. The powder was placed in light-protected, airtight containers with desiccant and stored at room temperature pending extraction.

Sample Extraction (Cassia alata L.)

Leaf powder of Cassia alata L. (500 g) was extracted by maceration in methanol (total 5 L) over 72 h, with sequential solvent charges of 2.5 L (day 1), 1.25 L (day 2), and 1.25 L (day 3) and agitation every ~8 h. The mixtures were filtered to separate residue and combined filtrates (1,017 mL). The filtrate was concentrated under reduced pressure using a rotary evaporator to afford a viscous crude extract (69.68 g), corresponding to a yield of ~13.94% w/w relative to the starting dry powder.

Flavonoids screening test

Weigh 0.5 g of extract into a test tube and add 5 mL of distilled water and 5 mL of chloroform. Shake vigorously (~30 s) and allow the mixture to stand until two layers form (aqueous and chloroform). Collect the aqueous layer, add 0.1 mL of HCl, and heat in a water bath for 15 min. Development of a pink to red coloration in the aqueous phase is interpreted as a positive reaction indicative of flavonoids. Run solvent blanks and replicates to confirm reproducibility[11,12].

Alkaloids screening test

Weigh 0.5 g of extract into a test tube, add 1 mL of 2 N HCl, and heat in a water bath for 2 min. Cool and filter/centrifuge to obtain the acidic filtrate. Add 2 drops of Dragendorff reagent to the filtrate. The appearance of an orange to brick-red precipitate is interpreted as a positive reaction indicative of alkaloids. Include solvent blanks and replicate runs to confirm reproducibility [13].

Saponins screening test

Place 0.5 g of extract in a test tube, add 10 mL of hot water, allow to cool to warm, and shake vigorously for ~10 s. Let the tube stand for ~10 min. The formation of a stable froth approximately 1–10 cm in height that persists during the standing period is interpreted as a positive reaction indicative of saponins. Include solvent blanks and replicate runs to confirm reproducibility[14].

Tannin screening test

Place 0.5 g of extract in a test tube, add 10 mL of hot water, and gently boil for 5 minutes. Allow to cool and filter to obtain a clear filtrate. Add 3–4 drops of $FeCl_3$ solution to the filtrate. Development of a greenish-black coloration is interpreted as a positive reaction indicative of tannins. Include a solvent blank and replicate runs to confirm reproducibility [15,16].

TLC (Thin Layer Chromatography) Test

The concentrated Cassia alata L. leaf extract was analyzed using Thin Layer Chromatography (TLC). The stationary phase was silica gel, while the mobile phase consisted of a mixture of n-hexane and ethyl acetate in different ratios of 4:1, 3.5:2.5, and 3:2. Each TLC plate was visualized under UV light at 254 nm. Once the desired spots were obtained, the Rf values were calculated using the formula: Rf = distance traveled by the compound / distance traveled by the solvent front [17,18].

Qualitative Testing Using UV-Vis Spectrophotometry

A 10 mg standard quercetin was dissolved in 10 mL methanol to prepare a 1000 ppm stock solution. From this, a 2 mL aliquot was further diluted to 20 mL for a 100 ppm solution. Serial dilutions were made from 100 ppm to concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm using methanol. For each, 0.5 mL was mixed

with 1.5 mL methanol, 0.1 mL $AlCl_3$ (10%), 0.1 mL sodium acetate (1%), and 2.8 mL distilled water. After 30 minutes, absorbance was measured using UV-Vis spectrophotometry, with a wavelength scan from 400-450 nm to establish the maximum wavelength and generate the calibration curve for quercetin [19,20].

3. Results and Discussion

Phytochemical screening of Cassia alata L. leaf extract using methanol as a solvent revealed the presence of several secondary metabolites. Flavonoids showed a pink to brick-red color upon reaction with magnesium and concentrated HCl. Alkaloids formed a brick-red precipitate after treatment with hydrochloric acid and Dragendorff reagent. Saponins were positive, indicated by foam formation after adding 10 mL of hot water. Tannins were detected by a green black color upon reaction with $FeCl_3$. However, steroids and terpenoids showed negative results as no color change (purple or blue-green) was observed after reaction with anhydrous acetic acid and concentrated sulfuric acid [17,21]. These results are presented in Table 1.

Table 1. Phytochemical Screening of Cassia alata L. Leaf Extract

Fraction	Metabolite Compound	Reagent	Positive Result	Test Result	Remarks
Methanol	Flavonoid	Mg-HCL	Brick Red	+	Color changed to Brick Red
	Alkaloid	Asam Klorida-Dragendrof	Brown Precipitate	+	Brown precipitate present
	Saponin	Hot Water-HCl	Stable Froth	+	Froth present
	Tannin	Hot Water-$FeCl_3$	Green-Black	+	Green-black color present
	Steroid	Lieberman-Burchard	Green	-	Kehitaman No color change
	Terpenoid	Lieberman-Burchard	Red or Purple	_	No color change

Based on the results of the phytochemical screening of Cassia alata L. leaf extract, several secondary metabolites were identified, which led to further confirmation through Thin Layer Chromatography (TLC). TLC is a separation technique where the stationary phase is a solid (silica gel), and the mobile phase is a liquid (eluent). This method is widely used for its simplicity and effectiveness in separating compounds. In this experiment, n-hexane and ethyl acetate (4:1) were used as eluents, with the differing polarities of the solvents allowing for effective separation on the TLC plate. The extract was dissolved in methanol and applied to a 4 cm × 1 cm

TLC plate, marked with upper and lower boundaries. The plate was then placed in a saturated chamber containing the eluent to facilitate the separation process. After the elution process, the plate was removed when the eluent reached the upper boundary, dried, and analyzed under UV light at a wavelength of 366 nm. The results showed the formation of five distinct spots on the TLC plate, with Rf values of 0.125, 0.25, 0.35, 0.45, and 0.75. These values and the corresponding spots can be seen in Table 2 and Figure 1. The TLC analysis provided clear evidence of the presence of different secondary metabolites in the leaf extract, supporting the findings from the initial phytochemical screening and confirming the complexity of compounds present in Cassia alata L. The results can be viewed in Table 2 and Figure 1.

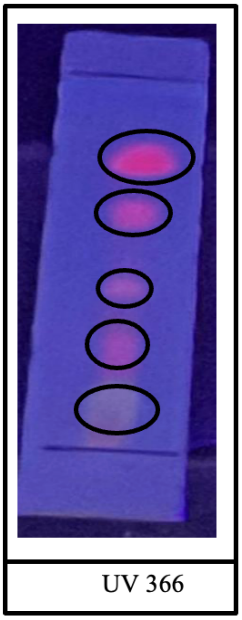

Figure 1. Thin Layer Chromatography (TLC) of Cassia alata L. Leaf Extract

Table 2. Thin Layer Chromatography Results of Cassia alata L. Leaf Extract

Extract	Eluent	Number of Spots	RF Values
Methanol	n-Hexane : Ethyl Acetate	5	Spot 1: 0.125 Spot 2: 0.25 Spot 3: 0.35 Spot 4: 0.45 Spot 5: 0.75

Table 3. Spektrofotometer Uv-Vis Results of Cassia alata L. Leaf Extract

Sample	Test	Wavelength (nm)	Absorbance
Cassia alata L. Leaf	Flavonoid	260,00	0,141
		365,88	0,229
	Alkaloid	213,81	0,015
		217,99	0,024
	Terpenoid	228,88	0,003
		338,36	0,004
	Steroid	345,24	0,004
	Saponin	202,56	3,549
	Tannin	240,88	0,085
		259,35	0,090
		299,01	0,083
		363,63	0,064

The Thin Layer Chromatography (TLC) analysis of Cassia alata L. leaf extract, followed by separation using silica gel, was then analyzed using UV-Vis spectrophotometry. The measurement ran from 200 to 400 nm, revealing a maximum absorbance wavelength at 365 nm, suggesting the presence of flavonoid compounds. Further analysis showed additional maximum wavelengths at 210 nm, 345 nm, 202 nm, and 363 nm, corresponding to secondary metabolites in the leaf extract. These findings suggest the presence of alkaloids, steroids, saponins, and tannins in the extract. The results align with previous research on the characteristic wavelengths of these compounds. The maximum absorbance wavelengths for each compound were observed at 365 nm for flavonoids, 210 nm for alkaloids, 345 nm for steroids, 202 nm for saponins, and 363 nm for tannins. These data indicate the complexity of secondary metabolites in Cassia alata L. and their potential medicinal value. The results can be further reviewed in Table 3, which display the corresponding UV-Vis absorption peaks.

4. Conclusion

Based on the results of the phytochemical screening and Thin Layer Chromatography (TLC) analysis of Cassia alata L. leaf extract, several secondary metabolites were identified. Flavonoids, alkaloids, saponins, and tannins were detected through specific color changes and precipitates, while steroids and terpenoids showed negative results. TLC analysis confirmed the presence of five distinct spots with Rf values ranging from 0.125 to 0.75. Further UV-Vis spectrophotometric analysis revealed maximum absorbance wavelengths for flavonoids, alkaloids, saponins, steroids, and tannins, confirming the complexity and potential medicinal value of the extract.

Aknowlegment

I would like to express my deepest gratitude to my first and second supervisors for their invaluable guidance, support, and expertise throughout this research. Their constructive feedback and encouragement were essential in completing this study. I also wish to thank all the individuals and sectors who contributed to this research, particularly the D3 Pharmacy Study Program at Universitas Negeri Gorontalo. Their continuous support, resources, and academic environment played a crucial role in the success of this project. I am truly grateful for the opportunity to learn and grow within this institution. Thank you to everyone who has been part of this journey.

Reference:

[1] Widodo H, Rohman A, Penelitian B, Tengah J, Bioteknologi PS, Pascasarjana S, et al. Pemanfaatan Tumbuhan Famili Fabaceae untuk Pengobatan Penyakit Liver oleh Pengobat Tradisional Berbagai Etnis di Indonesia 2018:65–88.

[2] Pranaka RN, Yusro F, Budiastutik I. Di Kabupaten Sambas The Utilization of Medicinal Plants by Melayu Ethnic in Sambas Regency 2020;13:1–24.

[3] Selegato DM, Monteiro AF, Vieira NC, Cardoso P, Pavani VD, Bolzani VS, et al. Update : Biological and Chemical Aspects of S enna spectabilis 2017;28:415–26.

[4] Francesca I, Mera G, Estefanía D, Falconí G, Córdova VM. Secondary metabolites in plants : main classes , phytochemical analysis and pharmacological activities . 2019.

[5] Zreen Z, Hameed A, Kiran S, Farooq T, Zaroog MS. Research Article A Comparative Study of Diospyros malabarica (Gaub) Extracts in Various Polarity-Dependent Solvents for Evaluation of Phytoconstituents and Biological Activities 2022;2022.

[6] Akinmoladun AC, Falaiye OE, Ojo OB, Adeoti A. Effect of extraction technique , solvent polarity , and plant matrix on the antioxidant properties of Chrysophyllum albidum G . Don (African Star Apple). Bull Natl Res Cent 2022. https://doi.org/10.1186/s42269-022-00718-y.

[7] Sharma S, Malik J, Chandra D. Preliminary Phytochemical Screening and HPTLC Fingerprinting of Leaf Methanolic Extract of Spermacoce hispida 2022;0990:22–5. https://doi.org/10.36349/easjpp.2022.v04i01.003.

[8] Rashed A, Shaeroun A, Ahmed AB, Alqamoudy H, Mohamed KS. Thin Layer Chromatography (TLC) and Phytochemical Analysis of Moringa Oleifera Methanol , Ethanol , Water and Ethyl Acetate Extracts 2019;4929. https://doi.org/10.36348/SJMPS.2019.v05i10.002.

[9] Extraction T, Analysis C, Cassia L. Ekstraksi dan Analisis Kimia Daun Gulinggang (Cassia alata Linn .) dengan Pelarut Air dan Etanol 2017:1–8.

[10] Extract E, Bar BS. Pemanfaatan Ekstrak Etanol Daun Gulinggang (Cassia alata Linn) sebagai Bahan Antijamur pada Produk Sabun Mandi 2018.

[11] Zabri H, Kodjo C, Benie A, Bekro JM, Alain Y. Phytochemical screening and determination of flavonoids in Secamone afzelii (Asclepiadaceae) extracts 2008;2:80–2.

[12] Ladan Z, Amupitan JO, Oyewale OA, Ayo RG, Temple E, Ladan EO. Phytochemical screening of the leaf extracts of Hyptis spicigera plant 2014;8:83–8. https://doi.org/10.5897/AJPAC2014.

[13] Rahmadi A, Zaidar E, Hardiyanti R, Zulham M. Phytochemical and toxicity test with the BSLT method on Kemuning Raya leaf extract (Muraya paniculata L .) Phytochemical and Toxicity Test Z ith W he BSLT Method on Kemuning Raya Leaf Extract (Muraya paniculata L .) 2021;080006.

[14] Inalegwu B. Antimicrobial and foam forming activities of extracts and purified saponins of leaves of Tephrosia vogelii 2015;5:49–53.

[15] Mailoa MN, Mahendradatta M, Laga A, Djide N. Tannin Extract Of Guava Leaves (Psidium Guajava L) Variation With Concentration Organic Solvents 2013;2:106–10.

[16] Javier R, Hidalgo D, Córdoba R, Grigoryan H, Vieira M, Melo MJ, et al. The making of black inks in an Arabic treatise by al - Qalalūsī dated from the 13th c .: reproduction and characterisation of iron - gall ink recipes. Herit Sci 2023:1–14. https://doi.org/10.1186/s40494-022-00823-1.

[17] Gambo NN, Okooboh MA, Ibejekwe SJ, Ogori BO, Chindo IY, Agada GOA. Phytochemical and Chromatographic Studies of the Polar Extracts of the Leaves of Cassia alata Linn . 2013;3:89–96.

[18] Narayanan S, Jadhav AP. Simultaneous Estimation of Aloe Emodin and Emodin from Rheum emodi , Cassia alata and Aloes by HPTLC 2015;77:783–7.

[19] Mukhopadhyay G, Jain D, Sodani A, Pramanick A, Das AK, Sahoo M. Standardization of quercetin in 2018;2018:575–8.

[20] Pavun L. Spectrofluorimetric Determination Of Quercetin 2014;33:209–15.

[21] Rahman MS, Ali MY, Ali MU. In Vitro Screening Of Two Flavonoid Compounds Isolated From Cassia Alata L . Leaves For Fungicidal Activities 2008:139–42.