STUDIES ON CHEMICAL CONSTITUENTS OF *CASSIAE ALATAE FOLIUM* COLLECTED IN CAN THO CITY, VIET NAM

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Abstract:
Cassia alata is a medicinal herb of the Fabaceae family. It is a general shrub tree growing wild in Viet Nam. Preliminary investigation of chemical composition found that triterpenoid, anthraquinone, coumarin, cardiac glycoside, flavonoid, saponin, and polyuronide in leaves of *Cassia alata*. The leaves were collected and extracted by percolation with ethanol 96%. The total extract was then fractionated by liquid-liquid extraction with solvents of different polarities to obtain fractional extracts: hexane, chloroform, ethyl acetate, and water. The chloroform extract was converted to phenolate to obtain CF2 extract. CF2 was isolated by classical column chromatography and three compounds were obtained: aloe-emodin (20 mg), kaemferol (10 mg), and rhein (56 mg). They were tested and determined that they are pure on the thin layer chromatography. The structure of the compounds was determined by NMR and compared with published documents. These studies will be the basis for conducting bioactivity surveys and isolating compounds in hexane and ethyl acetate fractions from *Cassia alata* leaves collected in Can Tho, Vietnam.

Keywords: *Cassia alata L., Folium Cassiae alatae*, *chemical constituents, anthraquinone, flavonoid.*

INTRODUCTION

Cassia alata also known as the plant, is a species of wild shrub in Vietnam. The plant is native to South America and is now grown throughout the tropics. In Vietnam, the tree is grown in many places, most in the South, Central and some Northern provinces (Thanh Hoa, Nghe An, Ha Tinh) [7]. The studies on chemical composition showed that, in the leaves of the burdock, there are many compounds such as anthraquinones [15], flavonoids [10], alkaloids [21], saponins, tannins [17] and essential oils [1]. According to scientific studies on the biological activity of the plant, several activities have been reported: antibacterial activity [8], antiviral activity [3], anti-diabetes [20], anti-cancer activity. [19], antioxidant [13], antiallergic [18], anti-inflammatory [16], heals burns [11], protects liver [2]. According to Vietnamese folklore, the plant is used to treat constipation, edema, liver pain, jaundice; In addition, it also has a good effect on skin diseases such as ringworm, Tokelo's disease, Herpes ringworm, scabies [6].

MATERIALS AND METHODS

Materials

Fresh *folium Cassiae alatae* was collected in February 2022 in O Mon district, Ho Chi Minh City. Can Tho is washed, dried, grinded, the moisture content is not more than 13% as prescribed [4]. Solvents, chemicals, reagents: *n*-Hexane, chloroform, ethyl acetate, ethanol, acetic acid, acetone, benzene, alcohol 96%, NaOH 10%, HCl 10%, NaOH 1%/ alcohol 96%; $FeCl_3$ 1%/ alcohol 96%, vanillin/ H_2SO_4 1% (Chemsol, Viet Nam). Nuclear magnetic resonance (NMR) spectrometer BRUKER – Ascend – 500 mHz, at the Institute of Chemistry – Viet Nam Academy of Science and Technology, Ha Noi.

Methods

Botany Studies

Plant morphology survey: Observation and description of morphological characteristics of parts of stems, leaves, flowers, fruits and seeds of Cassia alata L. in Can Tho.

Microscopic examination: The microsurgical specimen is a leaf piece cut from the third part of the midrib from where it meets the petiole and part of the leaf blade on both sides. The pieces were stained and observed directly on the microscope, recorded images and described microsurgery characteristics.

Powder screening: Folium Cassiae alatae is dried and finely ground to make powder slides. Observe on a microscope, record images and describe the found components.

Preliminary analysis of phytochemical composition

The compounds present in the folium Cassiae alatae were qualitatively carried out by chemical reactions according to the modified Ciuley procedure [5].

Extraction method

From 2 kg of dried *folium Cassiae alatae*, extracted by the exhaust method with 96% alcohol, the extract was obtained, evaporating under reduced pressure to obtain a high total yield. Add a suitable amount of distilled water to the total volume, conduct a liquid-liquid distribution with solvents of different polarities: n-hexane, chloroform, ethyl acetate and water.

Phenolate conversion in the chloroform fraction: The chloroform fraction was evaporated under reduced pressure, obtained a high concentration, transferred to a large decanter (2000 ml). Shake the distribution with 1000 ml of 10% NaOH, yielding a red to pink-red lye layer and a chloroform layer (CF1). Acidify the lye layer by adding 500 ml of 10% HCl (check the pH of the solution: pH = 2 – 3). Shake the distribution of the acidified solution with chloroform. Collecting the chloroform layer, evaporating under reduced pressure to obtain high CF2.

Compound isolation

Isolation of 10.8 g high CF2 by classical column chromatography with silica gel 60 (0.040 – 0.063 mm) stationary phase. The fractions were monitored by thin layer chromatography and checked by UV irradiation at 254 nm, UV 365 nm, dipping in 1% NaOH/ 96% alcohol solution and VS reagent. Proceed to crystallize and purify to obtain substances with symbols CA1, CA2 and CA3.

Purity test

The substances after isolation were checked for purity on thin layer chromatography with 6 solvent systems including: toluene – EtOAc (6:4); *n*-hexane – EtOAc (1:1); CHCl3 – EtOAc (5:1); benzene – EtOAc – acetic acid (6:4:0,1); benzene - acetone - acetic acid (5:5:0.1); $CHCl_3$ – EtOAc – acetic acid (6:4:0,1). After expansion, detect stains by UV 254 nm, UV 365 nm; Dip a solution of NaOH 1%/alcohol 96%; $FeCl_3$ 1%/alcohol 96% and record chromatograms.

Determine the structure of the isolated compound

The structures of the isolated compounds were determined based on the NMR spectral data and compared with known spectral data.

RESULTS AND DISCUSSION

Botany Studies: *Plant morphology*

Cassia alata L. is a shrub, about 1.5 m tall or more, with a stout trunk and horizontal branches. The compound leaves are feathery once even, consisting of 8 to 12 pairs of leaflets, rectangular or oval, rounded at the ends, gradually enlarged towards the apex. The petioles are short, slightly enlarged at the base, the veins are feathery. Both sides of leaves are smooth, margins are whole. Inflorescences grow between leaves and stem tops into large flowers. Flowers are yellow, consisting of 5 petals, tied at the base to a short and narrow nail. The fruit is a pea, flattened, winged at the edges, with many oval-shaped seeds.

Figure 1. Botanical morphology of *Cassia alata* L.

Botany Studies: *Microsurgery*

Leaf veins: Microsurgery has a flat upper surface, convex lower surface. The epidermis on both sides has short unicellular hairs, the tip is pointed, the lower surface has a denser hair density. Soft tissue cells are irregular in size, thin-walled. Tissue is 2-3 layers thick, arranged in clusters close to the upper epidermis. Bow libe – wood is in the middle, the two ends of the bow roll inward but not adjacent to each other. Libe lay in small continuous clumps. The wood consists of cells with a petrified wall on the upper surface and an arc of triangular bundles on the underside. Outside the libe-wood arch, there is a ring of hard, thick-walled tissue surrounding a heart-shaped closed ring. Solid soft tissue located inside the libe-wooden arch, polygonal, thin-walled. Cubic calcium oxalate crystals are scattered along the hard tissue ring.

Leaf blade: The epidermis is single-layer polygonal, irregular in size, with a thick cuitn layer on the upper epidermis, with cuticle protrusions on the lower epidermis, and stomata on both sides. The leaf blade has two layers of hedge tissue containing chlorophyll, oblong rectangular shape, accounting for 1/2 the thickness of the leaf blade. Soft tissue in leaf blade is the tissue with oval defect, irregular size.

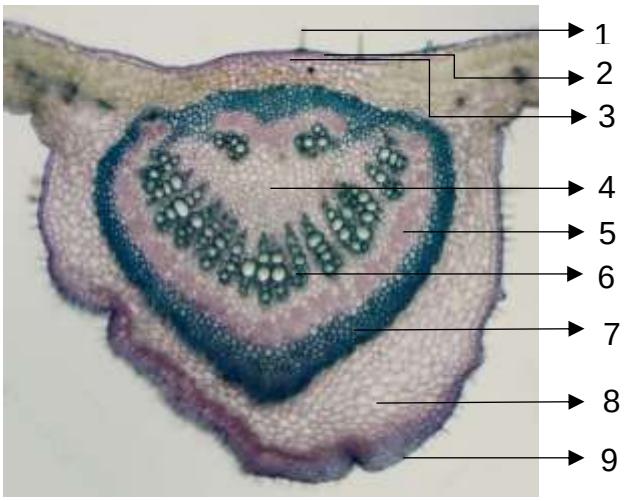


Figure 2. Microsurgery of *Cassia alata* veins.

1. Covering feathers; 2. Upper epidermis; 3. Tissue thickening angle; 4. Solid soft tissue; 5. Libe; 6. Wood; 7. Ring of hard tissue; 8. Soft tissue; 9. Lower epidermis.

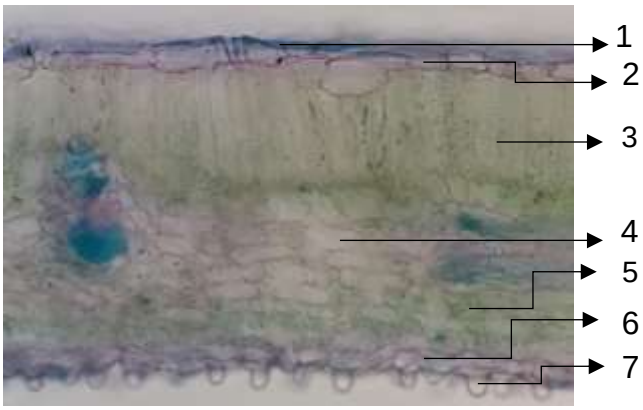


Figure 3. Microsurgery of *Cassia alata* leaf blade.

1. Cuticle layer; 2. Upper epidermis; 3. The hedges carry chlorophyll (above); 4. Defective soft tissue; 5. Hedge tissue (below); 6. Lower epidermis; 7. Convex cutin.

Botany Studies: *Check the dough*

Figure 4. Powder composition *folium Cassiae alatae*

1. Cuticle fragment; 2. Bicellular stomata; 3. Unicellular hair cover; 4. Cutaneous convex tumor; 5. Epidermis bearing unicellular hairs; 6. Piece of soft tissue; 7. Fiber with calcium oxalate crystals; 8. Point circuit; 9. Network circuit; 10. Twisted circuit; 11. Bar circuit; 12. Calcium oxalate crystals; 13. Colored plastic blocks; 14. Hard tissue cells.

Analysis of phytochemical composition

Preliminary qualitative results of the chemical composition showed that the leaves of *Cassia alata* contain compounds: triterpenoids, anthraquinones, coumarins, cardiac glycosides, flavonoids, saponins and polyuronides.

Extraction and isolation

After extraction, the folium *Cassiae alatae* extract was concentrated to recover the solvent under reduced pressure, yielding a high alcohol content (419.5 g). The total extract is added with the appropriate amount of distilled water, and the liquid-liquid distribution is carried out with solvents of different polarities. After evaporating the solvent under reduced pressure, the corresponding high 113.5 g high hexane was obtained; 15.5 g high chloroform; 27.9 g high ethyl acetate and 262.6 g high water. Select the chloroform fraction to convert phenolate, obtain CF1 and CF2 solutions, and recover the solvent.

Isolation of compounds from CF2

Select high CF2 (10.8 g) to conduct compound isolation. The column was developed with chromatographic conditions: wet column filling, dry sample loading and development solvent systems including: CHCl3 100%, $CHCl_3$ – EtOAc (5:1), $CHCl_3$ – EtOAc (1:1). The resulting fractions were carried out by thin layer chromatography with the solvent system Toluene – Ethylacetate (6:4).

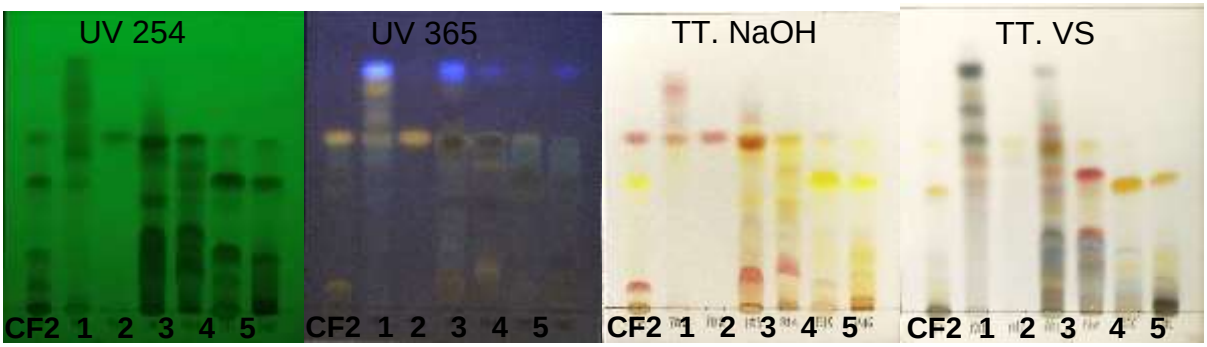


Figure 5. Chromatogram of high fractions chloroform (CF2).

Among the obtained fractions: Segment (2) has only 1 spot, select this fraction to crystallize, obtain a red-orange needle-shaped crystal with a mass of 20 mg, symbol CA1. Segment (3), has quite a few traces, but when the solvent is slowly evaporated, a precipitate is obtained in the form of a reddish-brown amorphous powder, with a mass of 56 mg, symbol CA3. Segment (5), which has a small number of traces and has a clear separation from the rest, selects this fraction for purification by column chromatography.

Compound isolation from fractions CF2 – 5

The CF2 - 5 fraction (5 ml) was purified by column chromatography with chromatographic conditions: wet column packing, wet sample loading and expansion solvent systems $CHCl_3$ 100%, $CHCl_3$ - EtOAc (5:1), $CHCl_3$ – EtOAc (1:1). The obtained fractions were carried out by thin layer chromatography with the solvent system $CHCl_3$ – EtOAc (5:1).

Fraction (2) of CF2-5 has a small number of traces, and it is purified to obtain a pure compound. The fractional purification stage was performed by column chromatography with chromatographic conditions: wet column packing, dry sample loading (3.7 g high) and expansion solvent systems $CHCl_3$ 100%, $CHCl_3$ – EtOAc (5:1). The fractions obtained, for solvent evaporation, slowly yielded a precipitate of a yellow-brown amorphous powder with a mass of 10 mg, symbol CA2.

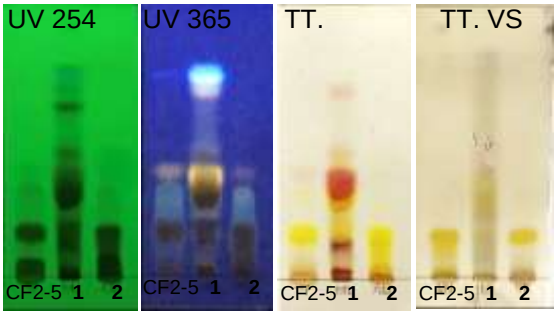


Figure 6. Fractional chromatogram CF2 – 5

Purity test

Compounds CA1 and CA2 were tested for purity with three different solvent systems. The results show that CA1 and CA2 show a spot on the thin plate, preliminary identification of pure CA1 and CA2 on thin layer chromatography.

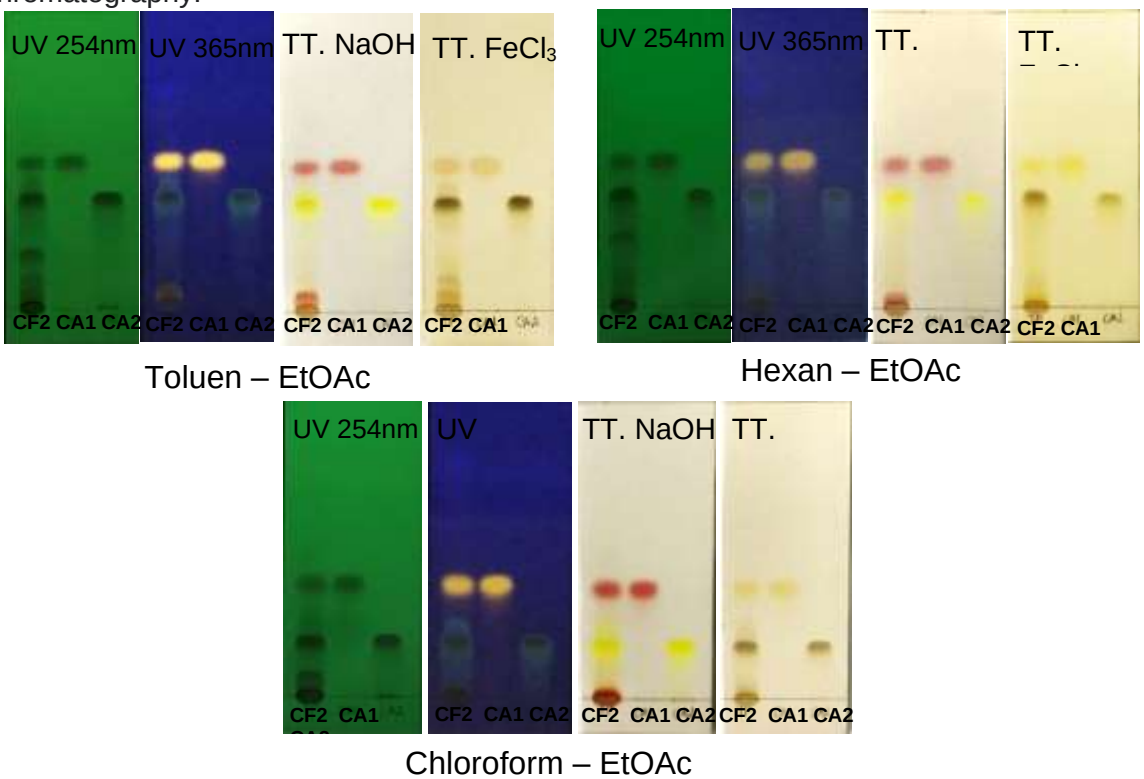
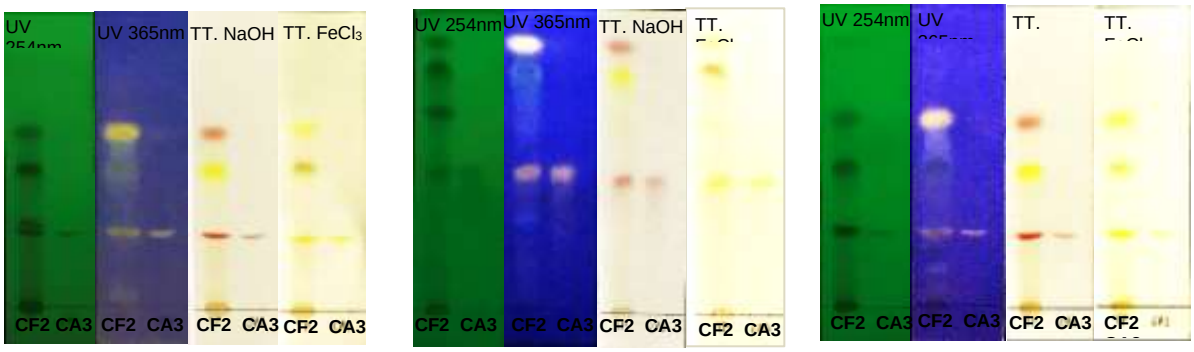


Figure 4. Chromatogram of compounds CA1 and CA2.

Compound CA3 was tested for purity with three different solvent systems. The results show that CA3 shows a stain on the plate, preliminary identification of pure CA3 on thin layer chromatography.



C_6H_6 – EtOAc – acid acetic (6:4:0,1) | C_6H_6 – aceton – acid acetic (5:5:0,1) | $CHCl_3$ – EtOAc – acid acetic (6:4:0,1)

Figure 5. Chromatogram of compound CA3.

Determine the structure of the isolated compound

Determine the structure of compound CA1

CA1 is a red-orange needle-shaped substance. Well soluble in CHCl3, MeOH, insoluble in less polar solvents. On the F254 silica gel plate, the spot is photosensitive at UV 254 nm, the spot is yellow at UV 365 nm, red with NaOH reagent 1%/alcohol 96%, yellow with FeCl3 reagent 1%/alcohol. 96%. 13C - NMR spectral data (DMSO-d6, 150 MHz) and DEPT show that compound CA1 has 15 carbon signals, of which there are 9 primary carbons, 5 tertiary carbons, 1 secondary carbon and no primary carbon.

Table 2. NMR data of CA1 compared with Aloe emodin.

C/H	CH_n	Compounds CA1 (150/600 MHz, DMSO-*d6*)		Aloe emodin [12] (125/500 MHz, DMSO-*d6*)	
		δC ppm	δH ppm	δC ppm	δH ppm (J, Hz)
1	C	161,6		161,6	
2	CH	120,6	7,28 *m*	120,7	7,31 *brs*
3	C	153,7		157,3	
4	CH	117,1	7,67 *t*	117,1	7,71 *brs*
5	CH	119,3	7,70 *dd*	119,3	7,74 *d* (7,5)
6	CH	137,3	7,79 *dd*	137,3	7,82 *t* (8,0)
7	CH	124,3	7,37 *d*	124,4	7,40 *d* (8,0)
8	C	161,3		161,3	
9	C	191,6		191,6	
10	C	181,4		181,5	
11	C	133,3		133,4	
12	C	115,8		115,9	
13	C	114,4		114,5	
14	C	133,0		133,1	
15	CH2	62,0	4,62 *d*	62,0	
	1 – OH		11,92		11,96 *br*
	8 – OH		11,92		11,96 *br*
	15 – OH		5,57 *t*		5,62 *t* (6,0)

Determine the structure of compound CA2

CA2 is a yellow-brown amorphous powder. Soluble in $CHCl_3$, EtOAc, MeOH, insoluble in hexane. On silica gel plate F_{254}, for UV 254 nm, for UV 365 nm, for yellow color with 1% NaOH reagent / 96% alcohol, moss green with 1% $FeCl_3$ reagent / 96% alcohol. ^{13}C - NMR spectral data (DMSO-d6, 150 MHz) and

DEPT show that CA2 compound has 15 carbon signals, of which there are 9 quaternary carbons, 6 tertiary carbons, no secondary carbon and no primary carbon.

Table 3. NMR data of CA2 compared with Kaempferol.

C/H	CH_n	Compounds CA2 (150/600 MHz, DMSO-*d6*)		Kaempferol [12] (125/500 MHz, DMSO-*d6*)	
		δC ppm	δH ppm	δC ppm	δH ppm (J, Hz)
1	O				
2	C	146,8		146,8	
3	C	135,6		135,7	
4	C	175,9		175,9	
5	C	160,7		160,7	
6	CH	98,2	6,19 *d*	98,2	6,20 *d* (2,0)
7	C	163,8		163,9	
8	CH	93,4	6,43 *d*	93,5	6,44 *d* (2,0)
9	C	156,1		156,2	
10	C	103,0		103,1	
1′	C	121,6		121,7	
2′	CH	129,5	8,04 *d*	129,5	8,05 *d* (9,0)
3′	CH	115,4	6,92 *d*	135,7	6,93 *d* (9,0)
4′	C	159,1		159,2	
5′	CH	115,4	6,92 *d*	135,7	6,93 *d* (9,0)
6′	CH	129,5	8,04 *d*	129,5	8,05 *d* (9,0)
	3-OH		9,35 *s*		9,39 *s*
	5-OH		12,47 *s*		12,48 *s*
	7-OH		10,75 *s*		10,79 *s*
	4′-OH		10,08 *s*		10,11 *s*

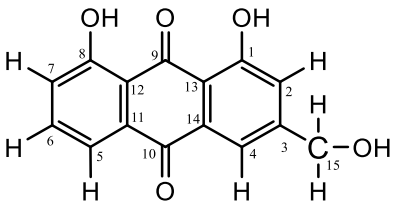
Determine the structure of compound CA3

CA3 is a red-brown amorphous powder. Soluble in $CHCl_3$, MeOH, insoluble in hexane. On the F254 silica gel plate, the spot is photosensitive at UV 254 nm, the spot is yellow at UV 365 nm, red with NaOH reagent 1%/alcohol 96%, yellow with $FeCl_3$ reagent 1%/alcohol 96%. ^{13}C – NMR spectral data (DMSO-d6, 150 MHz) and DEPT show that compound CA3 has 15 carbon signals, of which there are 10 quaternary carbons, 5 tertiary carbons, no secondary carbon and no primary carbon.

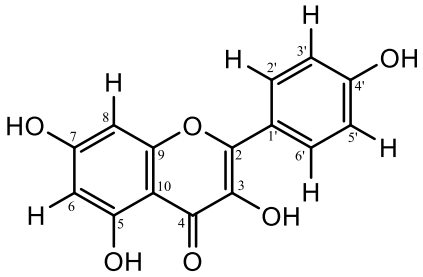
Table 4. CA3 NMR data compared with Rhein.

C/H	CH_n	Compounds CA3 (150/600 MHz, DMSO-*d6*)		Rhein [22] (300/300 MHz, DMSO-*d6*)	
		δC ppm	δH ppm	δC ppm	δH ppm (J, Hz)
1	C	161,38	11,87 *s*	162,39	
2	CH	119,37	7,75 *d*	120,42	7,79 *s*
3	C	137,97		138,95	
4	CH	124,54	8,11 *d*	125,59	8,16 *s*
5	CH	124,07	7,73 *dd*	125,10	7,76 *d* (7,5)

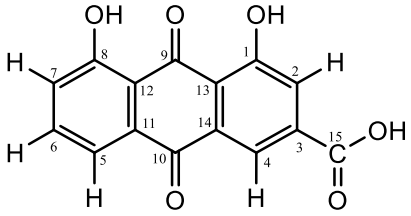
6	CH	137,55	7,82 *dd*	138,59	7,87 *t* (7,5)
7	CH	118,72	7,40 *dd*	119,74	7,44 *d* (7,5)
8	C	161,03	11,87 *s*	162,05	
9	C	191,27		192,19	
10	C	180,90		181,97	
11	C	133,76		134,82	
12	C	118,67		119,74	
13	C	116,12		117,16	
14	C	133,16		134,19	
15	COOH	165,34		166,37	



Aloe emodin



Kaempferol



Rhein

CONCLUSION

After conducting botanical surveys such as morphological survey, microsurgery and powder analysis, compared with reference materials [4], the medicinal herb was identified as *Cassia alata*. Samples of folium *Cassiae alatae* collected in Can Tho were conducted preliminary survey of chemical composition, determined that the leaves contain triterpenoid compounds, anthraquinones, flavonoids, coumarins, cardiac glycosides, saponins and polyuronides. For the first time, the study has extracted and isolated 3 compounds from the chloroform fraction, including aloe emodin (20 mg), kaempferol (10 mg) and rhein (56 mg) present in the folium *Cassiae alatae* collected in Can Tho, Viet Nam. The structures of the compounds were determined based on the analysis of NMR spectral data and comparison with reference materials.The above studies will serve as the basis for further studies on biological activities and isolation of compounds in hexane and ethyl acetate fractions from folium *Cassiae alatae* collected in Can Tho, Viet Nam.

Acknowledgment

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References

1. Agnaniet H., Bikanga R., Bessière J.M., Menut C., 2005. Aromatic Plants of Tropical Central Africa (Part XLVI): Essential Oil Constituents of *Cassia alata* (L.) from Gabon. Journal of essential oil research. Vol. 17/ No. 4. p. 410 – 412. 4.
2. Anandan R., Jayakar B., Manavalan R., 2009. Hepatoprotective activity of the alcoholic extract of the dried leaves of *Cassia alata* Linn. Biomedical and Pharmacology Journal. Vol. 2/ No. 6. p. 1107 – 1109.
3. Angelina M., Hanafi M., Syatna F.D., Wirawati T., Ratnasari S.S., Dewi B.E., 2017. Antiviral effect of sub fraction *Cassia alata* leaves extract to dengue virus serotype – 2 strain new Guinea c in human cell line huh – 7 it – 1. IOP Conference Series: Earth and Environmental Science. Vol. 101. p. 1 – 10.

4. Ministry of Health, 2018. Vietnam Pharmacopoeia V, volume 2. Medicine Publishing House, Hanoi. p. 1256 – 1257.
5. Department of Medicinal Materials, 2014. Textbook of Research Methods of medicinal herbs. Ho Chi Minh City University of Medicine and Pharmacy. p. 27–34, 71–83.
6. Do Huy Bich et al, 2006. Medicinal plants and medicinal animals in Vietnam, volume II. Science and Technology Publishing House, Hanoi. p. 319 – 322.
7. Đo Tat Loi, 2018. Vietnamese medicinal plants and herbs. Medical Publishing House, Hanoi. p. 460 – 461.
8. Khan M.R., Kihara M., Omoloso A.D., 2001. Antimicrobial activity of *Cassia alata*. Fitoterapia. Vol. 72. p. 561 – 564.
9. Lewis A. and Levy A., 2011. Anti-inflammatory activities of *Cassia alata* leaf extract in complete freund's adjuvant arthritis in rats. West Indian Medical Journal. Vol. 60. p. 615 – 621.
10. Liu A., Xu L.Z., Zou Z.M., Yang S.L., 2009. Studies on chemical constituents from leaves of *Cassia alata*. China Journal of Chinese Materia Medica. Vol. 34/ No. 7. p. 861 – 863.
11. Nasution S.L.R., Putri M., Hulu W., Girsang E., Nasution A.N., 2019. Optimization of *Senna alata* leaves extract for healing burns. Trends in Pharmaceutical Sciences. Vol. 9/ No. 5. p. 127 – 136. 23.
12. Ngo Thi Thuy Duong et al., 2013. Contributing to learning the chemical composition of leaves *Cassia alata* L., (Caesalpiniaceae). Science and Technology Development Magazine. No. 16. p. 26 – 31.
13. Okpuzor J., Ogbunugafor H., Kareem G.K., Igwo – Ezikpe M.N., 2009. In vitro investigation of antioxidant phenolic compounds in extracts of *Senna alata*. Research Journal of Phytochemistry. Vol. 3/ No. 4. p. 68 – 76.
14. Olarte E.I., Herrera A.A., Villasenor I.M., Jacinto S.D., 2013. In vitro antitumor properties of an isolate from leaves of *Cassia alata* L. Asian Pacific Journal of Cancer Prevention. Vol. 14/ No. 5. p. 3191 – 3196.
15. Panichayupakaranant P., Sakunpak A., Sakunphueak A., 2009. Quantitative HPLC determination and extraction of anthraquinones in *Senna alata* leaves. Journal of Chromatographic Science. Vol. 47/ No. 3. p. 197 – 200.
16. Patrick-Iwuanyanwu K.C., Onyeike E.N., Dar A., 2011. Anti-inflammatory effect of crude methanolic extract and fractions of ring worm plant *Senna alata* (L. Roxb) leaves from Nigeria. Der Pharmacia Sinica. Vol. 2/ No. 4. p. 9 – 16.
17. Raji P., Samrot A.V., Rohan D.B., Kumar M.D., Geetika R., Sharma V.K., Keerthana D., 2019. Extraction, characterization and invitro bioactivity evaluation of alkaloids, flavonoids, saponins and tannins of *Cassia alata*, *Thespesia populnea*, *Euphorbia hirta* and *Wrightia tinctoria*. Rasayan Journal of Chemistry. Vol. 12/ No.1. p. 123 – 137.
18. .Singh B., Nadkarni J.R., Vishwakarma R.A., Bharate S.B., Nivsarkar M., Anandjiwala S., 2012. The hydroalcoholic extract of *Cassia alata* (Linn.) leaves and its major compound rhein exhibits antiallergic activity via mast cell stabilization and lipoxygenase inhibition. Journal of Ethnopharmacology. Vol.141/ No.1. p.469 – 473.
19. Sugumar M., Doss V.A., Maddisetty P., Rangaraju M., Kandhasamy S., Munuswamy S., 2019. Pharmacological analysis of hydroethanolic extract of *Senna alata* (L.) for in vitro free radical scavenging and cytotoxic activities against HepG2 cancer cell line. Pakistan Journal of Pharmaceutical Sciences. Vol. 32. No. 3. p. 933 – 936.

20. Varghese G.K., Bose L.V., Habtemariam S., 2012. Antidiabetic components of *Cassia alata* leaves: Identification through α – glucosidase inhibition studies. Pharmaceutical Biology. Vol. 51/ No.3. p. 345 – 349.

21. Villasenor I.M., Sanchez A.C., 2009. Cassiaindoline, a new analgesic and anti inflammatory alkaloid from *Cassia alata*. Zeitschrift für Naturforschung C. Vol. 64/ No. 5 – 6. p. 335.

22. Yingngam B., Zhao H., Baolin B., Pongprom N., Brantner A., 2019. Optimization of ultrasonic – assisted extraction and purification of rhein from *Cassia fistula* pod pulp. Molecules. Vol. 24/ No. 10.