MATTER: International Journal of Science and Technology
ISSN 2454-5880

Tanty & Herlina

Volume 3 Issue 3, pp. 339 - 348

Date of Publication: 10th March, 2018

DOI-https://dx.doi.org/10.20319/mijst.2018.33.339348

This paper can be cited as: Tanty, H., & Herlina, T. (2018). Antidiabetic Activity Test for Leaves Extract of Cassia Siamea. Lamk. MATTER: International Journal of Science and Technology, 3(3), 339 - 348.

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ANTIDIABETIC ACTIVITY TEST FOR LEAVES EXTRACT OF CASSIA SIAMEA. LAMK

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Abstract

This study was investigated antidiabetic activity of n-hexane and ethylacetate fractions of Cassia siamea. Lamk by α-glucosidase inhibition method using α-glucosidase enzyme and p-nitrophenyl α-D-glycopyranoside (pNPG) as substrate. The result of alpha-glucosidase inhibition test showed that n-hexane and ethyl acetate fractions and ethanol extract were inhibited the alpha glucosidase enzyme with activity 52.319%, 42.85% and 19.100% respectively at concentration of 1000 ppm.

Keywords

Cassia Siamea, Lamk, Antidiabetic Activity, Alpha-Glucosidase Inhibitor, Invitro Test

1. Introduction

Cassia siamea. Lamk is a plant from Fabaceae family. It has been studied to have bioactivity as antimicrobe, antimalaria, antidiabetic, anticancer, antioxidant, antihypertension,

anti-inflammation, analgesic, antipyretic, antidepressant, and sedative (Mamadou, K. et all. .2014)

Qualitative analysis of *C. siamea* was done for various compounds including alkaloids, tannins, saponins, chromone, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins (Heruna,T. 2011., Lee, D.S.2001., Samia.1978., Usha, V.2011). *C. siamea* leaves contain antrona, flavona, triterpenoida, alkaloids and casiadinine (Heyne, K. 1987). Samia *et.al. (*1984) successfully isolated isoquinolones (Siaminine A, Siaminine B and Siaminine C) from *C. siamea* leaves extract. Shiori *et.al.* isolated new bischromone and chrobisiamone A from *C.siamea* leaves extract that showed antiplasmodial bioactivity, while Heruna isolated Chromone in methanol extracts of ethyl acetate fraction (Heruna.T.2011)

Methanol extracts of *C.siamea* leaves showed significant zone of inhibition against tested bacterial strains (Anuthida, et al .2014., Lakshmi, et al. 2013) . The ethyl acetate fraction of *C.siamea* leaves showed antimalaria activity against *P. falciparum* (Ekasari. 2014). while water extract showed activity to inhibit the growth of *P.berghei* on mice with ED_{50} value 83.77412 mg / kg BW *in vivo* (Ardhistia, R. 2014) Ethanolic, ethyl acetate and hexane extracts of *C.Siamea* leaves also have antidiabetic activity (Mamadou, K. et all. 2014).

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Julia.2011).

Various kinds of anti-diabetic therapies such as biguanides, glinides, sulphonylureas, and a-glucosidase inhibitor are widely used. α-Glucosidase inhibitors are drugs that delay digestion of complex carbohydrates by acting as competitive inhibitors of the intestinal α-glucosidase enzymes that hydrolyze oligosaccharides into monosaccharides In diabetic patients, alpha glucosidase causes inhibition of glucose absorption, decreases hyperglycemia and improve insulin sensitivity (Lebovitz, H.E.1998). The objective of the present study is to investigate the *in vitro* antidiabetic activity of *C. siamea* leaves extract.

2. Methodology

2.1 Sample Preparation

Samples were collected from Setu village, Cirebon district, West Java, Indonesia. Samples were washed several times with distilled water to remove the traces of impurities then were dried at room temperature for three weeks followed by grinding to get powdered materials.

2.2 Fractination and Extraction

Samples (454.99 g) were extracted with 4 L of ethanol 90% for 24 hours with 3 times replications (3x4Lx24 hours). The filtrates were filtered through filter paper and evaporated at 40^{o} C using Rotary Evaporator to get crude ethanol extract (15.1 g) .The extract were fractionated with mixture of water and *n*-hexane (1:9) to obtain *n*-hexane fraction and water fraction. Water fraction was fractionated further by ethyl acetate to obtain ethyl acetate fraction and water fraction. Each fraction is concentrated to provide n-hexane fraction (5.1 g) and ethyl acetate fraction (2.4 g). Extraction and fractionation procedures of sample can be seen in figure1.

2.3 Alpha-Glucosidase Inhibition Test

Enzyme solution for α-Glukosidase Inhibition Test was made by dissolving 1.0 mg of α-glucosidase enzyme in 100 mL phosphat buffer (pH 7.0) that contains 200 mg bovin albumin serum.[12,17]. Before use, 1 mL of α- glucosidase enzyme was diluted 25 times with phosphat buffer 0.1 M (pH 7.0) that contains 200 mg bovin albumin serum. The mixture contains 250 µL 0.5 mM p-nitrofenil α-D glukopiranoside as a substrate, 475 µl 0.1 M phosphat buffer (pH 7,0) and 25 µL solution sample in DMSO. After the mixture incubated at 37 ^{o}C for 5 minutes, 250 µL enzyme solution 0.04 U/mL is added and it was incubated for 25 minutes more. Enzymatic reaction was stopped by adding 1000 µL 0.2 M sodium carbonate, and the intensity of the *p*-nitrophenol color was measured at 400 nm. The complete enzyme reaction system can be seen in Table 1.

Table 1: *Enzyme reaction System for One Sample with 2 mL Total Volume*

	Control (µL)	**Blank (µL)**	**C_1 (µL)**	**C_0 (µL)**
Sample *)	-	-	25	25
DMSO 1%	25	25	-	-
Buffer fosfat 0,1 M	475	475	475	475
Subtrat pNPG 0,5 mM	250	250	250	250
Incubation in waterbath at 37^{o}C , 5 minutes				
Enzim α-glucosidase 0,04 unit/mL	250	-	250	-

Buffer fosfat 0,01 M	-	250	-	250
Incubation in waterbath at 37°C , 25 minutes				
Na_2CO_3 0,2 M	1000	1000	1000	1000

Samples are ethanol extract, n-hexane fraction extract and ethyl acetate fraction extract. Fraction extract of C.siamea leaves with 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm consentration variation and DMSO as a solvent. For each extract we performed alpha-glucosidase inhibition test with 2 times repetition. The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by equation:

$$\% \text{ Inhibition} = [(A - B) / A] \times 100$$

In which B = sample absorbance (C_1-C_0), C_1 = sample absorbance with enzyme addition, C_0 = sample absorbance without enzyme addition, A = absorbance control (DMSO) without samples (control-blank) (M. B. Narkhede, et al. 2011)

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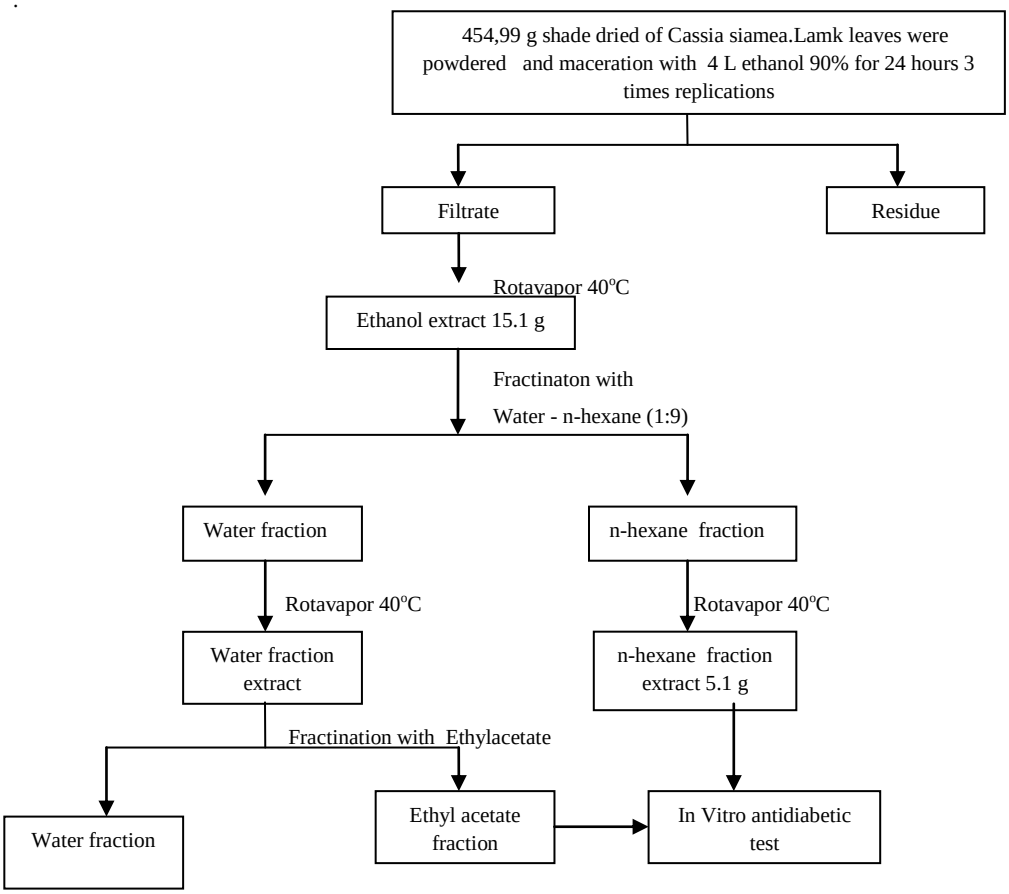

Figure 1: *Extraction and Fractination Chart of C. siamea leaves*

3. Results and Discussion

Antidiabetic activity test results of ethanol extract can be seen in Table 2 and the chart of ethanol extract consentration with inhibition percentage can be seen in Figure 2

Table 2: *Antidiabetic activity test results of ethanolic extract*

Concentration		Experiment		average		A	B	% inhibition
		1	2					
	control	0.280	0.310	0.295		0.270		
	blank	0.025	0.026	0.026				
50 ppm	C_1	0.295	0.310	0.303			0.262	2.968
	C_0	0.046	0.036	0.041				
100 ppm	C_1	0.298	0.294	0.296			0.253	6.308
	C_0	0.039	0.048	0.044				
250 ppm	C_1	0.300	0.291	0.296			0.253	6.308
	C_0	0.039	0.047	0.043				
500 ppm	C_1	0.313	0.308	0.311			0.249	7.607
	C_0	0.061	0.062	0.062				
1000 ppm	C_1	0.298	0.309	0.304			0.218	19.109
	C_0	0.082	0.089	0.086				

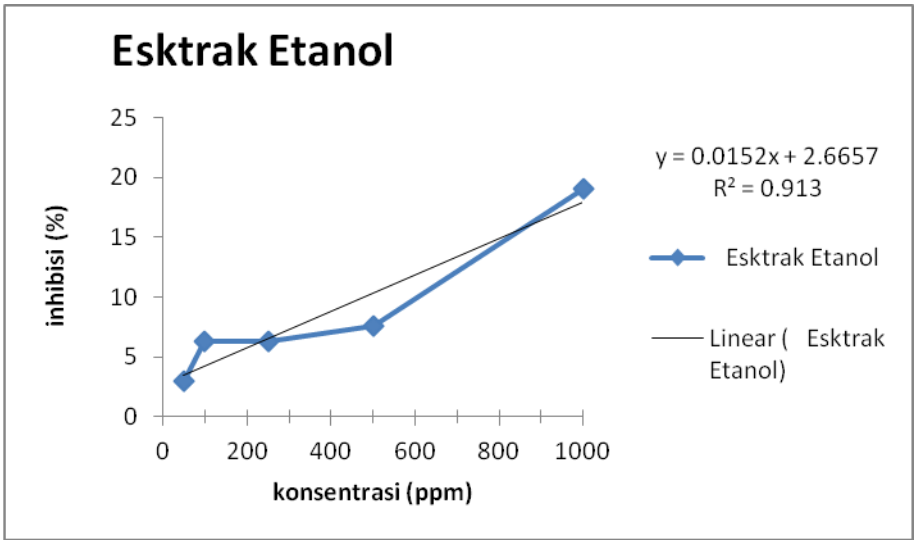

Figure 2: *Inhibition Percentage of ethanol extract at various concentration*

Table 2 and figure 2 shows the results of in vitro antidiabetic activity of ethanolic extract of *C.siamea* leaves by the use of *α*-glucosidase enzyme. At a concentration of 1000 ppm of the ethanolic extract have the inhibitory activity of 19.109% and IC_{50} is 3114.099.

Table 3: *Antidiabetic activity test results of ethyl acetate fraction*

Concentration		Experiment		average		A	B	% inhibition
		1	2					
	control	0.280	0.310	0.295		0.270		
	blank	0.025	0.026	0.026				
50 ppm	C_1	0.278	0.282	0.280			0.248	7.978
	C_0	0.028	0.036	0.032				
100 ppm	C_1	0.277	0.276	0.277			0.247	8.534
	C_0	0.027	0.033	0.030				
250 ppm	C_1	0.276	0.278	0.277			0.245	9.091
	C_0	0.022	0.042	0.032				
500 ppm	C_1	0.281	0.283	0.282			0.225	16.698
	C_0	0.057	0.058	0.058				
	C_0	0.062	0.063	0.063				
1000 ppm	C_1	0.297	0.136	0.217			0.154	42.857
	C_0	0.062	0.063	0.063				

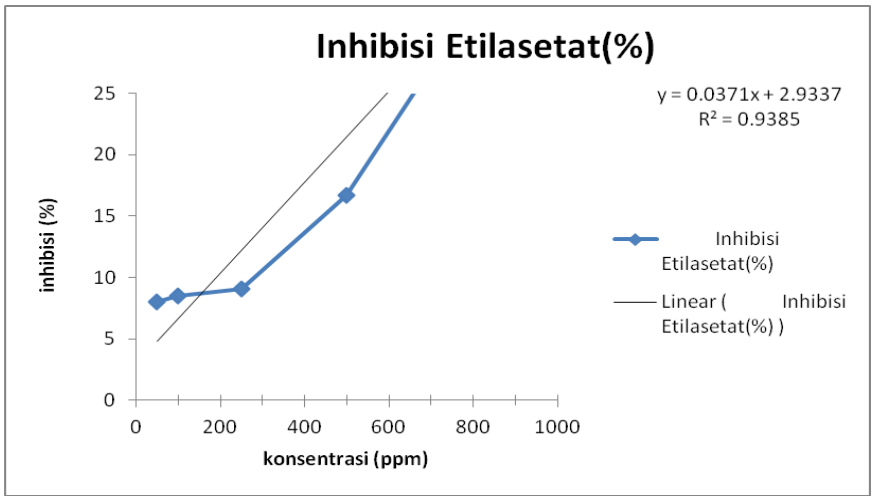

Figure 3: *Inhibition Percentage of ethyl acetate fraction at various concentrations*

Table 3 and figure 3 shows the results of in vitro antidiabetic activity of fraction ethylacetate extract of *C.siamea* leaves by the use of *α*-glucosidase enzyme. At a concentration of 1000 ppm of the ethanolic extract have the inhibitory activity of 42.857% and IC_{50} is 1268.633.

Table 4: *Antidiabetic activity test results of n-hexane fraction*

Concentration		Experiment		average	A	B	% inhibition
		1	2				
	control	0.280	0.310	0.295	0.270		
	blank	0.025	0.026	0.026			
50 ppm	C_1	0.191	0.221	0.206		0.154	42.857
	C_0	0.055	0.049	0.052			
100 ppm	C_1	0.208	0.219	0.214		0.139	48.609
	C_0	0.073	0.077	0.075			
250 ppm	C_1	0.179	0.198	0.189		0.138	48.794
	C_0	0.046	0.055	0.051			
500 ppm	C_1	0.192	0.185	0.189		0.134	50.464
	C_0	0.054	0.056	0.055			
1000 ppm	C_1	0.250	0.240	0.245		0.129	52.319
	C_0	0.118	0.115	0.117			

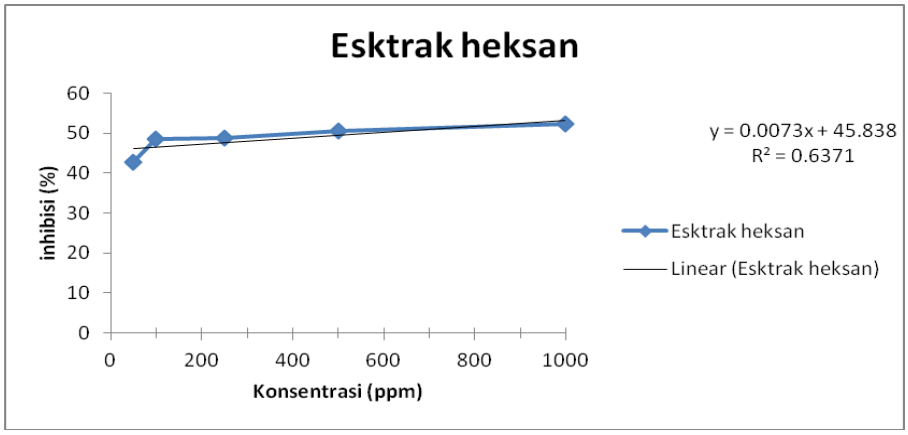

Figure 4: *Inhibition Percentage of n-hexane fraction at various concentrations*

Table 4 and Figure 4 showed that at concentration of 1000 ppm *n*-hexane fraction had inhibitory activity of 52.319% and IC_{50} 570.137.

n-Hexane fraction showed maximum inhibition percentage (52.319%) compared to ethyl acetate fraction (42.857%) and ethanol extract (19.109%) at concentration 1000 ppm. Further studies are needed to find and isolation which compound is actively involved in the antidiabetic activity of the *C.siamea* leaves.

The antidiabetic activity of plants studies have been done by several researchers. Era Rahmi *et.al.* revealead that ethanol extracts of *P. macrocarpa* fruits and *A. muricata* leaves showed activity at 261.34 and 428.79 µg/mL respectively in α- glucosidase inhibitory activity test (Era, R. et al. 2016). Ethanol extract of *Psidium guajava* leaves inhibited the alpha amylase and alpha glucosidase enzymes as 97.5% and 91.8% respectively (Lee, D.S .2001). Ethanol fraction of *Annona muricat*a showed antidiabetic activity at 73,426 µg/ml and 64,425 µg/ml for α-amylase and α-glucosidase respectively (Hassan, B.A.R. 2013). Ethanol extract of *Premna seratifolia*.Linn leaves revealed that the concentration of 2% of sample inhibited α-glucosidase enzyme with a percentage of 91.03% (Dini, H. 2017).

3. Conclusion

The ethanol extracts of *C.siamea* leaves have an antidiabetic activity in (in vitro) model useing α-glucosidase enzyme. *n*-hexane fraction showed highest antidiabetic activity at 52.319% and IC_{50} value 570.137. Further in vivo studies and compound isolation studies are needed.

References

Anuthida, et al (2014). Antioxidant and antibacterial activities of different solvent extractions from Cassia siamea Lamk leaves. Journal of Chemical and Pharmaceutical Research, 6(4), 655-662. Research Article ISSN : 0975-7384 CODEN(USA) : JCPRC5 655.

Ardhistia, R., Wiwied, E., & Achmad, F. H. (2014). Uji Aktivitas Antimalaria Ekstrak Air Daun Johar (Cassia siamea Lamk) Terhadap Plasmodium Berghei Secara In Vivo. Jurnal Farmasi dan Ilmu Kefarmasian Indonesia, Vol. 1, No. 1.

Dini, H. (2017). In Vitro α-Glucosidase Inhibitory Activity of Ethanol Extract os Buas-Buas (Premna serratifolia. Linn). Trad.Med vol 22(2), 80-83. ISSN-p: 1410-5918, ISSN e: 2406-9086. https://doi.org/10.22146/tradmedj.27917

Ekasari. (2014). Aktivitas Antimalaria Fraksi Etil Asetat Daun Johar (Cassia siamea Lamk) Terhadap Pertumbuhan Plasmodium Falciparum Strain G2300 (Resisten Klorokuin) In Vitro. E-Journal Planta Husada Vol. 2 No. 1.

Era, R. et al. (2016). Combination of Ethanolic Extract of A-Glucosidase Inhibitory Activity Of Phaleria Macrocarpa (Scheff.) Boerl. Fruits and Annona Muricata Linn. Leaves Aktivitas Penghambatan A-Glucosidase Oleh Kombinasi Ekstrak Etanolik Buah

Phaleria Macrocarpa (Scheff.) Boerl. Dan Daun Annona muricata Linn. Trad. Med. J., May - August Vol. 21(2), 63 .ISSN : 1410-5-68 918.

Hassan, B.A.R. (2013). Overview on Diabetes Mellitus (Type 2). J Chromat Separation Technique 4:114. https://doi.org/10.4172/2157-7064.1000114

Heruna,T. (2011). *Isolasi Chromone dalam Daun Juar (Cassia siamea. Lamk).* Prosiding Seminar Nasional Kimia UNJANI, ISSN : 977-208-9220-68-6.

Heyne, K. (1987). *Tumbuhan Berguna Indonesia, Jil. 2.* Yayasan Sarana Wana Jaya, Jakarta : 926-927.

Julia, & Dulce. (2011). *Wordpress.* Retrieved from https://dulcejulia91.files.wordpress.com/2011/07/kea_cassia_siamea_johar.pdf.

Kate, Kgasi. (2017). *CMS script Diabetes Mellitus Type 1. Issue 1.* Retrieved from http://www.medicalschemes.com/files/CMScript/CMScript1Of2017.pdf.

Lakshmi, et al. (2013). International Journal of Pharmacy and Pharmaceutical Sciences ISSN-0975-1491 Vol 5, Suppl 3, 2013.

Lee, D.S., Lee, S.H & Genistein. (2001). *A Story isoflavone is a potent α-Glucosidase inhibitor.* FEBS Lett : 501:84-86. https://doi.org/10.1016/S0014-5793(01)02631-X

Lebovitz, H.E. (1998). Research Gate. Retrived from https://www.researchgate.net/publication/279907756_Alpha-Glucosidase_inhibitors_as_agents_in_the_treatment_of_diabetes

Mamadou, K. et all. (2014). Ethnobotany, phytochemistry pharmacology and toxicology profiles of Cassia siamea Lamk. The Journal of Phytopharmacology 3 (1): 57-76.

M. B. Narkhede, et al. (2011). In vitro antidiabetic activity of Caesalpina digyna (R.) methanol root extract. ISSN : 2249 – 7412.

Mugiyanto, E., Simanjuntak, P., & Setyahadi. (2016). Aktivitas Inhibisi Enzim Alfa Amylase Dan Alfa Glukosidase Ekstrak Daun Sirsak (Annona Muricata Linn.) Secara Invitro. Proceeding The 4th University Research Colloquium (URECOL). Retreived from http://hdl.handle.net/11617/7879.

Prasanth, D. et al. (2001). α-Glucosidase inhibitory activity of Mangifera Indica bark. Fitoterapia; 72: 686-688. https://doi.org/10.1016/S0367-326X(01)00293-3

Perpustakaan Pengawas Obat dan Makanan. Retrieved from http://perpustakaan.pom.go.id/ebook.

Ramasamy, M., Vijaya, S. K., & Pushpa. (2016). Phytochemical and In vitro Antidiabetic Activity of Psidium Guajava Leaves. Pharmacognosy Journal, 6,8,4,392-394. doi : 10.5530/pj.2016.4.13.

Samia,et al. (1984). New Isoquinone Alkaloids from Leaves of Cassia Siamea. Journal of Natural Product, Vol 47, No. 4 : 708-718. https://doi.org/10.1021/np50034a025

Samia., Mohammed, E.S., Otto, S., & V. Mohan, C. (1978). Chemical Constituents of Cassia siamea Lamk., Planta Med 1978; 33(3): 258-261 https://doi.org/10.1055/s-0028-1097385

Shiori, Oshimia., et al. (2008). Chrobisiamone A, a new bischromone from Cassia siamea and a biomimetic transformation of 5-acetonyl-7-hydroxy-2-methylchromone into cassiarin A. Bioorganic & Medicinal Chemistry Letters, Volume 18, Issue 13 : 3761–3763.

Suzanne, M., D.L. Monte, M.D., M.P.H. & J. R. Wands, M.D. (2008). Alzheimer's Disease Is Type 3 Diabetes–Evidence Reviewed. Journal of Diabetes Science and Technology. doi : 10.1177/193229680800200619. https://doi.org/10.1177/193229680800200619

Usha, V., & A. K. Bopaiah . (2011). Preliminary phyto-chemical evaluation of the leaf extract of five Cassia Species Chem. Pharm. Res. 3(5):574-583 574.