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LC-MS/MS investigation of phytochemical ingredients and alpha amylase inhibition activity of *Cassia siamea* Lam leaves aqueous extracts

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Abstract
The main objective of the present investigation study was to analyze the phytochemical ingredients and alpha amylase inhibition activity of aqueous extract of *Cassia siamea* Lam plant leaves. Phytochemical ingredients analysis was performed by using LC-MS/MS analysis spectral technique and qualitative analysis through the well-known standard tests protocol available in the literature. Also study was aimed to investigate alpha amylase inhibition activity of leaves aqueous extract of *Cassia siamea* Lam as well established bioactive phytochemical ingredients responsible for such activity by porcine pancreatic alpha amylase enzyme inhibition *in vitro* assay. The LC-MS/MS analysis of aqueous extract reported the presence of a total 9 phytochemical ingredients in the leaves of *Cassia siamea* Lam, while phytochemical qualitative analysis of leaves aqueous extract also noted the presence of secondary metabolites like carbohydrates, proteins, amino acids, flavonoids, tannins, glycosides, steroids and phenolic compounds. IC_{50} values obtained by alpha amylase inhibition activity for *Cassia siamea* Lam aqueous extract was found to be 30.38 ± 0.03 µg/mL and acarbose as a standard reference was found to be 27.62 ± 0.02 µg/mL. The extract showed promising alpha amylase inhibition activity against tested porcine pancreatic alpha amylase enzyme has varying degree of alpha amylase inhibition activity ranging from lower to higher dose dependent activity correlated by the presence of bioactive phytochemical ingredients. The results of investigation study suggest that Cassia *siamea* Lam medicinal plant leaves has promising alpha amylase inhibition activity and could serve as potential source of natural alpha amylase inhibitor as an alternative medicine to treat disorders of diabetes mellitus.

Keywords: *Cassia siamea* Lam, aqueous extract, phytochemical ingredients, LC-MS/MS, alpha amylase enzyme, diabetes mellitus

1. Introduction
Cassia siamea Lam widely distributed plant and traditionally its leaves have been used as a folkloric medicine for the treatments of diabetes mellitus related disorders. Diabetes mellitus disease is a metabolic disorder of irregular secretary action of hyper glycaemia regulating organ in the human beings and becomes a serious threat to human being health. Currently exists many therapeutic treatments are control diabetes related disorders. In the treatment of metabolic disorders of diabetes mellitus especially in the developing countries medicinal plants has been plays significant roles due to their cost effectiveness. Naturally Plant found drugs are generally considered safe, easily available and are much effective [1].
For the control and treatment of many diseases enzyme activity inhibitors have been plays potential roles. The reviews of literature found that the traditionally used medicinal plants parts materials were possessing outstanding anti-diabetic potential, which could be possibly investigated further for the presence of alpha amylase inhibitor phytochemical ingredients [2]. Artificially synthesized enzyme activity inhibitory agents can be produce serious side effects on body and are unsuitable for use in pregnancy [3]. Therefore, more safer and effective enzyme activity inhibitor agents has been searching of new areas to active research, and after the recommendations made by WHO on diabetes mellitus [4] further research on hypoglycaemic agents from medicinal plants has been become an important aspect of the recent study.

Different parts of *Cassia siamea* Lam plant have been shown various pharmacological activities like antimalarial [5], antimicrobial, anti-diabetic, anticancer, anti-inflammatory, hypotensive, diuretic, antioxidant, analgesic, laxative, anxiolytic, antipyretic, antidepressant and sedatives. The phytochemical ingredients were reported in this plant parts as chromone, chromone alkaloids, bianthraquinones, anthraquinones, flavonoids and phenolics constituents are barakol was identified as the major phytochemical ingredients of flowers and leaves [6].

Root extracts of *Cassia siamea* Lam plant showed pancreatic lipase enzyme inhibition activity and its bioassay guided fractionation provided important information of cassiamin-A and a bianthraquinone, as most active ingredient of pancreatic lipase inhibition [7]. This inhibition activity of plant has been extensively used for the investigating the potency of natural phytochemical ingredients as an antiobesity agents [8].

The species of the genus *Cassia* has been also reported rich contents of bioactive phytochemical ingredients; which have remarkable pharmacological activities useful for the treatment of various health related disorders [9]. The review of literature survey were not more provided information on the *in vitro* alpha amylase inhibitory activity of the *Cassia siamea* Lam plant leaves aqueous extracts investigated [10]. Therefore, consider leaves potency of *Cassia siamea* Lam plant and growing demands as a source of alpha amylase inhibitor study for investigating their anti-diabetic property and appearance of phytochemical ingredients.

2. Materials and Methods

2.1 Collection of plant leaves material

Cassia siamea Lam plant Leaves were collected from local area and identified with the help of our institute botanists.

2.2 Extraction of plant leaves material

The leaves of *Cassia siamea* Lam plant were dried under shade and then grinded. 5 g of grind leaves material was poured out in 100 mL of aqueous solvent and kept on a magnetic stirrer for 1 hrs. Thereafter, mixture material was extracted sequentially using a soxhlet apparatus in aqueous solvent. The extract fractions were collected and the remaining solvent was evaporated out to dryness. The obtained material from fractions was stored at 4ºC in airtight bottles for investigation study.

2.3 Investigation of Alpha Amylase inhibitory activity

Alpha amylase inhibitory activity investigation study was adopted by using a modified 3,5-dinitrosalicylic acid (DNS) *in vitro* alpha amylase inhibition assay method to quantify reducing sugar maltose liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated in the course mixture [11-13].

2.4 Phytochemical ingredients investigation

The fractioned material of aqueous extract was qualitatively analysed for the bioactive phytochemical ingredients such as phenols, protein, amino acids, glycoside, steroids, carbohydrates, tannins, flavonoids, alkaloids, saponins, triterpenoids etc. according to the standard protocols of qualitative analysis [14-15].

2.5 LC-MS/MS analysis

LC-MS/MS analysis technique was used for identification of phytochemical ingredients separated by liquid chromatography. It provides separation of ingredients and detection by MS provides molecular weight of compounds. LC-MS analysis of aqueous solvent extracted material was carried out on Waters UPLC-TQD Mass spectrometer. The ingredients were identified by comparison of mass spectra with the inbuilt Metlin, Lipid and Mass Bank databases.

2.6 Statistical analysis

The investigation experimental study was performed out in triplicate and the results were expressed in mean ± SD.

3. Results and Discussion

The result of experimental investigation study showed that the *Cassia siamea* Lam plant leaves aqueous extract exposed dose dependent alpha amylase inhibitory activity by *in vitro* assay method using potato starch as a substrate. The detected phytochemical ingredients in the leaves extract of *Cassia siamea* Lam plant find themselves in the traditional preparation with several pharmacological active properties.

3.1 Investigation of Alpha Amylase inhibitory activity

The alpha amylase inhibitory activity was investigated through the inhibition of alpha amylase enzyme inhibitory assay that made the digestion of starch and so reduced the glucose absorption. Acarbose is used as a standard reference drug at a concentration range of 20-100µg/mL (Table 1) and *Cassia siamea* Lam leaves aqueous extract (20-100 µg/mL) expressed alpha amylase inhibitory activity in a dose dependent manner (Table 2).

Table 1: Alpha Amylase inhibitory activity of Acarbose (Standard Reference Drug) Absorbance of the sample at 540nm Absorbance of Control = 0.513

Sr. No.	Concentration in (µg/mL)	Absorbance	% Inhibition	IC_{50} value (µg/mL)
1	20	0.271	47.17 ± 0.02	27.62 ± 0.02
2	40	0.234	54.38 ± 0.01	
3	60	0.209	59.25 ± 0.04	
4	80	0.177	65.49 ± 0.03	
5	100	0.156	69.59 ± 0.02	

Table 2: Alpha Amylase inhibitory activity of Cassia siamea Lam leaves extract Absorbance of the sample at 540 nm Absorbance of Control = 0.513

Sr. No.	Concentration in (µg/mL)	Absorbance	% Inhibition	IC_{50} Value (µg/mL)
1	20	0.268	47.75 ± 0.04	30.38 ± 0.03
2	40	0.245	52.24 ± 0.01	
3	60	0.217	57.69 ± 0.02	
4	80	0.196	61.79 ± 0.05	
5	100	0.173	66.27 ± 0.03	

The data of experimental study leaves aqueous extract and acarbose as a standard reference were represents inhibition activity at higher concentration tested. Leaves of *Cassia siamea* Lam. plant exhibited significant activity i.e. it inhibits alpha amylase enzyme activity by about IC_{50} values of aqueous extract 30.38 ± 0.03 µg/mL and acarbose as a standard reference 27.62 ± 0.02 µg/mL at concentrations rising from 20 µg/mL to 100µg/mL as shown in fig. 1.

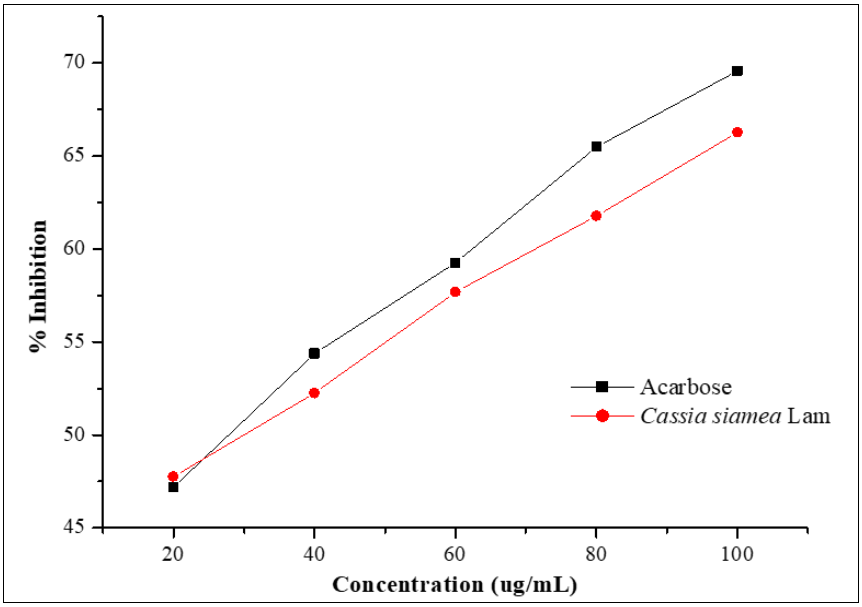


Fig 1: Alpha amylase inhibitory activity of Cassia siamea Lam leaves extract

3.2 Phytochemical Ingredients Investigation of Extract

The aqueous extract of *Cassia siamea* Lam leaves were investigated qualitatively for the bioactive phytochemical ingredients by using standard protocols used in reference literature. The investigation study result of the aqueous extract were reported the presence of carbohydrate, protein, amino acids, glycoside, tannins, flavonoids, steroids and phenolic constituents [16] as shown in table 3.

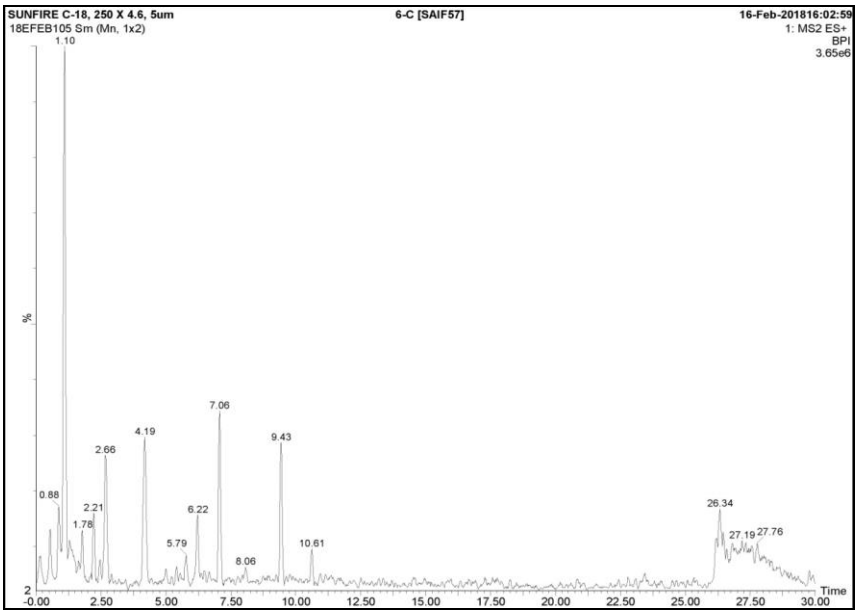
Table 3: Phytochemical Tests Performed for *Cassia siamea* Lam leaves extract

Phytochemicals	Result
Alkaloid	-
Carbohydrate	+
Protein and amino acids	+
Glycoside	+
Tannin	+
Saponin	-
Flavonoids	+
Steroids	+
Triterpenoids	-
Phenolic compounds	+

(+) for present and (-) for absent

3.3 LC-MS/MS investigation of aqueous extract

The LC-MS investigation of leaves aqueous extract of *Cassia siamea* Lam. plant was detected phytochemical ingredients intensity peaks chromatogram (BPI and EIC) as shown in fig. 2



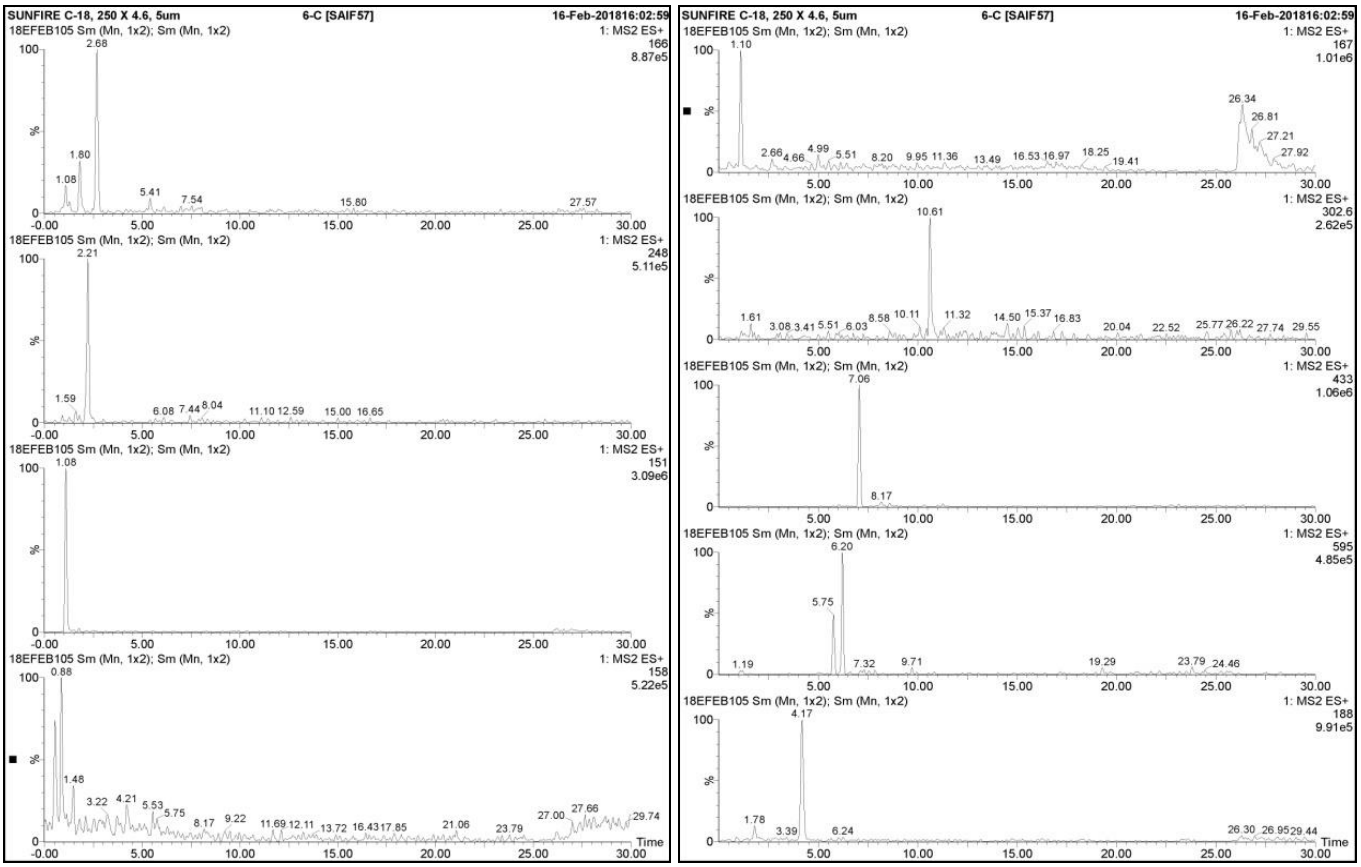


Fig 2: LC-MS/MS chromatogram (BPI and EIC) of aqueous extract

Table 4: Chemical constituents detected in leaves aqueous extract

Peak	R. Time	Name	Base m/z
1	0.88	N,N-diethyl-3-hydroxybut-2-enamide	158.2
2	1.10	(S)-2-Hydroxy-2-phenylacetic acid	151.3
3	2.21	β-sanshool (2E,6E,8E,10E)-N-(2-methylpropyl) dodeca-2,6,8,10-tetraenamide	248.3
4	2.66	Pyridoxal	166.3
5	4.19	N8-Acetylspermidine	188.3
6	6.22	1-(9Z-octadecenoyl)-2-(4-oxobutryl)-sn-glycero-3-phospho-(1'-sn-glycerol)	595.3
7	7.06	(5Z,7E)-(1S,3R)-1,3,25-trihydroxy-22-oxa-9,10-seco-5,7,10(19)-cholestatrien-24-one	433.3
8	10.61	Dobutamine	302.5
9	26.34	4-Hydroxy-3-methoxybenzoic acid	167.3

The phytochemical ingredients [17] tentatively reported in leaves aqueous extract of *Cassia siamea* Lam. plant which contributes to alpha amylase inhibitory activity as shown in above table 4. The naturally occurring health products contains bioactive phytochemical ingredients from plant origin were clearly indicates as a promising avenue for the prevention and treatments of metabolic chronic disorders. The aqueous extract of *Cassia siamea* Lam. leaves were tested for anti-diabetic activity in alloxan induced diabetes of diabetic rats; various extract doses produced significantly decreased the plasma blood glucose level as well as improving lipid metabolism and body weight in rats with induced diabetes problems [18, 19]. Good docking score has shown by in silico molecular docking studies, emodin and chrysophanol are present in *Cassia siamea* Lam leaves to be good inhibitors of angiotensin II receptor type 2 and possess good anti-diabetic property [20].

The C*assia siamea* Lam. plant leaves are a rich source of minerals [21] and contains numerous phytochemical ingredients like cassiamin, siameadin, lupeol, lupeone, chrysophanol, cassiamin A, chrysophanol-antrone, rhein, barakol, cassia chromone (5-acetonyl-7-hydroxy-2-methylchromone), p-coumaric acid, apigenin-7-o-galactoside, β-sitosterol, cassia chromonone and cassiadinine [22-25]; whereas Cassiarin A [26], chrobisiamone-A, bischromone [27], were isolated from the *Cassia siamea* Lam plant leaves and denoted promising anti-plasmodial activity.

The LC-MS/MS investigation was detected 9 bioactive phytochemical ingredients. Qualitative phytochemical investigation study of leaves aqueous extract also confirmed the presence of secondary metabolites like carbohydrate, protein, amino acids, glycoside, tannins, flavonoids, steroids and phenolic ingredients [28]. All the chemical ingredients identified a wide range of phytochemical ingredients in the leaves of *Cassia siamea* Lam plant find them in the traditional and pharmaceutical importance's [29-31]. *Cassia siamea* Lam plant different parts also reported significantly pharmacological activities and their uses [32]. Thus, our investigation study also suggested that the aqueous extract of leaves showed significant alpha amylase inhibitory activity at rising concentration tested due to the rich contents of bioactive phytochemical ingredients. The dose dependent inhibition of alpha amylase enzyme reported by

leaves extract than standard reference used *in vitro* assay [33-35]. Therefore, *Cassia siamea* Lam medicinal plant leaves have been used as potent alpha amylase inhibitor to treat and prevent diabetes mellitus complementary metabolic disorders.

4. Conclusion

The aqueous leaves extract of *Cassia siamea* Lam plant has remarkably reported effective alpha amylase inhibitory activity. The overall activity depends on contribution of bioactive phytochemical ingredients were present in the extract of leaves. It could be a main source of natural inhibitory agents, which have more significant role as therapeutic agent for prevention and management of type-II diabetes related complications. Therefore, it was concluded that aqueous leaves extract of *Cassia siamea* Lam plant showed potent alpha amylase inhibitory activity and more investigations are proposed to validate these claims by identifying bioactive phytochemical ingredients with potential therapeutic benefits for diabetes mellitus disorders.

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