AN INVITRO EVALUATION OF ANTIDIABETIC ACTIVITY OF ALPINIA GALANGAL AND ALPINIA CALCARATA

ABSTRACT

Background-The herbs, genus *Alpinia calcarata* and *Alpinia galanga* that underneath the family Zingiberaceae are rhizomatous and extremely aromatic. The study is to investigate the anti-diabetic activity of *Alpinia galanga* and *Alpinia calcarata* in-vitro. **Material and methods-**The inhibitory effect of Alpinia *galanga* and *Alpinia calcarata* on α-amylase and α-glucosidase activities were evaluated. **Results-**The results revealed that both *Alpinia galanga* and *Alpinia calcarata* inhibited α-amylase and α-glucosidase activities in a dose-dependent manner (200–1000 µg/mL). However, *Alpinia calcarata* possess better antidiabetic activity than *Alpinia galangal.* **Conclusion-**The presence of phenolic and other phytochemical content in the herbs might be the reason for their ability to inhibit α-amylase and α-glucosidase activities. Thus, the drug formulating from the herbs, *Alpinia galanga and Alpinia calcarata* could be part of the potential alternative for synthetic anti-diabetic drug.

Key words - *Ant diabetic, α-amylase, α-glucosidase*

INTRODUCTION

According to World Health Organization, the term Diabetes Mellitus (DM) is outlined as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in hypoglycemic agent (insulin) secretion hypoglycemic agent action, or both [1]. DM is one of the most prevalent disease that affects many folks round the world [2]. As expressed by World Health Organization, the population of diabetic patient can increase over three hundred million folks, most likely in the year 2025 [3]. DM is known as hypo insulinemia, hyperglycemia, hyperlipidemia and hyper aminoacidemia [4]. It is classified as type-1, type-2 and Gestational Diabetes Mellitus (GDM). Type-1 DM is additionally named as autoimmune diabetes, that due to extermination of β-cell islets in exocrine gland via response - mediate, resulting in deficiency of hypoglycemic agent (insulin). Type-2 DM is conjointly named as ketoacidosis-resistant diabetes, that due to resistance of hypoglycemic agent or uncommon secretion of hypoglycemic agent [5]. 90% of polygenic disorder cases add up to Type-2 DM and remainder of the ten percent cases add up to Type-1 DM and GDM [6]. Currently, insulin and some synthetic oral hypoglycemic agents such as sulfonylurea, meglitinides, incretin mimetics, thiazolidinediones and biguanides are used for treating hyperglycemia [7]. Although the synthetic drug act as the first line of therapy, they will turn out with some adverse impact and drug interaction [8] e.g., sulfonamides hinders excretion or metabolism of antidiabetic drug sulfonylurea thus causing hypoglycemia. Rifampicin speed up the rate of metabolism so as to decrease symptom impact [9]. On account of the high price and adverse impact of artificial medicine, medicine from natural plants is designed to treat the un wellness [2, 10] and it is appropriate for any age and sexes [11]. Medicinal plants have bioactive compound like polyphenols, phenol, flavanoid, terpenoid. These are helpful to prevent the damage by oxidation due to formation of reactive oxygen species (ROS) [12]. These phytochemicals within the medicinal plants have medical care worth like anti-diabetic, anti-inflammatory, anti-hyperglycemic etc [13]. Therefore the usage of the inhibitor is helpful for the treatment of diabetes, since it's a reasonably oxidative stress associated diseases [14]. So treating these diseases with the drug from medicinal herbs has been approved by World Health Organization [6, 15]. Several Indian plants are studied to treat different forms of diabetes and been reported in numerous scientific journals. The present study was done with plants extract of ***Alpinia galanga*** and ***Alpinia calcarata*** to prove their anti- diabetic properties.

Alpinia galanga and Alpinia calcarata

The herbs, genus *Alpinia calcarata* and *Alpinia galanga* that underneath the family Zingiberaceae, are rhizomatous and extremely aromatic [16]. In Indonesian and Thai cuisines, these herbs are used for preparation and that they posses healthful worth like antimicrobial, antiulcer, anti-spasmodic, anti-inflammatory and anti-diabetic [17]. *Alpinia galanga*, conjointly referred to as greater galanga, is a source of ascorbic acid, iron, fat-soluble vitamin and sodium. These herbs have bioactive compounds like galangin, saponins, terpenoids, phenolics, carbohydrates, quercetin, alkaloids, glycosides, emodin, phytosterols, galango, beta-Sitosterol, flavonoids. These bioactive compounds have some therapeutic worth like antineoplastic, hypoglycemic, gastro protective, hypo lipidemic, antifungal and anti-inflammatory activities. Studies have shown that ethanolic extract of *Alpinia galanga* possess sturdy inhibition action against α -amylase and α- glucosidase [18] whereas the methanolic extract of *Alpinia galanga* shows gentle inhibition action against α-amylase and α-glucosidase and scavenging activity against DPPH (2,2,1-diphenyl-1-picrylhydrazyl) radical [19]. The genus *Alpinia calcarata* rhizome have several medicinal value such as antibacterial, antifungal, anti-diabetic, antioxidant , aphrodisiac, antifungal, gastro protective, anthelmintic and anticancer activity [20]. Hence the decoction of this rhizome can be used to treat respiratory ailments, stomach ache, asthma, cough, rheumatism, bronchitis, diabetes [21]. Analysis have shown that the ethanolic and hot water extract of *Alpinia calcarata* has considerable repressing action against α-amylase and α-glucosidase [21] and strong free radical scavenging activity against DPPH [23]. Data are available that *Alpinia calcarata* and *Alpinia galanga* have ellagic acid and gallic acid. *Alpinia calcarata* has ellagic acid which is double the amount of ellagic acid present in *Alpinia galanga* whereas the *Alpinia galanga* has four times higher amount of gallic acid than *Alpinia calcarata* [24, 16].

α-amylase and α-glucosidase inhibitor

In the blood, the predominant supply for glucose is starch [25] .The dietary starch is hydrolyzed by two major enzymes. They are α-amylase and α-glucosidase. Alpha-amylase breakdown the starch into maltose. Then it is further breakdown to glucose [26]. The quicker digestion of starch to glucose can ends up with the elevation of glucose level in the blood which is termed as Post Prandial Hyperglycemia [27]. By inhibiting the hydrolyzing enzyme concerned with the digestion of carbohydrates like α-amylase and α-glucosidase, the digestion of sugar is delayed, this can eventually decrease Post Prandial Hyperglycemia [7, 28]. This is one of the effective ways to control type-2 diabetes mellitus [29]. The bioactive component present in the medicinal plants and herbs can act as inhibitors of alpha-amylase and alpha-glucosidase with minimal side effects [5, 30]. Because of the above reason and economical consideration, inhibitors from natural plants have earned popularity [31].

Aim of the Study: The aim of the study was to investigate the anti-diabetic effect of A*lpinia galanga* and *Alpinia calcarata* by in-vitro studies by inhibiting the alpha amylase and alpha glucosidase in order to minimize the toxicity and side effects of the inhibitors currently used to control hyperglycaemia.

Objectives: To determine the anti-diabetic activity of *Alpinia galanga* and Alpinia *calcarata* by In-vitro studies.

Materials and Methods: This study was conducted in the research laboratory at department of Biochemistry, Chettinad Academy of Research and Education (CARE), Kelambakkam.

Preparation of plant extract

Aqueous *alpinia galanga and alpinia calcarata* extract was prepared from locally available alpinia roots. The alpinia roots were peeled on crushed ice and 50 g of *alpinia galanga* and *alpinia calcarata* rhizome were cut into small pieces and homogenized in 75 ml cold, sterile 0.9% Sodium chloride in the presence of some crushed ice. The homogenization was carried out in a blender at high speed using 2 min bursts for a total of 12 minutes. The homogenized mixture was filtered three times by cheese cloth (very little

material was retained on the cheese cloth). The filtrate was centrifuged at 2000 relative centrifugal force for 10 min and the clear supernatant fraction was made up to 100ml with normal saline. The concentration of this extract preparation was considered to be 500mg/ml. The aqueous extract was stored in small samples at 0°c for further use [32].

METHODOLOGY [33]

I. Porcine pancreatic Alpha amylase inhibition assay by using starch

Table 1 - Reaction volume of alpha amylase inhibition assay

SI.NO	INGREDIENTS	TEST	CONTROL	BLANK
1.	Phosphate buffer	200 µl	200 µl	200 µl
2.	PPA	60 µl	60 µl	60 µl
3.	Sample	250 µl (plant extraction at different concentration)	250 µl (plant extraction at different concentration)	250 µl Milli-Q-water
Incubated at 37°c for 15 minutes				
4.	1% soluble starch	200 µl	200 µl	200 µl
Incubated at 37°c for 15 minutes				
5.	0.1 M HCL	120 µl	120 µl	120 µl
6.	Iodine reagent	600 µl	600 µl	600 µl
Read absorbance of test and control against the reagent blank at 620nm				

PPA - Porcine pancreatic amylase solution

The IC_{50} values were determined from plots of percentage inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. The entire test was performed in triplicate.

II. ALPHA – GLUCOSIDASE INHIBITION ASSAY

Table 2 - Reaction volume of alpha glucosidase inhibition assay

SI.NO	INGREDIENS	TEST	CONTROL	BLANK
1.	Phosphate buffer	50 µl	50 µl	50 µl
2.	PPA	10 µl	10 µl	10 µl
3.	Sample	250 µl (plant extraction at different	250 µl (plant extraction at different	250 µl Milli-Q-water

		concentration)	concentration)	
Incubated at 37°c for 15 minutes				
4.	PNPG	20 µl	20 µl	20 µl
Incubated at 37°c for 15 minutes				
5.	0.1 M Sodium carbonate	50 µl	50 µl	50 µl
Read absorbance of test and control against the reagent blank at 405nm				

PNPG - p-nitro phenol-α-D-glucopyranoside subtrate

The **IC_{50}** values were determined from plots of percentage inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All the test were performed in triplicate

Statistical analysis

All of the statistical analyses were performed using Graph Pad Prism statistical. The data were expressed as mean ± SEM for here experiments in each group. The IC50 values were estimated by nonlinear curve-fitting and presented as their respective 95% confidence limits using graph pad prism software

RESULTS

The ability of *Alpinia galanga* and *Apinia calcarata* to inhibit α-amylase and α-glucosidase activity in vitro was investigated and the result is presented in table 3 & 4 respectively. The results revealed that both *Alpinia galanga* and *Apinia calcarata* inhibited α-amylase and α-glucosidase activities in dose-dependent manner (200-1000µg/ml). It was observed that the Alpinia *calcarata* possess better anti- diabetic activity as compared to the *Alpinia galanga*. The study showed that both Alpinia *galanga* and *Apinia calcarata* inhibited α-amylase and α-glucosidase. The highest concentration 1000µg/ml of *Alpinia calcarata, Alpinia galanga* and Acarbose showed a maximum inhibition of [71.85±0411, 66.37±0.553 and 74.7±0.582] % againsts α-amylase and [89.47±0.142, 79.32±1.954 & 89.81±0.093] % againsts α-glucosidase while the lowest concentration 200µg/ml of *Alpinia calcarata, Alpinia galanga* and Acarbose showed a minimum inhibition of [39.89±0.18, 28.23±0.83 & 41.44±1.08] % againsts α-amylase and [25.3±0.197, 14.54±0.64 & 23.3±0.004] % againsts α-glucosidase. The IC50 values of the *Alpinia calcarata, Alpinia galanga* and Acarbose were found to be [534.39, 639.83 & 513.97 µg/ml] against α-amylase and [501.34, 596.78 & 482.68 µg/ml] againsts α-glucosidase respectively. Hence the Alpinia *calcarata* showed strong α-amylase and α-glucosidase inhibition as compared with *Alpinia galangal*.

Table 3 - The inhibition of alpha amylase enzyme assay.

SAMPLE	**INHIBITION OF ALPHA AMYLASE**			
	Concentration	**Absorbance at 620nm**	***Inhibition %***	**IC50**
Acarbose	200 µg/ml	0.094±0.001	41.44±0.8	**513.97µg/ml**
	400 µg/ml	0.113±0.003	52.89±1.64	
	600 µg/ml	0.147±0.001	55.14±0.32	

	800 µg/ml	0.184±0.002	70.36±0.26	
	1000 µg/ml	0.224±0.001	74.70±0.58	
Alpinia calcarata	200 µg/ml	0.091±0.001	39.89±0.18	**534.39µg/ml**
	400 µg/ml	0.108±0.003	44.77±1.42	
	600 µg/ml	0.142±0.005	61.26±0.16	
	800 µg/ml	0.176±0.005	68.71±1.80	
	1000 µg/ml	0.195±0.002	71.85±0.41	
Alpinia galanga	200 µg/ml	0.07±0.008	28.23±0.83	**639.83µg/ml**
	400 µg/ml	0.09±0.002	40.87±0.45	
	600 µg/ml	0.124±0.001	55.63±0.41	
	800 µg/ml	0.134±0.003	59.05±0.10	
	1000 µg/ml	0.163±0.002	66.37±0.55	

***values are expressed as mean ±SEM (n=5)**

Table 4 - The inhibition of alpha glucosidase enzyme assay

SAMPLE	**INHIBITION OF ALPHA GLUCOSIDASE**			
	Concentrationµg/ml	**Absorbance at 405nm**	***Inhibition %***	**IC50**
Acarbose	200 µg/ml	0.801±0.001	25.30±0.20	**482.68µg/ml**
	400 µg/ml	0.521±0.002	51.35±0.19	
	600 µg/ml	0.381±0.002	64.25±0.22	
	800 µg/ml	0.208±0.004	80.53±0.39	
	1000 µg/ml	0.105±0.002	89.81±0.09	

Alpinia calcarata	200 µg/ml	0.842±0.004	23.03±0.004	**501.34µg/ml**
	400 µg/ml	0.679±0.001	47.89±0.50	
	600 µg/ml	0.45±0.0017	62.91±0.17	
	800 µg/ml	0.345±0.001	78.64±0.29	
	1000 µg/ml	0.177±0.001	89.47±0.14	
Alpinia galanga	200 µg/ml	0.952±0.006	14.54±0.64	**596.78µg/ml**
	400 µg/ml	0.781±0.005	36.60±0.05	
	600 µg/ml	0.506±0.002	54.5±10.19	
	800 µg/ml	0.399±0.008	68.71±0.08	
	1000 µg/ml	0.024±0.001	79.32±1.95	

***values are expressed as mean ±SEM (n=5)**

DISCUSSION

Management of the blood glucose level is an essential approach in the control of diabetes complications. Inhibitors of carbohydrates hydrolysing enzymes have been helpful as oral hypoglycemic medicines for the control of hyperglycemia exclusively in patients with type-2 diabetes mellitus. Inhibition of these enzymes holds of carbohydrate digestion and extends the total carbohydrate digestion time, leading to a decrease in the postprandial plasma glucose rise [34].

As mentioned earlier, since the synthetic drugs for diabetes mellitus causes some adverse effects, natural herbs have gained some popularity. One of the herb which has enormous therapeutically values is genus *Alpinia*.

The study is to investigate the anti-diabetic activity of *Alpinia galanga* and *Alpinia calcarata* in vitro. It was shown that the *Alpinia calcarata* possess very well anti-diabetic activity as compared to the *Alpinia galanga*. This study shows that both *Alpinia galanga* and *Alpinia calcarata* exhibits appreciable inhibition againsts α-amylase and α-glucosidase. The IC50 values of the *Alpinia calcarata, Alpinia galanga* and Acarbose were found to be [534.39, 639.83 & 513.97 µg/ml] against α-amylase and [501.34, 596.78 & 482.68 µg/ml] againsts α-glucosidase respectively.

An effective strategy for type 2 diabetes management is the strong inhibition of α-glucosidase and mild inhibition of pancreatic α-amylase, which was achieved by plant extracts [35].

Studies have shown that the presence of these compounds may be the reason for the excellent antioxidant, anti-diabetic, and antinflammatory activity [36].

A study reveals that the Alpinia *calcarata contains 6* major compounds such as 2- octanone, camphene, 1, 8-cineole, αfenchyl acetate, 2 hexanone and 4 methyl- 2- hexanone [37].

Data reveal that *Alpinia galanga* contain polyphenols like Syringic acid, Pyrogallo, Benzoic acid and Protocatchuic and flavonoids like Hisperdin, Rutin, Naringin, 7-OH flavones, Narengenin and Kampherol [38].

CONCLUSION

The result of this study shows that the both *Alpinia galanga* and *Alpinia calcarata* exhibits appreciable inhibition againsts α-amylase and α-glucosidase but it indicates that the *Alpinia calcarata* possess better anti-diabetic activity than *Alpinia galanga.* *Alpinia calcarata* and *Alpinia galanga* are important medicinal plants of great deal and are useful for its various properties by a number of pharmaceutical companies and general public. Still a lot of scope is there for research on these plants to explore it further for the well-being of humans. Thus, it can be concluded from the above study that its anti-diabetic properties might be due to the presence of high content of phenolic compounds and other phytochemicals present in this plant. So the preparation of drug from these herbs might be a potential alternative for synthetic anti-diabetic drug.

Ethical Approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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