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In-vitro Anti-diabetic Evaluation of Sambirani Poo Kuligai by Alpha Amylase Enzyme Inhibition Assay

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Authors' contributions

This work was carried out in collaboration among all authors. Author AD conceived and designed the study, wrote the original draft. Author MM wrote the paper and prepared the visualizations. Author RM administered the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion. The Siddha system of medicine is a traditional medical system that uses a scientific and holistic approach to provide preventive, promotive, curative, rejuvenating and rehabilitative health care. According to Siddha literature, diabetes is known as Innippu Neer, Madhumegam and Neerizhivu. The various reasons for the cause of this disease are attributed to food, habits and lifestyle changes and also due to hereditary causes. The inhibition of alpha amylase enzyme involved in the digestion of

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carbohydrates can significantly reduce the postprandial increase of blood glucose and therefore can be an important strategy in the management of blood glucose level in type-2 diabetic and borderline patients.

Aim & Objective: This study aimed to evaluate the in vitro alpha-amylase inhibitory effect of Sambirani Poo Kuligai (SPK), a Siddha formulation, to assess its potential anti-diabetic properties.

Methods: Alpha-amylase inhibitory activity was assessed using a spectrophotometric assay.

Results: SPK exhibited alpha-amylase inhibitory potential, with a maximum inhibition of about 48.67% ± 4.097 and a corresponding IC50 value of 578.6 ± 44.75 µg/mL.

Conclusion: This in vitro study demonstrates that the Siddha formulation Sambirani Poo Kuligai (SPK) exhibits alpha-amylase inhibitory activity, suggesting its potential anti-diabetic properties.

Keywords: Siddha; sambirani poo kuligai; anti-diabetic; in-vitro alpha amylase inhibition assay.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion [1]. It is estimated that 537 million (10.5%) individuals (those aged 20–79 years) worldwide are currently managing the disease. In 2021, the International Diabetes Federation (IDF) approximated that there were 537 million individuals living with diabetes, making up 10.5% of the global population, resulting in global healthcare expenses amounting to $966 billion. This health cost is predicted to rise to more than $1054 billion by 2045. It is alarming that the prevalence of DM is anticipated to increase to 643 million (11.3%) by 2030 and 783 million (12.2%) by 2045 [2].

The Siddha system of medicine is a traditional medical system that uses a scientific and holistic approach to provide preventive, promotive, curative, rejuvenating and rehabilitative health care. The word siddha is derived from the Tamil word siddhi, which means “to achieve” or “perfection” or “heavenly bliss” [3]. According to Siddha literature, diabetes is known as Innippu Neer, Madhumegam and Neerizhivu. The various reasons for the cause of this disease are attributed to food, habits, and lifestyle changes and also due to hereditary causes.Vatham, Pitham and Kapham are the basic principles of Siddha medicine which play a vital role in the pathology of Madhumegam [4].

“Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorped by the body. Starches are complex carbohydrates that cannot be absorbed unless they are the first broken down by the digessive enzyme amylase and other secondary enzymes. Salivary and pancreatic amylases catalyze the hydrolysis of glycosidic linkages in starch and other related polysaccharides, their inhibition have been theorized to have beneficial therapeutic effects by reducing carbohydrate-induced hyprglycemia and hyperinsulinemia” [3].

The inhibition of alpha amylase enzyme involved in the digestion of carbohydrates can significantly reduce the postprandial increase of blood glucose and therefore can be an important strategy in the management of blood glucose level in type-2 diabetic and borderline patients [5]. In vitro study, extracts of piper betel and syzygium aromaticum(clove) have potential alpha amylase and alpha glucosidase inhibitory activity [6,7]. Based on the previous studies, SPK has the possibility of having the same pharmacological activity as alpha amylase inhibitor for potential anti-diabetic agent, but such tests have not been conducted. This research purpose is to investigate *In-vitro* alpha amylase enzyme inhibitor of SPK.

2. MATERIALS AND METHODS

a) Ingredients of the Test Drug

1) Sambirani (benzoin)
2) Kirambu (clove)
3) Korosanai (korochana)
4) Vetrilai (piper betle)

The reference for this preparation was taken from the classic siddha text, Agathiyar Paripooranam 400” [8]. The trial drug was prepared as per a Standard Operative Procedure (SOP).

b) Drug Authentication

The requisite raw drugs were procured from a well reputed indigenous raw drug shop. The

herbal raw drugs were authenticated by the Botanist of Government Siddha Medical College, Chennai and the mineral drugs were authenticated by the HOD of *Gunapadam* Department of Govt. Siddha Medical College, Chennai.

c) Purification of Raw Drugs

Herbal and mineral drugs underwent purification as per "*Sikitcha Ratna Deepam Ennum VaidhiyaNool*" [9] and "*Gunapadam Thathu Jeeva Vaguppu*" respectively.

d) Preparationof the Drug

After being well-powdered, the purified benzoin was added to a tiny saucepan. Another large pot had a piece of paper stuck to its inside. With their mouths facing one other, the large pot was positioned on top of the small pot. Seven layers of dirty, wet cloth were used to close the spaces between their jaws, and the material was left to dry. After that, it underwent a 12-hour sublimation process. To cut down on heat, the pot was left undisturbed once the sublimation process was complete. Following the seal's opening, the sublimed product was gathered and scrapped. After finely ground clove, it was sieved through a white cloth. Korochana also was powdered well. Clove powder and Korochana powder were added along with the sublimate. Then all these substances were grounded well with Piper betel leaf juice for 48 minutes. The paste was rolled into pills to the size of the seeds of Abrus precatorius which was equivalent to 130mg and dried in the shade and bottled up.

Table 1. Purification of raw drugs

S.No	Name of the Drug	Purification
1	Sambirani	The gums were purified by removing the sand, dust and odd particles
2	Kirambu	The flower buds were removed and fried slightly
3	Korosanai	The unwanted substances were removed Test for korosanai: On piercing a red hot needle into korosanai, it shows the deposition of yellow material and emission of yellow fumes
4	Vetrilai	The stalk and the middle vein were removed

Table 2. Raw drug's botanical name, family and part used

S.No	Name of the Raw Drug	Botanical/ Zoological Name [10]	Family	Part Used
1	Sambirani	*Styraxbenzoin,Dryand*	Styracaceae	Resin
2	Kirambu	*Syzygiumaromaticum,Linn*	Myrtaceae	Dried Flower buds
3	Korosanai	*Felbovinumpurifactum(purified ox bile or ox gall)*	NA	NA
4	Vetrilai	*Piper betle,Linn*	Piperaceae	Leaves

Table 3. Actions and chemical constituents of raw drugs

S. No	Name of the Drug	Actions	Chemical Constituents
1	Sambirani	Stimulant [10]	Cinnamic acid, benzoic acid, bensylbenzoate, lignans, vanillin, benzaldehyde [11]
2	Kirambu	Anti-diabetic, Hepatoprotective, [12] Anti spasmodic [10]	B caryophyllene, eugenol, acetophenone, eugenyl acetate, α humulene, γ cadinene, α phellandrene, rhammetin, kaempferol, gallic acid, vanillin [13]
3	Korosanai	Anti-spasmodic [14]	Cholic acid, chenodeoxycholic acid, biliverdin, phospholipids, cholesterol [15]
4	Vetrilai	Stimulant, Carminative, Anti-hypercholesterolemic, [16] Anti-oxidant, Anti-diabetic [17,18]	Arecoline, choline, eugenol, chavicol, caryophyllene, limonene, allylpyrocatechol [19]

Sample of 10 gram of study medicine was sent to the Noble Research Solutions, Chennai, to evaluate the alpha amylase inhibitory activity.

In-vitro Alpha Amylase Inhibition Study

Method Adopted: The spectrophotometric assay method.

The enzyme α-amylase (0.5 U/ml) was prepared by mixing 3.24 mg of α-amylase in 100 ml of phosphate buffer (pH 6.9). Test Sample (SPK) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml using DD water. Acarbose100 µg/ml used as a reference standard. About 600 µl of test sample were added to 30 µl of α-amylase enzyme solution and incubated at 37°C for 15 min. To this reaction mixture, 370 µl of substrate, 2-Chloro-4-Nitrophenyl-α-Maltotrioside ($CNPG_3$- 0.5 mg/ml) was added, mixed and for incubated 37°C for 10 min. Finally, absorbance was measured at 405 nm against blank in spectrophotometer. A control reaction was carried out without the test sample.

Percentage inhibition was calculated by the following formula.

Percentage inhibition

$$\%\text{inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Table 4. Percentage inhibition of test drug SPK on Alpha Amylase enzyme Inhibition Study

Concentration (µg/ml)	% Inhibition of SPK
100 µg/ml	16.77 ± 3.228
200 µg/ml	25.37 ± 8.351
300 µg/ml	35.39 ± 19.16
400 µg/ml	42.38 ± 11.04
500 µg/ml	48.67 ± 4.097
Standard Acarbose	94.02 ± 4.742

Data are given as Mean ± SD (n=3)

Table 5. IC50 Values for Alpha Amylase Enzyme inhibition by SPK and STD

Test Drug / Standard	IC50 Value of Alpha Amylase enzyme inhibition ± SD (µg /ml)
SPK	578.6 ± 44.75
Standard-Acarbose	26.41 ± 2.526

Data are given as Mean ± SD (n=3)

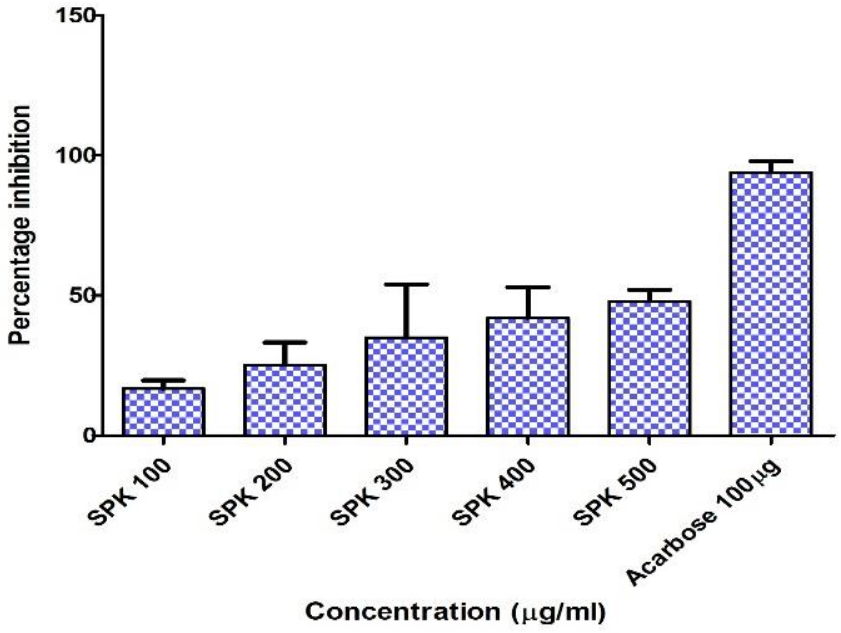

Fig. 1. Percentage inhibition of test drug SPK and Standard on Alpha Amylase Enzyme Inhibition Assay

3. RESULTS AND DISCUSSION

This study investigated the alpha- amylase inhibition activity of SPK as a potential therapeutic strategy for type 2 diabetes. Our findings demonstrated that SPK exhibited significant alpha-amylase inhibitory potential with the maximum inhibition of about 48.67 ± 4.097% and the corresponding IC_{50} is 578.6 ± 44.75 µg /ml. Standard acarbose exhibited significant inhibition in alpha amylase enzyme with the maximum inhibition of about 94.02 ± 4.742% and the corresponding IC_{50} is 26.41 ± 2.526 µg /ml.

The alpha amylases are the calcium metalloenzymes which can't function in the absence of calcium. There are many digestive enzymes in humans and among them the most important one is pancreatic alpha – amylase, that act as a catalysis in the reaction which involves the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylopectin, amylose, glycogen and numerous maltodextrins and is responsible for starch digestion. The large molecules like starch cannot cross the blood brain barrier as glucose has to reach the brain thus; to overcome this problem alpha-amylase cleaves the large starch molecules into smaller fragments of sugars in order to cross the blood brain barrier. If there will be excess conversion of starch to sugars, it will increase the sugar level in blood, then the role of insulin will come into action by ordering cells to metabolize the excess sugar moieties and store as energy sources i.e., glycogen. This cycle is endlessly happening in a healthy person. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, level of blood glucose arises which might results in hyperglycaemia [20].

In the management of post prandial hyperglycemia (PPH) enhancement of insulin secretion, insulin sensitivity or reducing glucose production in the liver are achieved by inhibiting the activity of alpha amylase and alpha glucosidase, the major risk factor for cardiovascular complication in DM patient is glycation end product(a metabolite), hence by reducing PPH reduces this metabolite [21].

Among four ingredients of Sambirani Poo Kuligai, styrax benzoin contains free balsamic acid chiefly cinnamic 10% and benzoic acid 6% and vanillin. In a study conducted by Veronica F Salau et al, the fructose-streptozotocin induced diabetic rats were given **vanillin** at a low (150mg/kg body weight) or high (300mg/kg body weight) dose of vanillin for 5 weeks intervention period and there levels of blood glucose observed significant reduction in blood given [22]. Rahman M Hafizur et al. conducted a study on mechanism of anti-diabetic activity of **cinnamic acid** in in-vitro and in-vivo non obese type 2 diabetic rats and finally concluded that cinnamic acid exerts antidiabetic activity by improving glucose tolerance in vivo and stimulating insulin secretion *In-vitro* [23].

Sabbir Ahmed et al conducted a study on molecular docking and dynamics stimulation of natural compounds from betel leaves (piper betlel.),In this study, a new molecule, apigenin-7-*O*-glucoside (screened out from 123 compounds of *Piper betle* L.), which inhibited both enzymes (alpha-amylase and alpha-glucosidase) activity by binding with its active sites, ASP-197, GLU-233, and ASP-300 and ASN-258, ASP-327, ILE-143, ASP-382, respectively [24]. Sindhu S Nair et al, conducted in-vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts, among them methanolic extracts of **piper betle** showed that (84.63µg/mL) exhibited 50% alpha amylase inhibition activity at the mentioned concentrations. The alpha glucosidase IC50 for the plant extracts Piper betle was found to be 96.56± 12.93 µg/mL respectively [6]. Hafiz Muhammad Arsalan conducted a study in 2019. In this study, ethanolic extract of Piper betle leaf was used (300 mg/kg). Diabetes was induced in male albino rats by using Alloxan (150 mg/kg). Glutathione (GSH), Catalase (CAT), Malondialdehyde (MDA), Nitric oxide (NO), micronutrients (Vitamin A, Vitamin C and Vitamin E), serum glucose level and Advance glycation end products (AGE's) were estimated. The results showed that levels of the Malondialdehyde (0.087, 0.151 and 0.104 respectively), Advance glycation end products (0.026, 0.053, 0.025) and Nitric oxide (0.07, 0.438, 0.190) were decreased while level of the GSH (0.184, 0.188, 0.207), CAT (0.087, 0.031, 0.091) and vitamin A (0.196, 0.136, 0.178), E (0.199, 0.140, 0.179) and C (0.163, 0.120, 0.164) were increased after administration of Piper betle leaf extract. Blood glucose level was also found to be decreased. These findings suggest that Piper betle leaves may be a potential antidiabetic agent due to their ability to lower blood glucose levels [25].

Stephen Adeniyi Adefegha et al. conducted a study on In vitro inhibition activity of polyphenol-

rich extracts from *Syzygium aromaticum* (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe^{2+}-induced lipid peroxidation in rat pancreas. The result revealed that both extracts inhibited alpha-amylase and alpha-glucosidase in a dose-dependent manner. However, the alpha-glucosidase inhibitory activity of the extracts were significantly (P<0.05) higher than their alpha-amylase inhibitory activity. The free phenolics (31.67 mg/g) and flavonoid (17.28 mg/g) contents were significantly (P<0.05) higher than bound phenolic (23.52 mg/g) and flavonoid (13.70 mg/g) contents [7]. Hafizah Umaira Tahir et al. conducted a study in 2016, to evaluate antidiabetic potential of Syzygium aromaticum essential oils and their emulsions by alpha amylase inhibition assay. Antidiabetic activity of S. aromaticum was examined in dose dependent mode (1 to 100 µg/mL). The maximum antidiabetic activity for S. aromaticum essential oils was noted at the highest dose (100 µg/mL). Five emulsions of different concentrations for S. aromaticum (A1 to A5) essential oil was formulated. Among different emulsions, A5 of S. aromaticum essential oil exhibited a maximum antidiabetic activity with 95.30 % inhibition of α-amylase. Moreover, the analysis of essential oils showed that eugenol (18.7%) was the major component of S. aromaticum essential oils [26].

Candra D. Hamdin et al conducted the study in 2019. The study was designed to investigate the effects of eugenol on the fasting blood glucose (FBG) and histological pattern of pancreas and liver of alloxan-diabetic rats. Eugenol was extracted and isolated from cloves leaves oil and was then screened by in vivo model of diabetic rats. Diabetic condition was induced with alloxan monohydrate (125 mg/kgbw, iv). Rats were divided into five groups: eugenol with 3 serial doses of 5 mg/kgbw (D1), 10 mg/kgbw(D2), 15 mg/kgbw (D3); positive control- glibenclamid 1.35 mg/kgbw (D4); normal control (D5) and negative control- CMC-Na 0,5% (D6). The treatments were performed daily for 15 days. Fasting blood glucose (FBG) level of D1 to D5 before treatment was404, 363, 319, 313, and 113 mg/dL, respectively; and after 15 days were reduced to be 294, 181, 97, 112, and 94 mg/dL, respectively. Statistically, blood glucose reduction of D1, D2, and D3 were significant (P>0,05) compared to positive control. D3 seems to be effective dose causing hypoglycaemic. Histological studies of pancreas and liver indicated that D3 may improve the morphology of Langerhans and liver into normal condition [27].

Studies mentioned in this review, reveal that among the four ingredients of Sambirani Poo Kuligai, Piper betle, syzygium aromaticum and styrax benzoin has potential anti-diabetic properties. In the present study, the results of alpha amylase inhibitory assay displayed a considerable inhibitory activity with the extract of Sambirani Poo Kuligai. Hence it can be concluded that Sambirani Poo Kuligai is a siddha formulation with multi-nodal antidiabetic actions and may serve as a potent anti-diabetic agent [28].

4. CONCLUSION

From this study, we can state that siddha formulation Sambirani Poo Kuligai showed significant inhibition of alpha amylase enzyme activity. Thus, the siddha formulation SPK may consider as a remedy for diabetes and other insulin resistance- related diseases like poly cystic ovarian syndrome; however, animal and human studies are needed to confirm this activity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

This study did not involve human subjects and therefore does not require informed consent.

ETHICAL APPROVAL

Ethical approval was not required for this study as it did not involve human participants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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