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SCREENING OF ANTI-DIABETIC POTENTIAL OF THE SIDDHA FORMULATION SAMBIRANI POO KULIGAI BY IN-VITRO ALPHA GLUCOSIDASE ENZYME INHIBITION ASSAY

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Abstract

Background:Non-insulin-dependent diabetes mellitus (NIDDM), also known as type 2 diabetes, is a prevalent endocrine disorder characterized by either impaired insulin secretion from pancreatic beta cells or reduced insulin sensitivity in target tissues. Originating in the Tamil tradition, the Siddha system of medicine represents a holistic and scientifically-informed approach to healthcare. It encompasses preventive, promotive, curative, rejuvenative, and rehabilitative practices. Siddha texts refer to diabetes as "InnippuNeer," "Madhumegam," and "Neerizhivu." The causes are attributed to food habits, lifestyle habits, hereditary factors, and imbalances in the tridoshic principles – Vatham, Pitham, and Kapham – which are fundamental concepts in Siddha medicine and play a crucial role in the development of Madhumegam (diabetes). One of the therapeutic methodologies is to decrease the postprandial hyperglycemia by hindering the absorption of glucose through the inhibition of carbohydrate-hydrolysing enzymes, such as α-amylase and α-glucosidase. α-Glucosidase are enzyme that catalyze the absorption of digested glucose from dietary polysaccharides in the small intestine. Aim & Objective: The aim of the present study is to evaluate the anti-diabetic potential of the siddha formulation of Sambirani Poo Kuligai(SPK) through alpha glucosidase enzyme inhibition assay. Method: The spectrophotometric assay method was used to find alpha glucosidase inhibitory activity. Results: SPK showed presence of alpha glucosidase inhibitory potential with tha maximum inhibition of about 41.74± 3.578 % and the corresponding IC_{50} is606.5 ± 49.51µg/ml. Conclusion: This in-vitro study exposed the existence of the alpha glucosidase activity in the siddha formulation Sambirani Poo Kuligai owns antidiabetic activity.

Keywords: Siddha Medicine, Sambirani Poo Kuligai, Anti-diabetic, In-Vitro alpha glucosidase inhibition assay.

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Introduction

Non-insulin-dependent diabetes mellitus (niddm), also known as type 2 diabetes, is a prevalent endocrine disorder characterized by either impaired insulin secretion from pancreatic beta cells or reduced insulin sensitivity in target tissues. Pharmacological interventions for NIDDM employ various mechanisms to manage blood glucose levels. These include stimulating insulin release, upregulating glucose transporter expression, inhibiting gluconeogenesis (glucose production in the liver), and delaying intestinal glucose absorption [1].Globally, the prevalence of diabetes among adults aged 20 to 79 was estimated to be 10.5% (536.6 million) in 2021 and would rise to 12.2% (783.2 million) in 2045. The prevalence of diabetes was highest in people 75–79 years old, and it was similar in men and women. According to estimates, prevalence in 2021 was greater in high-income nations (11.1%) compared to low-income countries (5.5%), and in urban areas (12.1%) than in rural regions (8.3%) [2].

Originating in the Tamil tradition, the Siddha system of medicine represents a holistic and scientifically-informed approach to healthcare. It encompasses preventive, promotive, curative, rejuvenative, and rehabilitative practices. The term "siddha" itself stems from the tamil word "siddhi," signifying "achievement," "perfection," or "heavenly bliss."[3]. Siddha texts refer to diabetes as "innippuneer," "madhumegam," and "neerizhivu." The causes are attributed to food habits, lifestyle habits, hereditary factors, and imbalances in the tridoshicprinciples – vatham, pitham, and kapham – which are fundamental concepts in siddha medicine and play a

crucial role in the development of madhumegam (diabetes) [4].

One therapeutic strategy for managing diabetes involves delaying glucose absorption through the inhibition of enzymes like alpha-glucosidase in the digestive system. Alpha-glucosidase, a widely distributed exo-type carbohydrase found in microorganisms, plants, and animal tissues, cleaves alpha bonds to liberate glucose from the non-reducing ends of complex carbohydrates. Inhibiting this enzyme activity slows the rise in blood sugar levels after consuming carbohydrate-rich meals. Alpha-glucosidase is specifically a membrane-bound enzyme located in the small intestine epithelium. It functions by catalyzing the breakdown of oligosaccharides into absorbablemonosaccharaides, facilitating intestinal glucose uptake [5].

Inhibition of intestinal alpha-glucosidase activity reduces the rate of oligosaccharide breakdown. Consequently, carbohydrate digestion extends to the lower small intestine. This spatial shift in the digestive process delays the overall rate of glucose absorption into the bloodstream. This approach has emerged as an effective strategy to mitigate postprandial blood glucose spikes, a key factor in preventing the development of complications associated with type 2diabetes [5].In vitro study, extracts of piper betel and Syzygiumaromaticum(clove) have potential alpha amylase and alpha glucosidase inhibitory activity [6, 7]. Previous research suggests that spk, a siddha formulation, might possess antidiabetic properties similar to alpha-amylase inhibitors. However, its potential in this area remains unexplored. This study aims to investigate the in vitro alpha-glucosidase inhibitory activity of SPK.

Material 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 literature, agathiyarparipooranam 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 siddhamedical 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

Raw drugs underwent purification as per"*sikitcharatnadeepamennumvaidhiyanool*"[9] and "*gunapadamthathujeevavaguppu*" respectively.

Table 1: name of the drug and purification

s.no	name of the drug	Purification
1	sambirani	the gums were cleaned 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, yellowsubstance builds up on the needle and releases yellow smoke.
4	vetrilai	the stalk and the middle vein were removed

Table2: raw drug's botanical name, family and part used

s.no	name of the raw drug	botanical/ zoological name (10,11)	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 [16]
2	kirambu	anti-diabetic, hepatoprotective, anti-spasmodic [10,12]	β caryophyllene, eugenol, acetophenone, eugenyl acetate, α humulene, γ cadinene, α phellandrene, rhammetin,

			kaempferol, gallic acid, vanillin [17]
3	korosana i	anti-spasmodic [11]	cholic acid, chenodeoxycholic acid [18]
4	vetrilai	stimulant, carminative, anti-hypercholesterolemic, anti-oxidant, anti-diabetic(13,14,15)	arecoline, choline, eugenol, chavicol, caryophyllene, limonene, allylpyrocatechol [19]

d) Preparation of the drug

The purified benzoin wasground well and sited in a small pot. a paper wasstuck on the inner surface of another big pot. The big pot wassited over the small pot with their mouths opposing each other. The gaps between their mouths werewrapped by seven layered muddy wet cloth and waspermitted to dry. Then it was subjected to sublimation process for 12 hours. After finishing sublimation process, the pot was left intact to reduce heat. then the seal wasunwrapped and the sublimed product was scrapped and collected. clove wasground well and sifted through a white cloth. Korochana also wasground well. Clovepowder 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 shaped into small pills, similar in size to the seeds of a plant called abrusprecatorius (which is roughly equivalent to 130 milligrams each). The pills were then dried in a shaded area and stored in a bottle.

Sample of 10 gram of study medicine was sent to the noble research solutions, chennai, to evaluate the alpha glucosidase inhibitory activity.

In-vitro α-glucosidase enzyme inhibition study

Method adopted: The spectrophotometric assay method.

Test solution: 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.

pnpg(p-nitrophenyl-α-d -glucopyranoside**)**: 20 mm pnpg prepared by dissolving 603 mg pnpg in 100 ml of pbs

Enzyme: To make the α-glucosidase enzyme solution, 0.5 mg of the enzyme was dissolved in 10 ml of phosphate buffer (ph 7.0) that contained 20 mg of bovine serum albumin. acarbose, which was utilized as a reference standard, at a concentration of 100µg/ml was added to 250 µl of 20 mm p-nitrophenyl-α-d-glucopyranoside and 495 µl of 100 mm phosphate buffer (ph 7.0) together with approximately 10 µlof the test sample. After a 5 minute pre-incubation period at 37°c, 250 µl of the α-glucosidase enzyme solution (0.5 mg of α-glucosidase in 10ml of phosphate buffer (ph7.0) with 20 mg of bovine serum albumin) was added to initiate the reaction. It was then incubated at 37°c for precisely 15 minutes. The reaction was then stopped by adding of 1000 µl of 200 mm na2 co3solution and the amount of p-nitrophenol released was quantified by measuring the absorbance of sample against a sample blank (containing pbs without any sample) at 405nm using uv visible spectrophotometer.

Percentage inhibition of test drug spk and std on α-glucosidase enzyme inhibition study

concentration (µg/ml)	% inhibition of Spk
100 µg/ml	12.66 ± 1.05
200 µg/ml	26.08 ± 2.146
300 µg/ml	30.95 ± 2.044
400 µg/ml	35.65 ± 0.8608
500 µg/ml	41.74 ± 3.578
standard- acarbose	96.07 ± 2.211

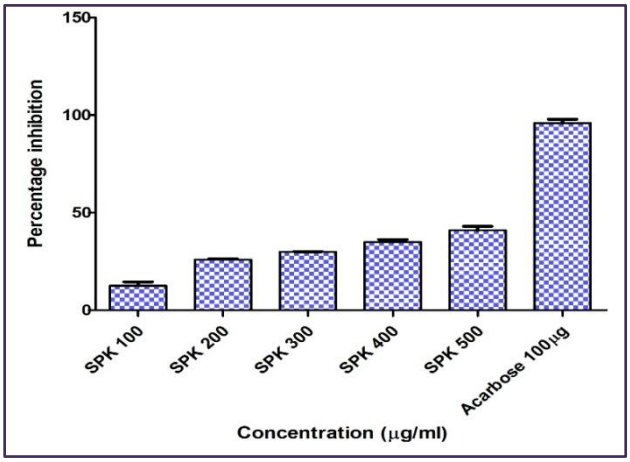
Data are given as mean ± sd (n=3)

Ic50 values for α-glucosidase enzyme inhibition assay by SPK and std

test drug / standard	ic50 value of α-glucosidaseenzyme inhibition ± sd (µg /ml)
spk	606.5 ± 49.51
standard- acarbose	29.03 ± 21.46

Data are given as mean ± sd (n=3)

Percentage inhibition of test drug SPK and standard on Alphaglucosidase enzyme inhibition assay



Discussion

This study explored the potential of **SPK** for type 2 diabetes management by investigating its alpha-glucosidase inhibitory activity. Our results revealed that SPK exhibited significant alpha- glucosidase inhibition activity with the maximum inhibition of about 41.74 ± 3.578 % and the corresponding IC50 is 606.5 ± 49.51µg/ml. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme activity with the maximum inhibition of about 96.07 ± 2.211 % and the corresponding ic5029.03 ± 21.46 µg /ml.

A major challenge in type 2 diabetes management is postprandial hyperglycemia, the rise in blood sugar levels after meals. Alpha – glucosidase inhibitors function by delaying carbohydrate breakdown in the small intestine, leading more gradual glucose absorption and a diminished postprandial glycemic response. The observed alpha-glucosidase inhibitory activity of SPK suggests its potential to contribute to enhanced glycemic control in type 2 diabetes patients. Further research is necessary to elucidate the specific mechanism by which SPK inhibits alpha-glucosidase activity. Potential mechanisms could involve competitive binding with the substrate at the enzymes active site, hampering its ability to break down carbohydrates.

One way to manage blood sugar after meals (postprandial hyperglycemia) is to slowdown glucose absorption. This can be achieved by inhibiting enzymes that break down carbohydrates, like alpha-amylase and alpha-glucosidase.Alpha -glucosidases are enzymes that catalyze the absorption of digested glucose from dietary polysaccharides in the small intestine [21].Alpha-glucosidase inhibitors exert their effect by impeding carbohydrate uptake from the small intestine. These drugs competitively inhibit key enzymes, namely glucoamylase, sucrase, maltase, and isomaltase, responsible for hydrolyzing complex, nonabsorbable carbohydrates into simpler, absorbable forms. This enzymatic inhibition translates to a delay in carbohydrate absorption, leading to a reduction in postprandial blood glucose rise by approximately 3 mmol/l [22].

Among four ingredients of sambirani poo kuligai, styrax benzoin contains free balsamic acid chiefly cinnamic 10% and benzoic acid 6% and vanillin [23]. Veronica fsalau et al. investigated the effects of **vanillin** on blood glucose levels in fructose-streptozotocin (stz)-induced diabetic rats. They administered vanillin at low (150 mg/kg body weight) or high (300 mg/kg body weight) doses for a five-week intervention period. Notably, both vanillin doses resulted in a significant reduction in blood glucose levels compared to the control group [24]. In a novel study by rahman m hafizur et al., **cinnamic acid's**antidiabetic mechanism was explored in non-obese type 2 diabetic rats. The research revealed that cinnamic acid exerts its effect through a combination of improved glucose tolerance in vivo and enhanced insulin secretion in vitro (25).Sabbirahmed et al. employed molecular docking and dynamics simulations to investigate the potential of natural compounds from betel leave (**Piper betle** l.) for inhibiting alpha-amylase and alpha-glucosidase activity. their study identified apigenin-7-o-glucoside as a promising candidate. This compound exhibited inhibitory effects against both enzymes by binding to their active sites. Specifically, apigenin-7-o-glucoside interacted with key residues: asp-197, glu-233, and asp-300 for alpha-amylase, and asn-258, asp-327, ile-143, and asp-382 for alpha-glucosidase [26].

Sindhu s nair et al. investigated the alpha-amylase and alpha-glucosidase inhibitory potential of various plants extracts using in vitro assays. Among these, the methanolic extract of **piper betle** displayed a significant inhibitory effect on both enzymes. Notably, a concentration of 84.63 µg/ml achieved 50% inhibition of alpha-amylase activity. Similarly, the extract exhibited an ic50 value of 96.56 ± 12.93 µg/ml for alpha-glucosidase inhibition (6).Stephen adeniyi adefegha et al. investigated the in vitro inhibitory effects of polyphenol-rich extracts from **syzygiumaromaticum** (clove) buds on enzymes linked to type 2 diabetes and oxidative stress in rat pancreas. Their findings revealed that both extracts dose-dependently inhibited alpha-amylase and alpha-glucosidase activity. Interestingly, the inhibition of alpha-glucosidase was significantly greater ($p < 0.05$) compared to alpha-amylase inhibition [7].

Studies mentioned in this review, reveal that among the four ingredients of Sambirani Poo Kuligai, Piper betle, Syzygiumaromaticum and Styrax benzoin has potential anti-diabetic properties. In the present study, the results of alpha glucosidase inhibitory assay displayed a considerable inhibitory activity with the extract of Sambirani Poo Kuligai. Hence it can be concluded that Sambirani Poo Kuligaiis a siddha formulation with multi-nodal antidiabetic actions and may aid as a potent anti-diabetic agent.

Conclusion

From this study, we can state that siddha formulation Sambirani Poo Kuligai showed significant inhibition of alpha glucosidase enzyme activity. These findings suggest its potential as a therapeutic agent for type 2 diabetes. However, further in vivo and clinical investigations are required to confirm its efficacy and safety for managing this chronic condition.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Informed Consent

This study did not involve human subjects and therefore doesn't require informed consent.

Ethical Statement

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

Author Contribution

A. Dharani conceived and designed the study, wrote the original draft. M. Muthupandiyan wrote the paper and prepared the visualizations.Dr.R. Menaka administered the study. All authors read and approved the final manuscript.

References

1. Thilagam E, Parimaladevi B, Kumarappan C, Mandal SC. α-Glucosidase and α-amylase inhibitory activity of Sennasurattensis. Journal of acupuncture and meridian studies. 2013 Feb 1;6(1):24-30.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JC, Mbanya JC, Pavkov ME. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes research and clinical practice. 2022 Jan 1;183:109119.
3. Dubey NP, Dubey N. Basic principles of integrated medicine. World Association of Integrated Medicine. 2013.
4. Logisha MS, Nivetha G, Karpagavalli K, Muthukumar NJ, Mahalakshmi MV, Meenakumari M. A Review on Anti-Diabetic Herbs of Siddha System Based on Their Organoleptic Characteristic. International Journal of Ayurveda and Pharma Research. 2023 Feb 5:87-91.
5. Kumar S, Narwal S, Kumar V, Prakash O. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. Pharmacognosy reviews. 2011 Jan;5(9):19.
6. Nair SS, Kavrekar V, Mishra A. In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. European journal of experimental biology. 2013;3(1):128-32.
7. Adefegha SA, Oboh G. In vitro inhibition activity of polyphenol-rich extracts from Syzygiumaromaticum (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe2+-induced lipid peroxidation in rat pancreas. Asian Pacific Journal of Tropical Biomedicine. 2012 Oct 1;2(10):774-81
8. R.C.Mohan, Agasthiyarparipooranam 400 page no: 62.
9. C. Kannuswamypillai, SikitchaRatnaDeepamEnnumVaidhiyaNool(2018)page no: 28,29,31.
10. VaithyaRatnamK.S.MurukaseMuthaliyar, Gunapadam (mooligaivaguppu), 2013
11. Dr.R.Thiyagarajan L.I.M, Gunapadam part 2&3 (Thathu-Jeevavaguppu), 2013.
12. Sammy J, Tamuno-Emine D, Nwachuku E. Evaluation of Anti-diabetic, Hepatoprotective and Antilipidemic Potentials of Syzygiumaromaticum (Clove) on Albino Rat. JOCAMR. 2020;1:38-50.
13. Venkadeswaran K, Muralidharan AR, Annadurai T, Ruban VV, Sundararajan M, Anandhi R, Thomas PA, Geraldine P. Antihypercholesterolemic and antioxidative potential of an extract of the plant, Piper betle, and its active constituent, eugenol, in triton WR-1339-induced hypercholesterolemia in experimental rats. Evidence-Based Complementary and Alternative Medicine. 2014 Jan 1;2014.
14. Perumal PA, Saravanabhavan K. Antidiabetic and antioxidant activities of ethanolic extract of Piper betle L. leaves in catfish, Clariasgariepinus. Asian J Pharm Clin Res. 2018;11(3):194-8.
15. Jing X, Sarker MM, Alam S, Gifari AJ, Akhter S, Khan F, Soma MA. Piper betel juice improved lipid profile and hepatic oxidative stress in high-fat-diet induced hyperlipidemic rats.
16. SohailAkhtar M, Alam T. Chemistry, Biological Activities, and Uses of Benzoin Resin. InGums, Resins and Latexes of Plant Origin: Chemistry, Biological Activities and Uses 2022 Mar 31 (pp. 1-22). Cham: Springer International Publishing.
17. Nassar MI, Gaara AH, El-Ghorab AH, Farrag A, Shen H, Huq E, Mabry TJ. Chemical constituents of clove (Syzygiumaromaticum, Fam. Myrtaceae) and their antioxidant activity. RevistaLatinoamericana de Química. 2007;35(3):47.
18. Hu PL, Yuan YH, Yue TL, Guo CF. Bile acid patterns in commercially available oxgall powders used for the evaluation of the bile tolerance ability of potential probiotics. PLoS One. 2018 Mar 1;13(3):e0192964.
19. 19 .Rekha VP, Kollipara M, Gupta BR, Bharath Y, Pulicherla KK. A review on Piper betle L.: nature's promising medicinal reservoir. American Journal of Ethnomedicine. 2014;1(5):276-89
20. 20 .Deutschländer MS, Van de Venter M, Roux S, Louw J, Lall N. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. Journal of ethnopharmacology. 2009 Jul 30;124(3):619-24.
21. Thilagam E, Parimaladevi B, Kumarappan C, Mandal SC. α-Glucosidase and α-amylase inhibitory activity of Sennasurattensis. Journal of acupuncture and meridian studies. 2013 Feb 1;6(1):24-30.
22. Akmal M, Patel P, Wadhwa R. Alpha glucosidase inhibitors. InStatPearls [internet] 2024 Feb 28. StatPearls Publishing.
23. Nurwahyuni I, Nababan B, Pangoloi S, Situmorang M. Cinnamic acid in frankincense sap as a criterion for determining the best mother plant for vegetative propagation of styrax benzoin (Sumatra Benzoin) in Sumatra, Indonesia. International Journal of Forestry Research. 2022;2022(1):4160241.
24. Salau VF, Erukainure OL, Olofinsan KO, Msomi NZ, Ijomone OM, Islam MS. Vanillin improves glucose

homeostasis and modulates metabolic activities linked to type 2 diabetes in fructose–streptozotocin induced diabetic rats. Archives of Physiology and Biochemistry. 2024 Mar 3;130(2):169-82.

25. Hafizur RM, Hameed A, Shukrana M, Raza SA, Chishti S, Kabir N, Siddiqui RA. Cinnamic acid exerts anti-diabetic activity by improving glucose tolerance in vivo and by stimulating insulin secretion in vitro. Phytomedicine. 2015 Feb 15;22(2):297-300.
26. Ahmed S, Ali MC, Ruma RA, Mahmud S, Paul GK, Saleh MA, Alshahrani MM, Obaidullah AJ, Biswas SK, Rahman MM, Rahman MM. Molecular docking and dynamics simulation of natural compounds from betel leaves (Piper betle L.) for investigating the potential inhibition of alpha-amylase and alpha-glucosidase of type 2 diabetes. Molecules. 2022 Jul 15;27(14):4526.