

IN VITRO EVALUATION OF ANTI-DIABETIC ACTIVITY OF VEPPAMPOO (NEEM FLOWER)**Dr. S. S. Selvapriya^{1*}, Dr. P. Lavanya², Dr. S. M. Chitra³**¹Pg Scholar, Department of Siddhar Yoga Maruthuvam, Government Siddha Medical College, Chennai, Tamilnadu, India.²Pg Scholar, Department of Siddhar Yoga Maruthuvam, Government Siddha Medical College, Chennai, Tamilnadu, India.³Head of Department, Department of Siddhar Yoga Maruthuvam, Government Siddha Medical College, Chennai, Tamilnadu, India.***Corresponding Author: Dr. S. S. Selvapriya**

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ABSTRACT

Introduction: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and long-term complications. Plant-derived bioactive compounds are increasingly investigated as safer alternatives to conventional antidiabetic drugs. *Azadirachta indica* (Neem) is widely recognized in traditional medicine for its diverse pharmacological properties. **Aim:** The aim of the study is to evaluate the anti-diabetic activity of Veppampoo (Neem Flower) through invitro analysis. **Methods:** The present study assessed the in vitro antidiabetic activity of Veppampoo (Neem Flower) extracts using α -amylase and α -glucosidase inhibition assays, key enzymatic targets in carbohydrate digestion. **Results:** Veppampoo extracts demonstrated dose-dependent inhibitory activity against both enzymes, indicating their potential to reduce postprandial glucose levels by delaying carbohydrate hydrolysis and absorption. **Conclusion:** These findings suggest Veppampoo as a promising natural source of antidiabetic agents. Further studies involving bioactive compound isolation, mechanistic evaluation, and in vivo validation are required to establish clinical relevance.

KEYWORDS: Veppampoo (Neem Flower), in vitro assay, α -amylase inhibition, α -glucosidase inhibition, anti-diabetic activity.

INTRODUCTION

The Siddha system of medicine, one of the oldest traditional practices in India, emphasizes preventive health through lifestyle, diet, exercise, and natural interventions. A distinctive feature of Siddha medicine is *Kayakarpam*, a group of formulations aimed at rejuvenation, longevity, and disease prevention.^[1] *Kayakarpam* is classified into *Pothu karpam* for healthy individuals and *Sirappu karpam* for patients suffering from diseases. Among the plants described in Siddha literature, *Azadirachta indica* (neem, Vembu) holds a prominent place due to its wide therapeutic applications.^[2]

Neem is revered in Indian tradition and extensively cultivated across tropical regions. All parts of the tree have been used in folk and classical medicine systems

including Siddha, Ayurveda, Unani, and Homeopathy. Phytochemical studies reveal that neem contains over 135 bioactive compounds, including Azadirachtin, Nimbin, Coumarins, Tannin, Nimbidinin, Nimbidic acid, limonoids, flavonoids, and phenolic constituents, which contribute to its diverse pharmacological activities such as antimicrobial, antimalarial, antihypertensive, antioxidant, antidiabetic, anti-inflammatory, immunostimulant and anticancer effects.^{[3][4]}

In Siddha medicine, *Madhumegam* is correlated with diabetes mellitus,^[5] a chronic metabolic disorder characterized by impaired insulin secretion or action. According to the ICMR INDIAB-17 study, more than 100 million people in India are estimated to have diabetes, and another 136 million are living with prediabetes.^[6,7] Based on the International Diabetes

Federation (IDF) Diabetes Atlas 11th Edition (2025), approximately 589 million adults (1 in 9) worldwide are living with diabetes, with projections to reach 853 million by 2050.^[8] The burden of diabetes includes vascular complications, renal failure, blindness, and non-healing ulcers, underscoring the urgent need for effective and accessible therapies.

Given the accessibility and traditional use of Veppampoo, this study aims to evaluate its *in vitro* antidiabetic activity using enzymatic inhibition assays. By targeting α -amylase and α -glucosidase, key enzymes in carbohydrate metabolism, Veppampoo extracts may offer a cost-effective natural therapeutic option for managing postprandial hyperglycemia.

AIM AND OBJECTIVE

The aim of the study is to evaluate the anti-diabetic activity of Veppampoo (Neem Flower) through *in vitro* Alpha amylase and Alpha glucosidase enzyme inhibition assay.

MATERIALS AND METHODS

STUDY DRUG SELECTION AND RATIONALE

Vembu is a traditional plant used in Siddha system of medicine, Veppampoo is flower of neem, its (Anti-diabetic) *Nirizivu Pokki* action is mentioned in Siddha Pharmacopoeia of India, Part-I.^[9]

INGREDIENT

Veppampoo (*Azadirachta indica A.Juss*)- Quantity - 250gms used in this study.

RAW DRUG COLLECTION AND AUTHENTICATION

The raw drug was collected in Aringar Anna Government hospital for Indian Medicine and Homeopathy campus, Arumbakkam, Chennai-106.

The study drug was identified and authenticated by the pharmacologist of Siddha Central Research Institute (Central Council For Research In Siddha, Chennai, Ministry of Ayush, Government of India), Aringar Anna Government Hospital for Indian Medicine and Homeopathy Campus, Arumbakkam, Chennai-106.

PURIFICATION OF STUDY DRUG

After collection of Veppampoo (Neem Flower), unwanted leaves twigs were taken out, washed in water and allowed to dry completely in shade for 2 weeks.

PREPARATION OF VEPPAMPOO POWDER

The cleaned and dried Veppampoo was made into a fine powder and sieved with a white clean cloth (Vasthirakayam).



Fresh Veppampoo

Dried Veppampoo

Veppampoo Powder

Figure 1: Preparation of Veppampoo (Neem Flower) powder.

IN VITRO ANTI-DIABETIC ACTIVITY

In-vitro Alpha Amylase Inhibition Study

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 (NF) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 μ g/ml using DD water. Acarbose 100 μ g/ml used as a reference standard. About 600 μ l of test sample were added to 30 μ l of α -amylase enzyme solution and incubated at 37°C for 15 min. To this reaction mixture, 370 μ l of substrate, 2-Chloro-4-Nitrophenyl- α -Maltotrioxide (CNPG₃, 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^[10]

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

In-vitro α -Glucosidase Enzyme Inhibition Study

Test Solution: Test Sample (NF) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 μ g/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: The α -glucosidase enzyme solution was prepared by dissolving 0.5 mg α -glucosidase in 10 ml

phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin. About 10 μ l of the test sample at varying concentration along with Acarbose 100 μ g/ml used as a reference standard were added to 250 μ l of 20 mM p-nitrophenyl- α -D -glucopyranoside and 495 μ l of 100 mM phosphate buffer (pH 7.0). It was pre-incubated at 37°C for 5 min and the reaction started by addition of 250 μ l of the α -glucosidase enzyme solution prepared by 0.5 mg α -glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin, after which it was incubated at 37°C for exactly 15 min. 250 μ l of phosphate buffer was added instead of enzyme for blank. The reaction was then stopped by addition of 1000 μ l of

200 mM Na₂CO₃ solution and the amount of p-nitrophenol released was measured by reading the absorbance of sample against a sample blank (containing PBS with no sample) at 405 nm using UV visible spectrophotometer.^[11]

RESULTS

The Study Drug Veppampoo was subjected to in Vitro Anti-Diabetic Assays such as Alpha Amylase and Alpha Glucosidase Enzyme Inhibition Activities to evaluate its Percentage of Inhibition of Both Alpha Amylase and Alpha Glucosidase and to estimate its IC₅₀ values. The results are mentioned in following tables and figures.

Table 1: Percentage inhibition of NF (Veppampoo)(Neem Flower) on Alpha Amylase enzyme Inhibition Study.

Concentration (μ g/ml)	% Inhibition of NF
100 μ g/ml	14.07 \pm 2.622
200 μ g/ml	25.78 \pm 1.212
300 μ g/ml	31.88 \pm 1.898
400 μ g/ml	43.75 \pm 2.395
500 μ g/ml	50.81 \pm 3.037
Standard Acarbose	98.2 \pm 0.8246

Data are given as Mean \pm SD (n=3)

Table 2: IC₅₀ Values for Alpha Amylase Enzyme inhibition by NF and STD.

Test Drug / Standard	IC ₅₀ Value of Alpha Amylase enzyme inhibition \pm SD (μ g /ml)
NF	484 \pm 23.38
Standard- Acarbose	27.02 \pm 9.355

Data are given as Mean \pm SD (n=3)

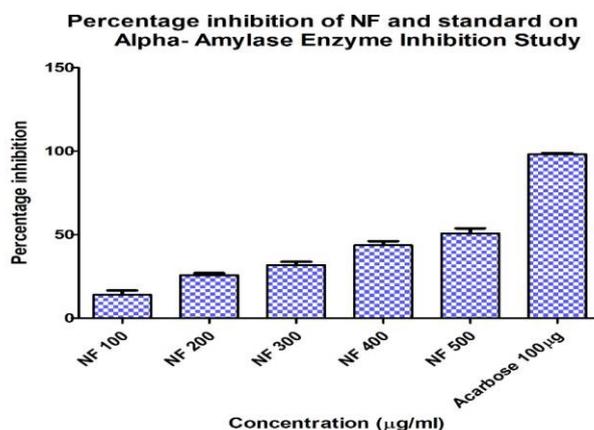


Figure 2: Percentage inhibition of Veppampoo and Acarbose on Alpha-Amylase enzyme Inhibition assay.

Table 3: Percentage inhibition of NF and STD on α -Glucosidase Enzyme Inhibition Study.

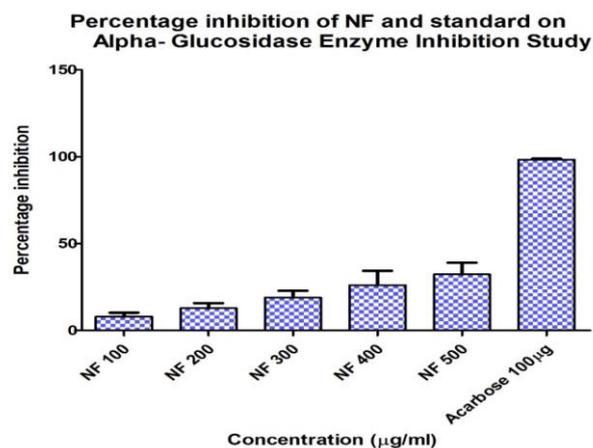
Concentration (μ g/ml)	% Inhibition of NF
100 μ g/ml	7.994 \pm 2.253
200 μ g/ml	12.86 \pm 2.875
300 μ g/ml	18.96 \pm 3.897
400 μ g/ml	26.16 \pm 8.249
500 μ g/ml	32.32 \pm 6.74
Standard- Acarbose	98.38 \pm 0.5451

Data are given as Mean \pm SD (n=3)

Table 4: IC₅₀ Values for α -Glucosidase Enzyme Inhibition Assay by NF and STD.

Test Drug / Standard	IC ₅₀ Value of α -Glucosidase enzyme inhibition \pm SD ($\mu\text{g/ml}$)
NF	822 \pm 212.6
Standard- Acarbose	25.74 \pm 18.27

Data are given as Mean \pm SD (n=3)

**Figure 3: Percentage inhibition of Veppampoo and Acarbose on Alpha- Glucosidase enzyme Inhibition assay.**

It was observed from the results of the present investigation that the Veppampoo powder shown promising alpha amylase enzyme inhibition potential with the maximum inhibition of about $50.81 \pm 3.03\%$ and the corresponding IC₅₀ is $484 \pm 23.38 \mu\text{g/ml}$. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about $98.2 \pm 0.82\%$ and the corresponding IC₅₀ is $27.02 \pm 9.355 \mu\text{g/ml}$.

It was observed from the results of the present investigation that the Veppampoo powder shown pronounced glucosidase enzyme inhibition activity with the maximum inhibition of about $32.32 \pm 6.74\%$ and the corresponding IC₅₀ is $822 \pm 212.6 \mu\text{g/ml}$. Standard acarbose exhibited significant inhibition in alpha amylase enzyme activity with the maximum inhibition of about $98.38 \pm 0.545\%$ and the corresponding IC₅₀ $25.74 \pm 18.27 \mu\text{g/ml}$.

DISCUSSION

In the management of diabetes mellitus and in prevention of secondary diabetes complications such as diabetic retinopathy, diabetic neuropathy, cardiovascular diseases, etc., most important factor considered is post prandial blood glucose level. Conventional treatments involves drugs like acarbose, migitol, voglibose which are enzyme inhibitors for controlling post prandial hyperglycemia. Though they are effective they are not desirable for long term use due to their expensive nature and their gastrointestinal side effects.^[12] Prospective inhibitors against postprandial hyperglycemia should have the ability to inhibit both alpha amylase and alpha glucosidase with higher percentage of inhibition and lower IC₅₀ values. Therefore, in this study Veppampoo (Neem Flower) was tested invitro for its ability to inhibit both alpha amylase and alpha glucosidase enzymes.

Various studies had been undergone to find out the mechanism of action, through which the Veppampoo (Neem Flower) shows its anti-diabetic activity. It is found that Veppampoo (Neem Flower) extracts inhibit the enzyme alpha-glucosidase, increase insulin sensitivity, allowing glucose to enter cells more efficiently, reducing blood sugar levels, protect pancreatic beta-cells from damage, preserving insulin secretion and reducing the risk of developing diabetes.^[13,14] Animal studies have demonstrated the anti-diabetic effects of Veppampoo (Neem Flower) extracts, including reduced blood sugar levels, improved insulin sensitivity, increased glucose uptake in muscles, protection against pancreatic beta-cell damage.^[15] The enzymes Alpha amylase aids in starch digestion and converts them to maltose. Similarly, the enzyme alpha glucosidase expressing at brush borders of small intestines involved in the conversion of maltose and sucrose into glucose and fructose. It plays crucial role in monitoring postprandial blood glucose levels in human. Compounds that inhibit alpha glucosidase activity may possess better regulation in controlling postprandial hyperglycemia and considerably used for the treatment of type II diabetes mellitus. Postprandial hyperglycemia are an important contributor to glycemic control.

In the present study, calibration plot sequence of Veppampoo reveals alpha amylase inhibition potential with an IC₅₀ value of $484 \pm 23.38 \mu\text{g/ml}$ when compared to that of acarbose with an IC₅₀ value of $27.02 \pm 9.355 \mu\text{g/ml}$. And alpha glucosidase inhibition potential with an IC₅₀ value of $822 \pm 212.6 \mu\text{g/ml}$ when compared to that of acarbose with an IC₅₀ value of $25.74 \pm 18.27 \mu\text{g/ml}$. Therefore this study highlighting the therapeutic potential of traditional Siddha medicine in diabetes management.

CONCLUSION

Many scientific reports support the hypoglycemic activity of *Azadirachta indica* in diabetes is known. Numerous compounds have been isolated from different parts of neem tree. The various studies on the bioactive secondary metabolites of *Azadirachta indica* had shown its potential anti-diabetic activity. In this study, Acarbose showed 98% inhibition of alpha amylase and alpha glucosidase under same experimental conditions, Veppampoo showed enzyme inhibition and IC50 but was not par with acarbose.

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Conflicts of interest

There are no conflicts of interest.

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