# IN- VITRO ANTIDIABETIC ACTIVITY OF NUTRACEUTICAL POWDER FORMULATION (NPF 30)

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## Abstract

**In accordance with the 3Rs as outlined by the ICH, which outline the replacement, reduction, and refinement of the use of animals in research, large-scale in-vitro assays have been developed and validated for early-stage screening aimed toward filtering out molecules with the potential for Pharmacological and toxicological screening. The goal of the current study is to evaluate Nutraceutical Powder formulation (NPF 30) for potential -amylase and -glucosidase inhibitory activities, glucose uptake potential in yeast, glucose adsorption activity, nitric oxide and DPPH scavenging activity.Different concentrations of the Nutraceutical Powder Formulation (NPF 30) were used in the current study. The concentrations of 20, 40, 60, 80, and 100 g/ml are examined for a variety of in-vitro experiments using the appropriate target chemicals. Comparable to the commercially available substance, acarbose, a statistically significant substantial dose dependent inhibition of the enzymes was observed. NPF 30 and acarbose were reported to have IC 50 values on fungus - Amylase of 84.14 g/ml and 65.38 g/ml, respectively. NPF30 and acarbose were found to have IC 50 values on -glucosidase of 94.22 g/ml and 53.58 g/ml, respectively.As a result of its substantial glucose absorption capacity and complementing glucose adsorption activity, NPF 30 has the potential to lower postprandial hyperglycemia.Furthermore, as oxidative stress plays a vital role in treatment objectives and is established by the NO and DPPH assays, antioxidant potential can slow the progression of disease. The study's findings make it very evident that Nutraceutical Powder.**

**Key words:** Postprandial hyperglycemia, Nutraceutical Powder formulation (NPF 30), In-vitro pharmacological screening -amylase, -glucosidase, anti-diabetic, IC 50,

## 1)Introduction: -

Diabetes, a disease of modern society that has a negative impact on people's quality of life everywhere, requires intensive research to find molecules that prevent and retard the progression 3. Diabetes mellitus is a significant long-term metabolic condition that affects how proteins, fats, and carbohydrates are metabolized. It comprises a class of metabolic illnesses known as hyperglycemia, in which blood sugar levels are raised due to either insufficient insulin production by the pancreas or improper cell response to the released insulin. Diabetes comes in three different kinds. Type 1, type 2, and gestational diabetes are the three primary kinds of the disease. Diabetes can strike either a woman or a

man at any age[1]. Due to the fact that monosaccharides are the type of carbohydrates that are absorbed via the mucosal border in the small intestine, the inhibitory activity of these enzymes results in a fall in blood glucose levels. Inhibiting the activity of the -amylase enzyme, which is responsible for the breakdown of starch into more simple sugars (dextrin, maltotriose, maltose, and glucose), is another efficient way to manage diabetes [2]. A-amylase inhibitors, which limit the rate of glucose absorption and maintain the serum blood glucose in hyperglycemic people, contribute to this [3].

Drugs influence these pathways by interacting with specific molecules along the pathway, either increasing or decreasing their activity or completely altering them. Finding a novel therapeutic agent to treat a complex disease is a difficult task that requires the application of a variety of computational, experimental, translational, and clinical models.The gift sample of Nutraceutical Powder Formulations (NPF20) provided by local yoga and ayurvedic practioners is composed of Jowar, foxtail mellet ,Little mellet ,finger mellet saggu bhiyyam, barle bhiyyam, barnayard mellet , proso mellet, green gram, soyabean, black eyed beans, bengal gram, horse gram, red gram, black gram,toor dal, maize, sunflower seeds,flax, seeds pumpkin seeds,fenugreek,almons,ground nut,cashew nut, dry dates, elachi, pepper, [4].This powdered formulation's in-vitro anti-diabetic diabetes activity was assessed.DPPH and NO radical scavenging assays were used to measure the NPF 20's effects on alpha-amylase, alpha-glucosidase inhibition, glucose uptake potential, glucose adsorption activity, and anti-oxidant activity with the goal of promoting its regular inclusion in our diets to have both preventive and therapeutic effects on diabetes and related metabolic disorders.

## 2) MATERIALS AND METHODS: -

**Procedure**:Following Steps are involved in the formulation of NC30 nutraceutical formulation.

### 2.1 Procurement of high standard raw materials:

When sourcing raw material, the most important step in sourcing good products is to get safe and high-quality raw material. [5].

### 2.2 Separation:

Raw material separation processes are technical methods used to separate products from impurities or other products. Separation can also include separation of dust from recycled materials. Removal of the foreign body, if present any.

### 2.3 Weighing:

Weighing Consider each material separately. Transfer to suitable containers such as steel, aluminium, pin go or plastic containers.

### 2.4 Soaking/Dipping:

This is the process of moistening and softening the seeds to facilitate the removal of the seed coat. This is the intake of water to activate the germinal process in the nucleus. Soak the materials by adding a measured amount of solvent (water). Overnight /12-24 h.

### 2.5 Drying:

Drying is a mass transfer process that involves the removal of water by evaporation. Drain the solvent using a filter device such as a steel strainer / meat cloth / coconut cloth.

**2.6 Spreading:**

Spread the material evenly on a wide cloth to dry or air dry. Turn the material every 3-6 hours and let it dry. Check the humidity regularly. After complete drying, collect all materials in suitable containers.

Heat the materials at the appropriate temperature. Weigh the given amount of material and transfer it to a heating vessel with a low flame. Transfer to a suitable container and leave to cool.

**2.7 Pulverising/grinding:**

Weigh the material and grind/grind to a coarse powder. We sift them together and collect and weigh the raw powder. And transfer them to airtight container.

## METHODS :

Purchase of Nutraceutical Formulation NPF30: The Powder Formulation Was Provided By Our Principal PROF. Chakka Gopinath-M.Pharm.

**2.8 Bernfeld et al.'s "-Amylase inhibition assay by DNSA method"**

Multiple steps are involved in the digestion of starch in humans. Initial breakdown of polymeric substrates into shorter oligomers is caused by partial digestion by salivary amylase. These are later further degraded into maltose, maltotriose, and tiny malto-oligosaccharides in the stomach by pancreatic -amylases. Maltose, a dietary starch, is hydrolyzed by the digestive enzyme (-amylase), converting it into glucose before being absorbed.The quantification of the reducing sugar (maltose equivalent) released by the hydrolysis of starch under test conditions is used to determine the inhibition of -amylase, and it is expressed as a decrease in the amount of maltose released. By hydrolyzing starch in the presence of the -amylase enzyme, alpha-amylase activity can be evaluated in vitro.The monosaccharide hydrolysis of starch caused by enzymes is inhibited, as seen by the orange yellow color's diminished intensity. In other words, the amount of orange-yellow color in the test sample is closely correlated with the activity of the enzyme that inhibits amylase [6].Ebrahimzadeh- Attari et al

**2.9. Studies on the uptake of glucose by** Yeast cells[7].**Materials:**

Glucose 25 mM was used as the substrate. The positive control was metronidazole (Flagyl, Pfizer).Commercial baker's yeast was purchased from a nearby bakery and used to analyze glucose uptake while being affected by FBPRD and the common medication metronidazole. Repeated centrifugation at 3000 rpm for 5 minutes in distilled water was used to repeatedly wash the yeast until the supernatant liquids were clear. In distilled water, a 10% (v/v) yeast suspension was made. To 1 mL of 25 mM glucose solution, different doses of FBPRD (25, 50, 100, 200, 400, and 800 g/mL) were added. The mixture was then incubated at 37 °C for 10 minutes. Adding 100 L of yeast suspension to the reaction, vortexing it, and then continuing to incubate it at 37 °C.There were three duplicates of each experiment.The following formula was used to determine the percentage increase in glucose absorption by yeast cells:

$$\text{Increaseinglucoseuptake}(\%) = \frac{\text{absorbance(Sample)} - \text{Absorbance(Control)}}{\text{Absorbance(Sample)}} \text{ fX } 100$$

**2.10.Assay for glucose adsorption:**

The Ou et al. method was used to calculate the extract's capacity to adsorb 102 glucose molecules. 100 mL of a glucose solution with five different concentrations (5, 10, 15, 20, and 25 mM) were mixed with approximately 10 ml of FBPRD. Each of these combinations was thoroughly combined, agitated, then incubated for six hours at 37 degrees Celsius in a shaker water bath, respectively. After

incubation, the mixture was centrifuged at 4800 rpm for 20 minutes, and then, using a glucose oxidase peroxidase testing kit, the glucose content in the supernatant was ascertained. The formula G1-G6, where G1 is the glucose concentration in the initial solution and G6 is the glucose concentration after 6 hours, was used to calculate the quantity of bound glucose.

Glucose adsorbed = Initial Glucose concentration- Glucose concentration after 6 Hours

**2.11.DPPH radical scavenging activity**[8].**:**

The reduction of DPPH, a stable free radical, is the basis for the DPPH test method. The highest absorption of the free radical DPPH with an odd electron occurs at 517 nm (purple color).When antioxidants react with DPPH, it pairs off in the presence of a hydrogen donor (such as an antioxidant that scavenges free radicals) and is reduced to the DPPHH, which lowers its absorbance relative to DPPH. Yellow coloration is the product of decolorization, which is radical to the DPPH-H form. The potential to reduce is greater the more coloration there is. The most often used method for assessing a novel drug's capacity to scavenge free radicals is this test. When a substance's solution and a solution of DPPH are combined that can donate a hydrogen atom, leading to the reduced form (Diphenylpicrylhydrazine; non radical), which loses the violet color (although there should still be some trace amounts of the picryl group's pale yellow color).

**Procedure:**

To make DPPH solution, 3.3 ml of methanol was mixed with 4.3 mg of 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the test tubes were covered with aluminum foil to keep light out. 3 ml of methanol was added to 150 l of DPPH solution, and an instantaneous absorbance measurement was made at 517 nm for the control reading. The volume was consistently increased to 150 l using methanol after 50 pi of various concentrations of the plant sample extracts (40, 80, 120, 160, 200, 240, 280, 320, and 360 ug/ml) and the standard chemical (Ascorbic acid) were taken. Following a further methanol dilution of each sample to a final volume of 3 ml, 150 l of DPPH was added to each tube. At 517 nm, absorbance was measured after 15 minutes with methanol serving as the blank.

% scavenging = Absorbance of control - Absorbance of test
sampleAbsorbance of control] X 100

According to a dose-response curve with the percentage of inhibition and concentrations shown on it, linear regression analysis was used to determine the effective sample concentration (IC50 value) needed to scavenge the DPPH radical by 50%.calculating the 50% inhibitory concentration (IC50) for an enzyme An indicator of a substance's ability to block a particular biological or biochemical activity is the half maximum inhibitory concentration (IC50). This quantitative measurement shows the amount of a specific medicine or other substance (inhibitor) required to completely stop a given biological process or component of a process, such as an enzyme, cell, cell receptor, or microbe. It is frequently applied as a pharmacological researcher's gauge of antagonist medication potency. the FDA, IC50 denotes the amount of a medicine that will produce 50% of the desired effect.

$$IC_{50} = \frac{50 - A}{B - A} \times (D - C) + C$$

**Where**

A =Percentage of inhibition, that is immediately less than50%

B = Percentage of inhibition, that is immediately greater than or equal to
50%C = The concentration of inhibitor that gives A% inhibition
D = The concentration of inhibitor that gives B % inhibition

## 3)RESULTS & DISCUSSION:

**1.In- vitro assay of α-amylase inhibition by Acarbose ( DNSA method)**

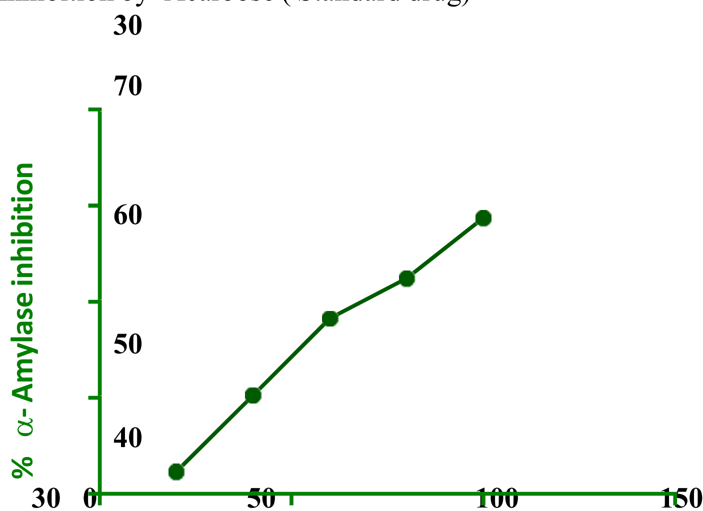
**Table`3.1 :Percentage inhibition of α-amylase activity by Acarbose**.

| S.No. | Concentration of Acarbose (µg/ml) | % α-amylase inhibition |
|---|---|---|
| 1 | 20 | 32.347±0.06 |
| 2 | 40 | 40.270±0.116 |
| 3 | 60 | 48.287±0.183 |
| 4 | 80 | 52.450±0.275 |
| 5 | 100 | 58.753±0.068 |

Acarbose has also shown dose dependent inhibition of of α- amylase inhibition of 32.52%,4027%,48.28%,52.45% and 58.75% at the concentration ranges of 20, 40,60,80 and 100µg/ml respectively.

**Graphical representation of α-amylase inhibitory activity by Acarbose**

% -Amylase Inhibition by Acarbose ( Standard drug)




**1.1 Concentration of Acarbose (g/ml)**

**3.2. Effect of NPF(30) incubation on in -vitro glucose adsorption capacity**

| S. No. | Concentration of Glucose (Mm/L) | Glucose Adsorption Capacity(mg/dL) |
|---|---|---|
| 1 | 5 | 6 |
| 2 | 10 | 8 |
| **3** | **15** | **16** |

| 4 | 20 | 19 |
|---|---|---|
| 5 | 25 | 19 |

**Graphical representation of glucose adsorption capacity by NPF(30):**

NPF(30) affects the in vitro adsorption of glucose. The current study's findings showed that the extract had a sizable capacity for glucose adsorption at all tested doses. Furthermore, the test sample's capacity to absorb glucose increased directly with its molar concentration. Therefore, the minimum and maximum glucose adsorption levels were 5 mM and 25 mM, respectively. Additionally, it was demonstrated that the test extract has the ability to bind glucose even at lower concentrations. Further research is needed to determine whether the NPF 30's adsorption ability is caused by the soluble and insoluble dietary fibers found in juice, which may reduce the postprandial rise in blood glucose levels.

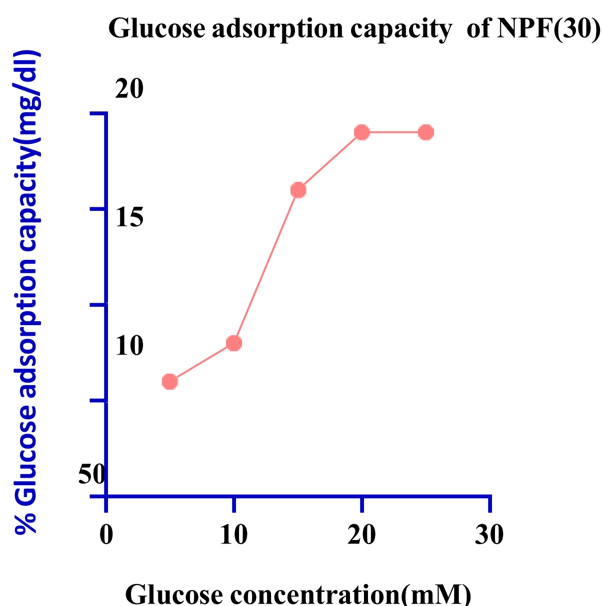



**Fig No 3.1 Glucose adsorption capacity by NPF(30)**

**3. 3.DPPH scavenging assay by NPF(30) & Ascorbic acid :**

| S. No. | Concentration of sample (µg/mL) | % DPPH scavenging by ASCORBIC ACID | % DPPH scavenging by NPF(30) |
|---|---|---|---|
| 1 | 40 | 43.35±0.45 | 34.36±3.21 |
| 2 | 80 | 51.52 ±0.32 | 46.25± 2.35 |
| 3 | 120 | 58.25±1.23 | 48.76± 2.56 |
| 4 | 160 | 70.85 ±2.21 | 54.65±5.43 |
| 5 | 200 | 78.41±3.25 | 64.52±2.34 |
| 6 | 240 | 86.43±4.26 | 71.65±0.65 |

| 7 | 280 | 96.43±4.32 | 82.34±2.54 |
|---|---|---|---|
| 8 | 320 | 98.29±2.54 | 84.32± 3.45 |
| 9 | 360 | 98.63 5.63± | 84.76± 2.65 |

**DPPH scavenging assay NPF( 30) & Ascorbic acid**

Graphical representation of DPPH scavenging assay by NPF (30) & Ascorbic acid

DPPH Scavenging activity of N P F ( 3 0 ) vs ASCORBIC ACID

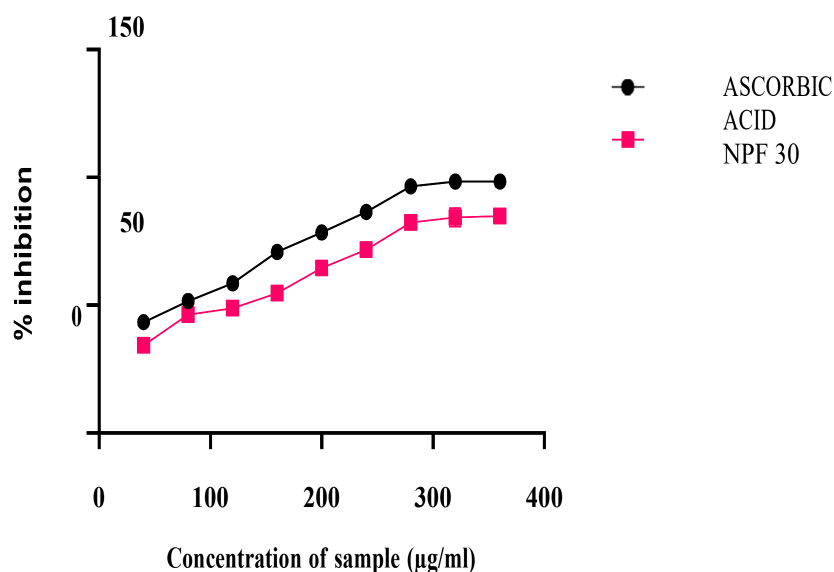



**Fig No4.12 DPPH scavenging Activity of NPF 30) & Ascorbic acid**

The earliest method for determining the antioxidant capacity of a substance, an extract, or other biological sources is the, -diphenyl--picrylhydrazyl (DPPH) free radical scavenging method created by Blois (1958). A stable free radical with a nitrogen core called DPPH emits the color violet. With the addition of the fractions, it was converted to a yellow-colored product called diphenylpicryl hydrazine in a concentration-dependent manner. The decrease in the amount of DPPH molecules is related to the availability of hydroxyl groups. When compared to Ascorbic acid, the DPPH scavenging activity was dramatically reduced in FLJMS, but at a relatively low percentage.

## 4.CONCLUSION

According to historical records, several plant foods contain pharmacologically active chemicals at levels high enough to provide a drug-like effect when ingested in moderation. In line with the numerous in-vitro pharmacological assays that produced statistically significant results with regard to in-vitro anti diabetic assays, numerous search engines and databases, including Google Scholar, ScienceDirect, PMC, Research Gate, and Scopus, also asserted the promising therapeutic potential of NPF 30.However, these

studies are insufficient to support the claim, so a thorough, stringent battery of pharmacological, photochemical, and bioanalytical studies, followed by observational studies in humans, must be conducted to bolster the alleged dictum of Hippocrates, the father of medicine, who is credited with saying, "Let food be your medicine and thy medicine be food," in 400 BC.

## 5.Acknowledgement: -

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