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In-vitro exploration of anti-oxidant and anti-diabetic properties of *Ipomea nil*

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ABSTRACT

This study aims to evaluate the antioxidant and *in-vitro* anti-diabetic potential of hydroalcoholic extract of *Ipomea nil*. Shade dried plants of *Ipomea nil* were processed for hydroalcoholic extraction. Further, they were processed for preliminary phytochemical evaluation followed by quantitative evaluation of phenolic compounds, flavonoids and alkaloids by colorimetric method. Anti-oxidant studies were investigated by 1,1-diphenyl-2-picrylhydrazyl, ferric reducing method and thiobarbituric acid method. In-vitro anti-diabetic activity was evaluated by measuring alpha-glucosidase inhibition, alpha-amylase inhibition and membrane filter method. Various phytoconstituents, phenols, flavonoids, and alkaloids were majorly present. Some of the phytochemical constituents were further quantified as 2.76mg/GAV/g of dried drug powder and 7.11mg QEV/g of dried plant. The scavenging activity of the extract was expressed as percentage inhibition and compared with standard antioxidant ascorbic acid. The IC_{50} value of ascorbic acid was found to be 12 ± 1.13 μg/ml but whereas the extract exhibited 20 ± 2.14 μg/ml. The inhibitory activity on alpha-amylase varied from 24 to 82% in the concentration range of 20 to 100μg/ml compared to standard drug acarbose. Due to its inhibitory effect on alpha-glucosidase and alpha-amylase enzymes, it can be used as an anti-diabetic drug in the long run with a controlled diet. But still, detailed animal studies are required for its anti-diabetic potential and toxicity profile to be evaluated in detail before proceeding for human consumption.

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INTRODUCTION

Herbs are an important part of the drug delivery system. From ancient times they have been part of this integral medicinal domain. Some plants well known for their curative powers are used as a treatment regime for a spectrum of diseases, from dengue to malaria (Khan *et al.*, 2016). Diabetes a so-called disorder with an abnormality of body metabolism due to high blood glucose levels. Often the condition worsens up as part of a chronic disorder known as post diabetic complications. There are so many drugs available for the treatment of diabetes

in allopathy, ayurveda, Unani, Siddha, homeopathy and modern medicine (Pal *et al.*, 2014).

The problems of hyperglycemia are increasing day by day and remain the main cause of changing the human lifestyle (Chaudhary, 2011). Hyperglycemia is associated with so many complications (Pal, 2015). These traditionally important plant species have anti-diabetic properties by lowering blood glucose levels and oxidative stress while also upregulating insulin and glucose transporter protein expression in the pancreas (Panda, 2004). In this regard, the species of Ipomea nil (Convolvulaceae) are the potential natural source to cure a global problem of hyperglycemia (Naomi *et al.*, 2021). *Ipomoea nil* is commonly understood as species of *Ipomoea* morning glory known by several common names (Akhtar *et al.*, 2018). The flowers open in the morning and close in the afternoon (Tahir *et al.*, 2018).

This plant is abundantly found in the forest region of the Chittoor district of Andhra Pradesh. Various works are reported with this medicinal plant, such as anti-microbial, dyslipidemia, and anti-diabetic anti-oxidant activities (Li *et al.*, 2009). Although numerous fascinating pharmacological characteristics of this plant's anti-oxidant, anti-inflammatory, and anti-microbial capabilities have been revealed in prior investigations (Hasanpour *et al.*, 2020). The activity of the α-amylase enzyme can be evaluated in vitro by hydrolyzing starch in the presence of the enzyme. This process was measured using iodine, which generates a blue tint when combined with starch. In other words, α-amylase activity is proportional to the blue colour intensity in the test sample.

As there were no detailed studies available for ipomea nil and its anti-diabetic potency, so this study has been designed to explore the anti-oxidant as well as anti-diabetic potency of hydro-alcoholic extract of ipomea nil. This study evaluates the inhibitory effect of this extract on the levels of alpha-amylase and alpha-glucosidase enzyme levels, being an indicator of anti-hyperglycemic activity.

MATERIALS AND METHODS

Plant material

Ipomea nil, a member of the Convolvulaceae family, was collected from a forest in Andhra Pradesh's Chittoor district. The plant was validated by Dr K Madhava Chetty (Voucher number 1005), Assistant Professor of Botany at Sri Venkateswara University in Tirupathi.

Qualitative phytochemical screening

Estimation of Total phenolic content

The total phenolic content was calculated using Folin Ciocalteu's technique. Add standard gallic acid of rising concentrations to 1 ml of extracts in each test tube and mark them with respect to the amounts added. Add 5 mL distilled water and 0.5 ml Folin Coicalteu's reagent to each test tube before shaking to mix the contents thoroughly. After 5 minutes, add 1.5 ml of 20 percent sodium carbonate to these test tubes and top up with distilled water to make the volume 10 ml.

After 2 hours of room temperature incubation, the mixture produces strong blue colour. Measure the absorbance of the extracts in triplicates after incubation at a 750 nm spectrophotometer using UV visible. Estimate the blank and gallic acid as the standard using reagent blanks with solvent. Using the standard gallic acid, plot the calibration curve. The findings, including total phenolic content data for polyherbal formulations, are reported in "mg of gallic acid equivalent weight" (GAE).

Estimation of total flavonoid content

The total flavonoid content was evaluated using a colorimetric assay. 1 ml of standard quercetin solution of increasing concentration was added to each 1 ml aliquot and test tubes were labelled accordingly. 4 mL distilled water and 0.3 mL 5 percent sodium nitrite solution was added to the combinations and left aside for 5 minutes. After 5 minutes, 0.3 ml of 10% aluminium chloride and 2 ml of 1 M sodium hydroxide were added at the 6-minute mark. Containers were filled with distilled water to 10 ml and stirred thoroughly. A yellowish-orange color developed as a result of this. Using a UV-visible spectrophotometer, the absorbance was measured at 510 nm. Quercetin was used as a standard (Vijayalakshmi *et al.*, 2014).

The tests were carried out in triplicates, with a calibration curve drawn using standard quercetin. "mg of quercetin equivalents/100 g of dry mass" is how total flavonoids in polyherbal formulations are expressed.

Determination of antioxidant activity

DPPH free radical scavenging assay method

The DPPH test was used to determine the extract's ability to scavenge free radicals using the Burits and Bucar technique. Add newly made 1 ml DPPH solution (0.004 percent w/v in 99 percent ethanol) to a 3 ml sample (100 g/ml ethanol) and incubate the combination in the dark at room temperature for 20 minutes. The mixture was vortexed after the incubation period and the absorbance was measured with a spectrophotometer at 517 nm. Ascorbic acid was employed as a reference, while 99 percent ethanol

Table 1: Phytochemical results of hydroalcoholic extract of *Ipomea nil*

Sl. No.	Phytochemical Test	Result
1	Test for tannins	Positive
2	Test for saponin's	Positive
3	Test for flavonoids	Negative
4	Test for steroids	Negative
5	Test for alkaloids	Positive
6	Test for phenol	Positive
7	Test for glycoside	Positive

Table 2: Effect of hydroalcoholic extract of *Ipomea nil* on DPPH scavenging activity

	DPPH scavenging activity	
Drug Conc. (μg/ml)	Percentage inhibition of scavenging activity of test drug	Percentage inhibition of scavenging activity of Std. drug
20	18.4±0.0030	9.708±0.0058
40	19.41±0.0026	10.67±0.0057
60	20.40±0.0037	14.56±0.0021
80	23.38±0.0042	15.53±0.0025
100	27.18±0.0047	20.38±0.0073

Values are expressed as mean ± S. D; n=3; IC_{50} =12±2.14 (Ascorbicacid); IC_{50} =20±1.13(*Ipomea nil* extract)

Table 3: Effect of hydroalcoholic extract of *Ipomea nil* on ferric reducing method

	Ferric reducing ion method	
Drug Conc. (μg/ml)	Percentage inhibition of scavenging activity of test drug	Percentage inhibition of scavenging activity of Std. drug
20	42.39±0.258	13.38±0.025
40	47. 60±0.137	32.99±0.029
60	57. 90±0.248	60.36±0.065
80	62.81±0.249	61.38±0.067
100	65.20±0.137	68.53±0.106

Values are expressed as Mean ± S. D; n=3; IC_{50} = 45±2.13(Ascorbic acid); IC_{50} = 58± 1.69 (*Ipomea nil* extract)

Table 4: Effect of hydroalcoholic extract of *Ipomea nil* on Thiobarbituric acid

	Thio Barbituric Acid Method	
Drug Conc. (μg/ml)	Percentage inhibition of scavenging activity of test drug	Percentage inhibition of scavenging activity of Std. drug
20	27.21±0.058	13.63±0.005
40	45.40±0.030	22.21±0.055
60	54.50±0.015	22.72±0.074
80	62.51±0.052	27.27±0.085
100	86.3±0.066	38.26±0.106

Values are expressed as Mean ± S. D; n=3; IC_{50}=22±1.01(Ascorbic acid); IC_{50} = 50±1.89 (*Ipomea nil* extract)

Table 5: Effect of hydroalcoholic extract of *Ipomea nil* on alpha amylase inhibition activity

Inhibition of alpha amylase activity		
Drug Conc. (μg/ml)	Percentage inhibition of alpha amylase activity of test drug	Percentage inhibition of alpha amylase activity of Std. drug
20	20.81±1.09	36.22±0.89
40	33.1±1.10	42.16±0.87
60	58.26±0.86	47.21±0.65
80	80.87±0.64	51.51±0.83
100	84.66±0.95	66.7±0.77

Values are expressed as Mean ± S.D; n=3; IC_{50}=63.5±2.01(Acarbose); IC_{50} = 53.6±2.95(*Ipomea nil* extract)

Table 6: Effect of hydroalcoholic extract of *Ipomea nil* on alpha glucosidase inhibition activity

Inhibition of alpha glucosidase activity		
Drug Conc. (μg/ml)	Percentage inhibition of alpha glucosidase activity of test drug	Percentage inhibition of alpha glucosidase activity of Std. drug
20	23.96±1.09	4.12±0.89
40	34.7±1.10	4.9±0.87
60	56.1±0.86	28.26±0.65
80	80.99±0.64	44.87±0.83
100	84.26±0.95	70.66±0.77

Values are expressed as Mean ± S. D; n=3; IC_{50}= 82.46±1.01(Acarbose); IC_{50} = 52.81±1.95(*Ipomea nil* extract)

was used as a blank. The formula used to calculate free radical scavenging activity is

% Inhibition=

$$\frac{(Absorbance\ of\ control - Absorbance\ of\ sample) \times 100}{Absorbance\ of\ control}$$

Ferric reducing assay method

The in vitro antioxidant test was done using the ferric reducing antioxidant power method. In a water bath, 1 ml of 0.2 M phosphate buffer pH 6.6 and 1 ml of 1 percent potassium ferricyanide were added to the 2.5 ml of extract and incubated for 20 minutes at 50°C. After that, the liquid was rapidly chilled 2.5 mL of 10% trichloroacetic acid was added and centrifuged for 10 minutes. 2.5 mL of aliquot after pipetting was mixed in 2.5 mL distilled water and 0.5 mL ferric chloride solution at 0.1 percent. 10 minutes after setting it aside, the color of the mixture turned green before measuring the absorbance. Absorbance was measured at 593 nm with a UV-visible, and blank was performed with the reagent blank and solvent, the standard being Ascorbic acid. Tests were done in a set of threes.

Thiobarbituric acid method

To 1 ml of sample solution, add 2 ml of 20% trichloroacetic acid and 2 ml of 0.67 percent 2-thiobarbituric acid according to the FTC technique. Place the mixture in a boiling water bath for a few minutes before removing it to cool. Centrifuge the mixture at 3000 rpm for 20 minutes after it has cooled. The absorbance of the supernatant was measured at 552 nm. The absorbance on the final day of the FTC technique was used to determine antioxidant activity.

In-vitro and diabetic activity

α-amylase inhibition method

The DNS (3, 5 dinitrosalicylic acids) technique was used to assess the inhibitory activity of α-amylase. This approach measures the amount of reducing sugar (glucose equivalent) released during the experiment. The enzyme inhibitor activity is measured by the decrease in units of glucose liberated. Add 1 ml of 1 unit PPA (Porcine Pancreatic alpha-Amylase) enzyme to various plant extract quantities (varying from 0.2 to 1 mg/ml) and incubate the mixture at 37°C for 30 minutes. After 10 minutes of incubation, add 1 mL of 1 percent buffered starch and continue to incubate at room temperature for another 10 minutes. To stop the reaction, add DNS reagent and heat the mixture for 5 minutes in a boiling water bath. Replace the plant extract and enzyme with an equal amount of phosphate buffer to make the blank. The control was an enzyme solution without plant extract, with 100 percent enzyme activity. The absorbance was measured using a UV Spectrophotometer at 540 nm. The anti-diabetic medication Acarbose was employed as a positive control. Antidiabetic efficacy was mea-

sured by inhibiting α-amylase and represented as a percentage of inhibition.

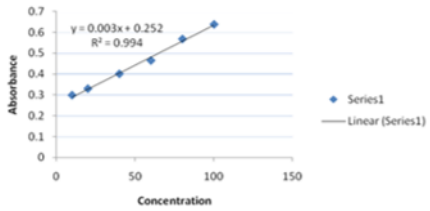

Figure 1: Standard curve of Gallic acid

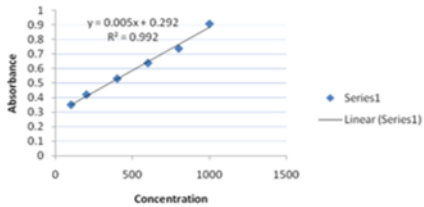

Figure 2: Standard curve of Quercetin

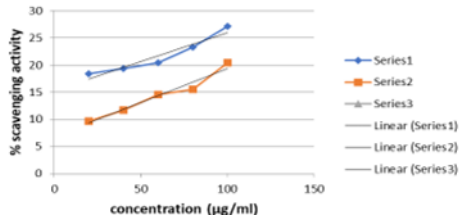

Figure 3: Effect of hydroalcoholic extract of *Ipomea nil* on DPPH scavenging activity

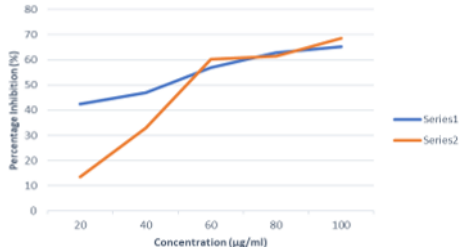

Figure 4: Effect of hydroalcoholic extract of *Ipomea nil* on Ferric reducing ion method

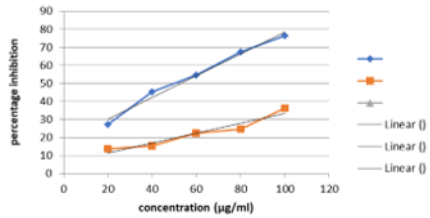

Figure 5: Effect of hydroalcoholic extract of *Ipomea nil* on TBA method

α-glucosidase inhibition method

Various quantities of ethanolic plant extracts were made, and 1 ml of starch substrate solution (2 percent w/v sucrose) and 0.2 M Tris buffer pH 8.0 were

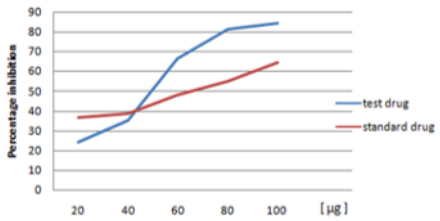

Figure 6: Effect of hydroalcoholic extract of *Ipomea nil* on alpha amylase inhibition activity

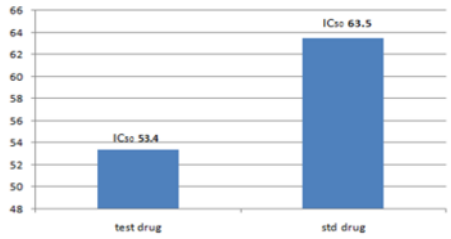

Figure 7: Effect of hydroalcoholic extract of *Ipomea nil* on alpha amylase inhibition activity [IC_{50}]

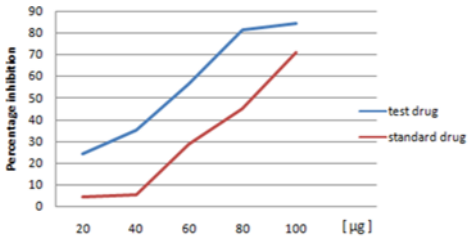

Figure 8: Effect of hydroalcoholic extract of *Ipomea nil* on alpha glucosidase inhibition activity

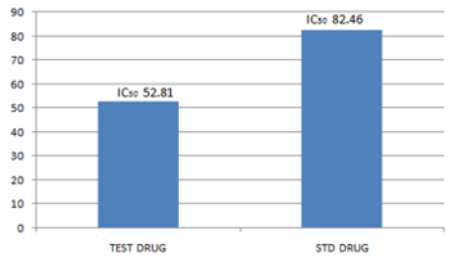

Figure 9: Effect of hydroalcoholic extract of *Ipomea nil* on alpha glucosidase inhibition activity [IC_{50}]

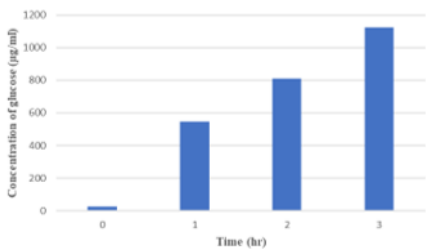

Figure 10: Effect of hydroalcoholic extract of *Ipomea nil* on glucose diffusion assay

added to test the inhibitory activity of the alpha-glucosidase enzyme. The concentrate mixes were then incubated at 37°C for 5 minutes. Add 1 ml of -glucosidase enzyme (1U/ml) to the concentrate mixtures after incubation and incubate for another 40 minutes at 35°C. 2 ml 6N HCl was added to finish the reaction (Singh *et al.*, 2016).

Membrane penetration method

15 ml of a glucose and sodium chloride (0.15 M) solution was placed in a dialysis tube (6 cm15 mm) and the presence of glucose in the external solution was assessed to see if extracts could prevent glucose diffusion into the external solution at defined time intervals. The sealed tube was introduced into a centrifuge tube holding 45 ml of 0.15 M NaCl, and then the entire centrifuge tube was placed on an orbital shaker at room temperature. The glucose concentrations were determined using a glucose oxidase kit method, and the incremental areas under the glucose curves were calculated using the trapezoidal rule (AUC).

% Increase in glucose uptake =

$$\frac{(Absorbance\ of\ control - Absorbance\ of\ sample) \times 100}{Absorbance\ of\ control}$$

Data analysis

All samples were analysed in triplicate and the results were expressed as mean±S.D. using SPSS software.

RESULTS AND DISCUSSION

Qualitative phytochemical investigation

TPC= C*V/m

C= Concentration

V=Volume taken for analysis

m= mass of drug taken for extraction

=2.76mg/GAV/g of dried drug powder

Total phenolic content estimation

TFC= C*V/m

C= Concentration

V=Volume taken for analysis

m= mass of drug taken for extraction

= 7.11mg QEV/g of dried plant

Extract of *ipomea nil* was obtained using ethanol: water (70:30). Followed by filtration, condensed to drying to obtain crude extracts. To identify the phytoconstituents, various chemical tests were done using established techniques. The presence of numerous plant elements was discovered using these screening methods. Phenols, flavonoids, and alkaloids were abundant among the numerous phytoconstituents (Table 1). These phytochemicals were measured as 2.76 mg/GAV per gram (Figure 1) of dried drug powder and 7.11 mg QEV per gram (Figure 2) dried plant.

Antioxidant activity of the crude extracts was investigated by the DPPH scavenging method (Table 2), ferric reducing assay method (Table 3, Figure 4) and thiobarbituric acid method (Table 4, Figure 5). The scavenging activity of the extract was expressed as percentage inhibition and compared with standard antioxidant ascorbic acid (Figure 3). The IC_{50} value of ascorbic acid was found to be 12±1.13 μg/ml but whereas the extract exhibited 20±2.14 μg/ml. The results of antioxidant activity revealed percentage inhibition of DPPH radicals in the particular concentration. Many studies have described the antioxidant activity of medicinal plants as co-related to the presence of polyphenolic compounds. Natural anti-oxidants are well known for their important role in preventing geriatric disorders. But due to the complexity of the anti-oxidants mechanism, it is always preferred to approach multi method analysis to test the potency. The anti-oxidant activity of anti-oxidants is solely dependent on total phenolic components.

In vitro, the inhibitory activity of the α-amylase enzyme can be assessed by hydrolysis of starch in the presence of the α-amylase enzyme. The process is measured with iodine, which creates a blue tint when added to starch. As a result, the intensity of the blue hue in the test sample is directly proportional to α-amylase activity. α-amylase is an enzyme that hydrolyzes alpha-bonds in alpha-linked polysaccharides like starch to produce a high concentration of glucose and maltose. α-amylase inhibitors bind to the α-bond of polysaccharides and prevent the carbohydrate from being broken down into mono and disaccharide. For all of the solvent extracts of the selected plants, and in vitro inhibitory test of α-amylase was performed. When compared to other medications, the hydroalcoholic extract of Ipomea nil showed a strong inhibitory effect, according to the current investigation. In the concentration range of 20 to 100g/ml (Table 5), the inhibition ranged from 24 to 82 percent (Figure 6). The results clearly show that the hydroalcoholic extract of Ipomea nil is capable of efficiently suppressing the activity of -amylase. The hydrolysis of alpha-1,4-glycosidic bonds of starch, glycogen, and other oligosaccharides is catalysed by alpha-amylase, while alpha-glucosidase further breaks down the disaccharides to simple sugars. Inhibition of α-glucosidase activity (Table 6) in the human digestive tract is thought to be an effective diabetes control approach.

As a result, effective and safe α-amylase and α-glucosidase inhibitors have long been sought. The main finding of this study was that a hydroalcoholic extract of *Ipomea nil* demonstrated effective suppression of alpha-amylase and alpha-glucosidase enzyme activity (Figure 7, Figure 8, Figure 9).

The level of blockage of glucose transport by the plant extract was measured and compared to the control in the absence of plant extract at various time intervals (Figure 10). Compared to the control, the hydroalcoholic extract of *Ipomea nil* dramatically reduced glucose transport across the membrane.

Diabetic patients' treatment goal is to maintain near-normal glucose control in fasting and postprandial situations. Suppression of glucose synthesis from carbohydrates in the gut or glucose absorption from the intestine has been studied using various natural sources. As a result of the in vitro study, it is clear that enzyme systems play a key role in diabetic situations. The intestine's α-amylase and α-glucosidase enzymes affect glucose absorption and catalyse starch and glycogen. Disaccharides are converted to simple sugars by alpha-glucosidase.

CONCLUSION

The activity of alpha amylase and alpha glucosidase enzymes has been inhibited by a variety of natural phenolic compounds. As a result of its inhibitory action on alpha-glucosidase and alpha-amylase levels, the hydroalcoholic extract of Ipomea nil could be used as a monotherapy and a diabetes-specific diet and exercise to maintain excellent health or in combination with approved anti-diabetic medications.

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Conflict of Interest

As a corresponding author, I certify that this manuscript is original and its publication does not infringe any copyright. The manuscript does not participate in any publishing process. I also declare there is no conflict of interest.

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