In vitro Antidiabetic and Antioxidant Activity of *Dioscorea oppositifolia*

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Abstract

Dioscorea oppositifolia, commonly known as Chinese yam, is a member of the Dioscoreaceae family which comprises of over 600 species. Dioscorea plants are known to contain various bioactive compounds including steroids, clerodane diterpenes, quinones, cyanidins, phenolics, diarylheptanoids and nitrogen compounds. The tubers and roots of Dioscorea species are particularly rich in steroidal sapogenins, primarily diosgenin along with volatile compounds. This study aimed to assess the in vitro antidiabetic and antioxidant properties of Dioscorea oppositifolia extract. The antidiabetic potential was evaluated using α-amylase and α-glucosidase inhibition assays while antioxidant activity was assessed through DPPH radical scavenging and ferric-reducing antioxidant power (FRAP) assays.

The extract demonstrated significant inhibition of α-amylase and α-glucosidase enzymes, indicating its potential to modulate postprandial blood glucose levels. Additionally, the extract exhibited strong antioxidant activity by effectively scavenging DPPH radicals and increasing the FRAP value. These findings highlight the potential of Dioscorea oppositifolia as a natural source of antidiabetic and antioxidant agents, supporting further investigation into its therapeutic applications for diabetes and oxidative stress-related disorders.

Keywords: Antidiabetic, Antioxidant, *Dioscorea oppositifolia*, α-amylase inhibition, α-glucosidase inhibition.

Introduction

Diabetes is a chronic endocrine disorder characterized by persistent hyperglycemia and associated carbohydrate, protein and lipid metabolism disruptions. This condition results from decreased insulin secretion and increased cellular resistance to insulin. Diabetes has significant implications for global health due to its detrimental effects on both microvascular and macrovascular systems, leading to organ and tissue damage, particularly affecting the eyes, kidneys, nerves, heart and blood vessels[9]. The prevalence of diabetes is increasing rapidly, with projected numbers expected to rise from 171 million in 2000 to 366 million by 2030[21]. Effective control of blood glucose levels is critical for preventing diabetic complications and improving patient health. However, currently available diabetes medications have limitations including side effects such as hypoglycemia, cell death and high rates of secondary failure[5].

In response to these challenges, recent research has focused on exploring functional foods and their bioactive components as complementary treatments for diabetes[12]. Medicinal plants recognized for their therapeutic properties and pharmacological effects, represent a promising avenue for developing novel antidiabetic agents[10]. The family Dioscoreaceae, comprising of over 650 species distributed worldwide, includes the genus Dioscorea which is particularly rich in species and is commonly known as yam. Among these, *Dioscorea oppositifolia* (known as "Vethalaivalli") is noteworthy for its medicinal potential[13,14].

Dioscorea species, including *D. oppositifolia*, is valued for their secondary metabolites with pharmacological significance, utilized in traditional medicine and the pharmaceutical industry. These plants exhibit antioxidant and anti-diabetic activities attributed to their bioactive compounds such as diosgenin, dioscin and trillin[3,22]. The presence of phytochemicals like phenolic antioxidants and aromatic phenanthrene derivatives further contributes to their medicinal properties[1,7]. In particular, Dioscorea appositively is recognized for its potential to manage diabetes and improve overall health due to its anti-diabetic, anti-hypercholesterolemia, hepatoprotective, nephron-protective and antioxidant activities.

Studies have also explored the immunomodulatory effects of Dioscorea species in aquatic animals, highlighting their broader physiological impacts[20,22]. The chemical profile of yam includes various phytochemicals such as amylase, saponins, amino acids, polyphenol oxidase, choline, starch, carbohydrates, proteins and vitamin C[6,20]. These compounds contribute to the diverse physiological functions exhibited by Dioscorea species, emphasizing their potential as valuable resources for developing novel therapeutic agents.

In summary, *Dioscorea oppositifolia* and other Dioscorea species hold promise as natural sources of bioactive compounds with anti-diabetic and antioxidant properties. Further research into their molecular mechanisms and clinical applications could pave the way for innovative treatments for diabetes and related disorders.

Material and Methods

Collection of Plant Materials: Fresh samples of *Dioscorea*

oppositifolia were randomly collected from Yercaud, Salem, Tamil Nadu. The plant materials were washed with running tap water, air-dried, homogenized into a fine powder and stored in airtight bottles in the refrigerator.

Preparation of Extract: Crude *Dioscorea oppositifolia* extract was prepared using the Soxhlet Extraction method. Approximately 20 grams of powdered material were packed into a thimble and extracted with 250 ml each of hexane and methanol separately. The extraction process continued for 24 hours or until the solvent in the siphon tube of the extractor became colorless. The resulting extract was transferred to a beaker, placed on a hot plate set at 30-40ºC and heated until all the solvent evaporated. The dried extract was stored in the refrigerator at 4ºC for future use.

Phytochemical Screening: Preliminary phytochemical analysis was conducted on both methanol and hexane extracts of *Dioscorea oppositifolia* following standard methods.

- **Detection of Alkaloids:** Extracts were dissolved in dilute hydrochloric acid, filtered and then subjected to Mayer's test. The formation of a yellow cream precipitate indicated the presence of alkaloids.
- **Detection of Flavonoids:** Extracts were treated with sulfuric acid (H_2SO_4). The formation of an orange color indicated the presence of flavonoids.
- **Detection of Steroids:** Liebermann-Burchard test was performed by adding acetic anhydride and sulfuric acid to the extracts. A color change from violet to blue or green indicated the presence of steroids.
- **Detection of Terpenoids:** Salkowski's test involved mixing the extract with chloroform and concentrated sulfuric acid to observe reddish-brown coloration, indicating the presence of terpenoids.
- **Detection of Anthraquinones:** Borntrager's test was conducted by boiling the extract with hydrochloric acid, filtering and adding ammonia and chloroform to observe a pink color, indicative of anthraquinones.
- **Detection of Phenols:** Ferric chloride test was performed by adding ferric chloride solution to the extracts and observing the formation of a bluish-black color, indicating the presence of phenols.
- **Detection of Saponins:** Froth test involved shaking the extract with distilled water to observe frothing, indicating the presence of saponins.
- **Detection of Tannins:** Ferric chloride test was conducted by mixing the extract with water, heating, filtering and adding ferric chloride solution to observe a dark green color, indicative of tannins.
- **Detection of Carbohydrates:** Fehling's test was performed by boiling the filtrate with Fehling solutions A and B to observe a red precipitate indicating the presence of sugars.
- **Detection of Oils and Resins:** Spot test involved applying the test solution on filter paper to observe a transparent appearance, indicating the presence of oils and resins.

***In vitro* Antioxidant Activity**

DPPH Assay: DPPH radical scavenging activity was assessed using the method described earlier. Various concentrations of the test sample in methanol were added to a DPPH solution and incubated in the dark. Changes in coloration were measured spectrophotometrically at 514 nm. Ascorbic acid was used as a reference compound and the percentage of inhibition was calculated. IC_{50} value was calculated using Graph pad Prism 5.0.

Reducing Power Assay: The reducing power assay was performed by adding phosphate buffer, potassium ferricyanide, trichloroacetic acid and ferric chloride to the sample solutions. The absorbance was measured at 700 nm and higher absorbance indicated higher reducing power.

***In vitro* Anti-Diabetic Activity**

Determination of α-Amylase Inhibitory Activity: The α-amylase inhibitory activity was assessed by incubating the test samples with alpha-amylase enzyme and potato starch solution in acetate buffer. The absorbance was measured at 565 nm to determine the inhibition percentage.

Determination of α-Glucosidase Inhibition Assay: The α-glucosidase inhibitory activity was evaluated using p-nitro-phenyl-a-D glucopyranoside (p- NPG) substrate solution described by Tao et al[19]. The absorbance was measured at 405 nm and the inhibitory activity was expressed as percent inhibition. These methods were conducted to evaluate the potential antioxidant and anti-diabetic properties of *Dioscorea oppositifolia* extracts.

Results and Discussion

Extraction of crude from *Dioscorea oppositifolia* Leaves: We extracted the crude from *Dioscorea oppositifolia* leaf powder with solvents such as methanol and hexane through using soxhlet apparatus. 40g of leaf powder was mixed with 40 ml of methanol (1: 1 ratio) and 40g of leaf powder was mixed with 40 ml of hexane (1:1 ratio) in Soxhlet apparatus. Yield obtained in *Dioscorea oppositifolia* is:

Methanol extract in %	Hexane extract in %
26.54	13.67

Phytochemical Analysis: Each of these tests exploits specific chemical reactions or interactions characteristic of the targeted phytochemical class[11]. Positive results in these tests provide evidence for the presence of alkaloids, flavonoids, steroids, terpenoids, phenols, or tannins in methanol extract and flavonoids, steroids, terpenoids, saponins, carbohydrates, oils and resins in hexane extract aiding in the qualitative analysis of plant constituents. These tests are valuable tools in phytochemical screening and can help in preliminary compound identification based on their chemical properties[2,8].

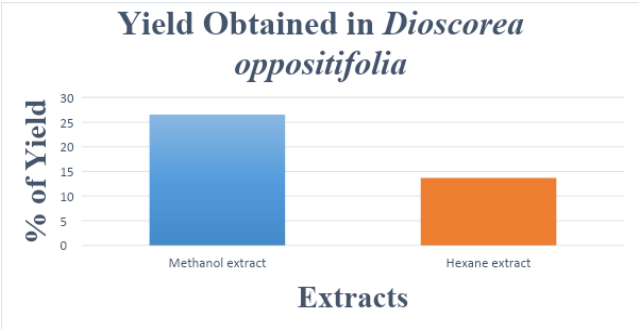

Figure 1: Quantity of Crude Extracts of Dioscorea oppositifolia in Methanol Extract and Hexane Extract.

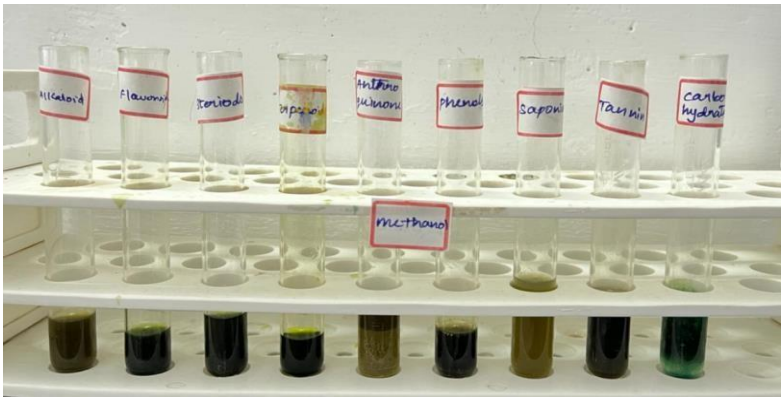

Figure 2: Phytochemical Analysis of *Dioscorea oppositifolia* in Methanol Extract

Table 1
Phytochemical Analysis of *Dioscorea oppositifolia* in Methanol Extract

S.N.	Phytochemicals	Observations	Methanol Extract
1.	Alkaloids Mayer's test Wagner's test	Cream color Reddish brown solution/ precipitate	+ +
2.	Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange color precipitate	 +
3.	Steroids Liebermann-Burchard test	Violet to blue or green color formation	+
4.	Terpenoids Salkowski test	Reddish brown precipitate	+
5.	Anthraquinone Borntrager's test	Pink color	-
6.	Phenols Ferric chloride test Lead acetate test	Deep blue to Black color formation White precipitate	 +
7.	Saponin	Stable persistent	-
8.	Tannin	Brownish green / Blue black	+
9.	Carbohydrates	Yellow / brownish / blue / green color	+
10.	Oils and Resins	Filter paper method	-

Figure 3: Phytochemical Analysis of *Dioscorea oppositifolia* in Hexane Extract

Table 2
Phytochemical Analysis of *Dioscorea oppositifolia* in Hexane Extract *in vitro* antioxidant activity

S.N.	Phytochemicals	Observations	Hexane Extract
1.	Alkaloids Mayer's test Wagner's test	Cream color Reddish brown solution/ precipitate	-
2.	Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange color precipitate	+
3.	Steroids Liebermann-Burchard test	Violet to blue or green colour formation	+
4.	Terpenoids Salkowski test	Reddish brown precipitate	+
5.	Anthraquinone Borntrager's test	Pink color	-
6.	Phenols Ferric chloride test Lead acetate test	Deep blue to Black color formation White precipitate	-
7.	Saponin	Stable persistent	+
8.	Tannin	Brownish green / Blue black	-
9.	Carbohydrates	Yellow / brownish / blue / green color	+
10	Oils and Resins	Filter paper method	+

Table 3
% IC_{50} and concentration of Reducing Power Assay of *Dioscorea oppositifolia*

S.N.	Concentration	%IC_{50}	IC_{50}
1.	50	37.41	627.72
2.	250	42.18	
3.	500	47.62	
4.	750	51.70	
5.	1000	58.50	

Reducing Power Assay of *Dioscorea oppositifolia* in Methanol Extract: The reducing power assay data indicates the antioxidant potential of the sample at different concentrations. The increasing reducing power with higher concentrations suggests dose-dependent antioxidant effects. The IC50 value of 6.2772×10^2µg/mL indicates the concentration required to achieve significant antioxidant activity. This information contributes to the characterization of the sample's biological effects and potential therapeutic applications as a natural antioxidant agent. Understanding the dose-dependent relationship between concentration and reducing power helps to assess the sample's potential health benefits in combating oxidative stress-related disorders**.**

DPPH Assay of *Dioscorea oppositifolia* in Methanol Extract: The detailed analysis of the DPPH assay data reveals the concentration-dependent antioxidant activity of the sample. The IC50 value of 6.1098×1026.1098×102 µg/mL indicates the concentration required to scavenge 50% DPPH radicals. The increasing percentage inhibition with higher concentrations underscores the antioxidant potential of the sample. This information is valuable for elucidating the biological effects and potential health benefits associated with the sample's antioxidant properties[16].

By comparing, the DPPH assay (IC50 = 610.98 µg/mL) demonstrates superior antioxidant efficacy compared to the reducing power assay (IC50 = 627.72 µg/mL) in terms of potency and effectiveness. The reducing power assay focuses on electron donation and reduction of oxidized compounds, while the DPPH assay assesses direct scavenging of free radicals, reflecting different mechanisms of antioxidant action. A lower IC50 value (as observed in the DPPH Assay) implies better potential for therapeutic applications, particularly in combating oxidative stress-related conditions[4]. Each assay has its merits and demerits, highlighting the need for a balanced approach in antioxidant research to capture the diverse mechanisms of antioxidant action in biological systems[19]. Integration of multiple assays and experimental conditions is recommended to obtain a comprehensive understanding of antioxidant properties and their potential health benefits of the extract.

***In vitro* anti-diabetic activity**

Alpha amylase Inhibitory Activity of *Dioscorea oppositifolia* in Methanol Extract: Understanding the concentration-dependent inhibition of alpha-amylase helps in elucidating the mechanism of action of the sample and its potential role in glycemic control. The detailed analysis of the alpha-amylase inhibitory activity assay data reveals the concentration-dependent inhibitory effect of the sample on alpha amylase enzyme activity.

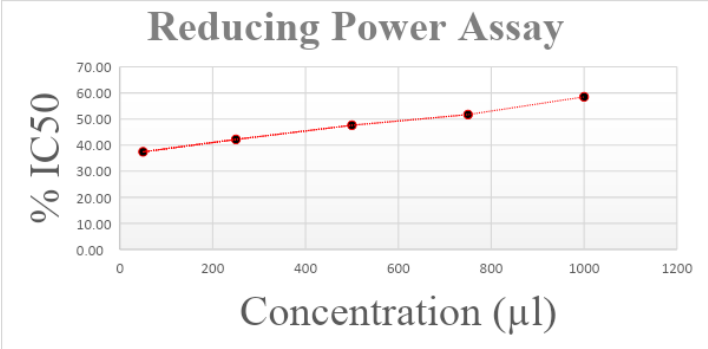

Figure 4: This graph shows the %IC50 and Concentration of Reducing Power Assay of *Dioscorea oppositifolia*.

Table 4
%IC50 and Concentration of DPPH Assay of *Dioscorea oppositifolia*

S.N.	Concentration	%IC50	IC50
1.	50	32.17	610.98
2.	250	37.76	
3.	500	45.45	
4.	750	56.64	
5.	1000	61.54	

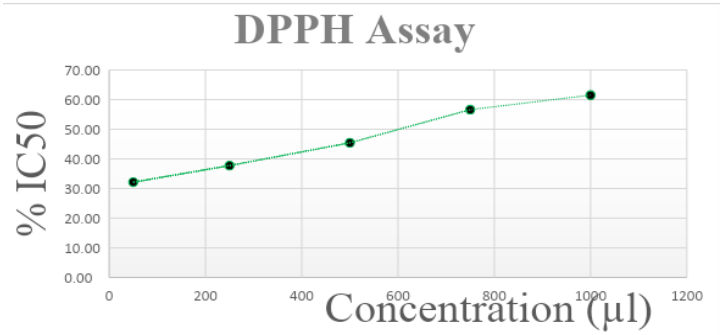

Figure 5: This graph shows the %IC50 and Concentration of DPPH Assay of *Dioscorea oppositifolia*.

Table 5
Alpha Amylase Inhibitory Activity of *Dioscorea oppositifolia*

S.N.	Concentration	%IC50	IC50
1.	50	37.50	636.68
2.	250	42.76	
3.	500	48.03	
4.	750	52.63	
5.	1000	56.58	

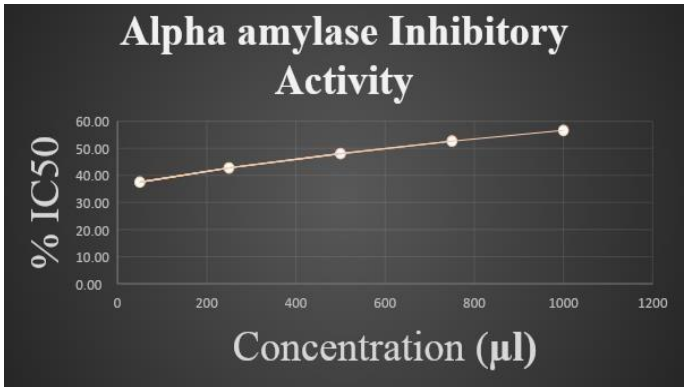

Figure 6: This graph shows the Alpha amylase inhibition activity of Dioscorea oppositifolia

Table 6
A-Glucosidase Inhibitory Activity of Dioscorea oppositifolia

S.N.	Concentration	%IC50	IC50
1.	50	37.82	694.69
2.	250	42.31	
3.	500	46.79	
4.	750	51.28	
5.	1000	55.13	

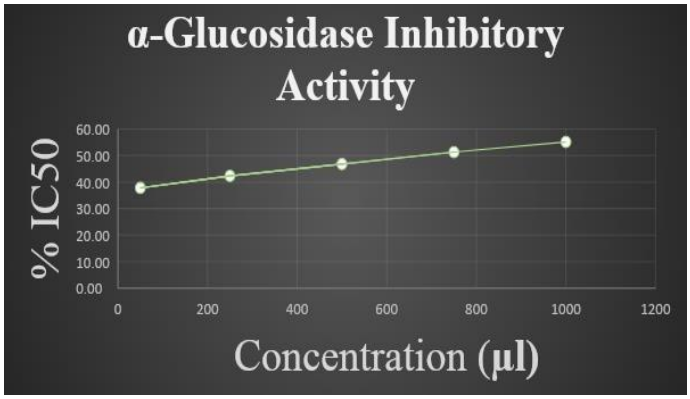

Figure 7: This graph shows the α-Glucosidase inhibition activity of Dioscorea oppositifolia.

The IC50 value of 6.3668×1026.3668×102 µg/mL indicates the concentration required to achieve significant enzyme inhibition. This information contributes to the evaluation of the sample's therapeutic potential in managing diabetes and related metabolic disorders.

α-Glucosidase Inhibitory Activity of *Dioscorea oppositifolia* in Methanol Extract: The percentage inhibition values represent the extent to which the sample inhibits the activity of α- glucosidase enzyme at different concentrations. As the concentration of the sample increases, the percentage inhibition of α-glucosidase also increases, indicating a dose-dependent response. The efficacy of the sample in inhibiting α-glucosidase can be compared with standard inhibitors to assess its therapeutic potential. The IC50 value of 694.69 µg/mL for α-glucosidase inhibitory activity signifies the concentration at which the sample exhibits significant inhibition of α-glucosidase enzyme. This value underscores the potential of the sample as a natural or synthetic α-glucosidase inhibitor and highlights its relevance in the development of antidiabetic therapies aimed at controlling postprandial hyperglycemia.

While comparing and discussing the IC50 values of α-glucosidase inhibitory activity (694.69) and alpha-amylase inhibitory activity (636.68), we analyze their implications in the context of managing blood glucose levels and potential therapeutic applications in diabetes treatment. A slightly lower IC50 value (636.68 µg/mL) compared to α-glucosidase inhibitory activity suggests that the sample is more potent in inhibiting alpha-amylase. Inhibition of alpha-amylase can delay starch digestion and can reduce glucose release, which is beneficial for controlling blood sugar levels. Both assays assess the potential of the sample in managing postprandial hyperglycemia, a critical aspect of diabetes management[9].

Alpha amylase inhibition (lower IC50) may have more direct effects on reducing immediate glucose spikes after carbohydrate consumption. Understanding their differential effects on enzyme inhibition provides insights into the potential therapeutic applications of the sample in managing blood glucose levels and mitigating hyperglycemia associated with diabetes. Further studies are needed to elucidate the mechanisms of action and to optimize the use of these inhibitors for diabetes treatment and prevention[12].

These results indicate the presence of antidiabetic and antioxidant plant compounds in *Dioscorea oppositifolia* extract. The antidiabetic and antioxidant activities of *Dioscorea oppositifolia* may be due to the presence of bioactive compounds such as saponins, alkaloids and flavonoids. The methanol extract of *Dioscorea oppositifolia* demonstrated significant *in vitro* antioxidant activity in the DPPH test, indicating its potential as a source of natural antioxidants. These results suggest that *Dioscorea oppositifolia* could be further explored for its therapeutic applications in combating oxidative stress- related disorders[15]. The ointment's electron transfer ability and important evidence of its antioxidant effects allowed researchers to study the substance's reducing ability by converting Fe^{3+} to Fe^{2+}. With increasing concentration, the reducing activity of the ointment and standard increases.

Dioscorea oppositifolia extract had slightly higher antioxidant activity than the reference used in this study. The methanol extract of *Dioscorea oppositifolia* exhibits

significant *in vitro* antioxidant activity, as evidenced by its strong reducing ability in the test[18]. This suggests the potential of *Dioscorea oppositifolia* as a source of natural antioxidants, which may be useful in fighting diseases related to oxidative stress. The results of this study showed that *Dioscorea oppositifolia* leaf extract has significant antidiabetic activity, as evidenced by its inhibition of alpha-amylase activity. The methanol extract of *Dioscorea oppositifolia* demonstrated significant antidiabetic activity *in vitro* through its potent inhibition of alpha amylase. This shows its potential as a natural remedy for controlling diabetes by regulating post-meal glucose levels[7].

Conclusion

The methanol extract of *Dioscorea oppositifolia* leaves has demonstrated promising phytochemical constituents that exhibit significant potential for anti-diabetic and antioxidant activities. Through this study, it has been revealed that the extract possesses valuable bioactive compounds that could serve as effective agents in managing diabetes and combating oxidative stress. The extract may slow down the breakdown of carbohydrates into glucose, thus reducing postprandial blood glucose levels.

The findings underscore the importance of exploring natural sources like *Dioscorea oppositifolia* in the search for novel therapeutics for diabetes and related complications. Further research is warranted to elucidate the precise mechanisms of action and to validate its therapeutic efficacy for clinical applications.

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