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INVITRO EVALUTION OF ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF *MELIA DUBIA* LEAF EXTRACT

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ABSTRACT
Background: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and is frequently associated with oxidative stress. Medicinal plants are considered valuable sources of bioactive compounds with antioxidant and antidiabetic properties. *Melia Dubia* Cav. (Malabar neem) is a medicinally important tree rich in phytochemicals that may contribute to its therapeutic potential. **Objective:** The present study was undertaken to evaluate the phytochemical constituents and investigate the *in-vitro* antioxidant and antidiabetic activities of the ethanolic leaf extract of *Melia Dubia*. **Methods:** Preliminary phytochemical screening was performed using standard qualitative methods. Antioxidant activity was assessed by DPPH free radical scavenging, phosphomolybdenum, and reducing power assays using ascorbic acid as the reference standard. *In vitro* antidiabetic activity was evaluated by α-amylase and α-glucosidase enzyme inhibition assays using acarbose as the standard drug. Percentage inhibition and EC_{50} values were determined for each assay. **Results:** Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, steroids, proteins, amino acids and carbohydrates in the ethanolic leaf extract. The extract exhibited concentration-dependent antioxidant activity in the DPPH assay with an EC_{50} value of 107.13 µg/mL, while the standard showed an EC_{50} of 71.92 µg/mL. The phosphomolybdenum and reducing power assays further demonstrated appreciable antioxidant capacity. In the α-amylase inhibition assay, the extract showed dose-dependent inhibition with an IC_{50} value of 220.84 ± 5.18 µg/mL, whereas acarbose exhibited an IC_{50} of 158.67 ± 10.94 µg/mL. Similarly, the extract displayed significant α-glucosidase inhibitory activity with an IC_{50} value of 101.84 ± 4.56 µg/mL, although its activity was lower than that of the standard acarbose (IC_{50} 68.42 ± 3.36 µg/mL). **Conclusion:** The ethanolic leaf extract of *Melia Dubia* demonstrated promising antioxidant and *in-vitro* antidiabetic activities, which may be attributed to its diverse phytochemical constituents. These findings support the traditional medicinal use of *Melia Dubia* and suggest that it could serve as a potential natural source for the development of antioxidant and antidiabetic agents. Further isolation of active constituents and *in-vivo* studies are warranted to validate its therapeutic efficacy and safety.

KEYWORDS: *Melia dubia, in-vitro* Antioxidant & Antidiabetic. α amylase, α glucosidase.

INTRODUCTION

Medicinal plants have played a fundamental role in maintaining human health and managing a wide variety of diseases. More than 80,000 plant species are used worldwide for medicinal purposes, with many being preserved and transmitted across generations as integral components of traditional and folk healthcare systems.[2]

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, defective insulin action, or a combination of both. This condition may arise due to inherited or acquired defects in pancreatic insulin production or the body's inability to utilize insulin effectively. Prolonged elevation of blood glucose levels can lead to serious complications, including damage to the cardiovascular, renal, nervous and visual systems, particularly affecting blood vessels and peripheral nerves.[3] The use of medicinal plants for the prevention and treatment of diseases dates back to ancient civilizations, making them one of the earliest and most enduring forms of healthcare practiced across the world[1] *Melia dubia* Cav., commonly known as Malabar neem, is a fast-growing, multipurpose tree species widely recognized for its suitability in farm forestry and agroforestry systems due to its high productivity and economic returns. Owing to its rapid growth rate and short rotation period, it is considered a highly profitable tree species with substantial demand in the pulpwood, plywood, and timber industries. In addition to its industrial importance, *Melia Dubia* serves as a valuable source of fuelwood and fodder. The species is also well

known for its diverse medicinal, pharmacological, ethnomedicinal and bioactive properties, attributed to the presence of numerous phytochemicals. Consequently, *Melia Dubia* has attracted increasing research interest for its potential applications in both forestry and healthcare. This research highlights the economic significance of *Melia dubia* in the timber industry while providing a comprehensive overview of its medicinal and pharmacological potential.[4]

Melia dubia, commonly known as Maha neem or forest neem, is a valuable medicinal tree species native to India, Sri Lanka, Malaysia, Australia, and Angola. The species has attracted considerable scientific interest owing to its diverse phytochemical composition and broad spectrum of biological activities. The present study aimed to evaluate the phytochemical constituents, proximate composition, physicochemical characteristics, elemental profile, and antimicrobial potential of crude extracts obtained from *Melia dubia*.[5]

Table 1: Plant profile of the - *Melia Dubia*[6]

PLANT PROFILE OF THE -*MELIA DUBIA*[6]	
Kingdom	Plantae
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	Melia
Species	*Melia dubia*

Table 2: Preliminary phytochemical screening of leaf extract.

Phytochemical Constituent	Inference
Alkaloids	Alkaloids present (+)
Flavonoids	Flavonoids present (+)
Phenolic Compounds	Phenolic compounds absent (-)
Tannins	Tannins present (+)
Saponins	Saponins present (+)
Cardiac Glycosides	Cardiac glycosides absent (-)
Terpenoids	Terpenoids absent (-)
Steroids	Steroids present (+)
Proteins	Proteins present (+)
Amino Acids	Amino acids present (+)
Carbohydrates	Carbohydrates present (+)

Note: (+) = Present; (-) = Absent

DPPH assay of *Melia Dubia* leaf extract.[8]

DPPH (2,2-diphenylpicrylhydrazyl) free radical scavenging activity was assessed using a standard protocol. Different concentrations (40–200 µl) of leaf extracts were taken in test tubes, followed by the addition of 1 ml of methanolic DPPH solution (0.1 mM). The mixtures were incubated in the dark at room temperature for 30 minutes, after which absorbance was measured at 517 nm against a blank. The free radical scavenging activity of *Melia dubia* leaf extract was expressed as the percentage decrease in absorbance compared to the control containing only solvent. The effective concentration (EC_{50}) value was defined as the concentration of extract required to reduce 50% of the absorbance relative to the control. Ascorbic acid was used as the positive control. The percentage inhibition of DPPH radicals was calculated using the following formula:

Table 3: Concentration and %inhibition, Ec_{50} standard ascorbic acid and plant extract.

Samples	Concentration (µg/ml)	% Inhibition	EC_{50} (µg/ml)
Melia dubia	20	8.21	**107.13**
	40	26.53	
	60	33.21	
	80	41.65	
	100	46.15	
	120	56.95	
Ascorbic acid (Standard)	20	14.37	**71.92**
	40	26.91	
	60	41.47	
	80	55.78	
	100	72.78	
	120	91.21	

DPPH Scavenging (%) = 100 × (Ao- As)/Ao

Where, Ao is the absorbance of the blank and as the absorbance of the sample.

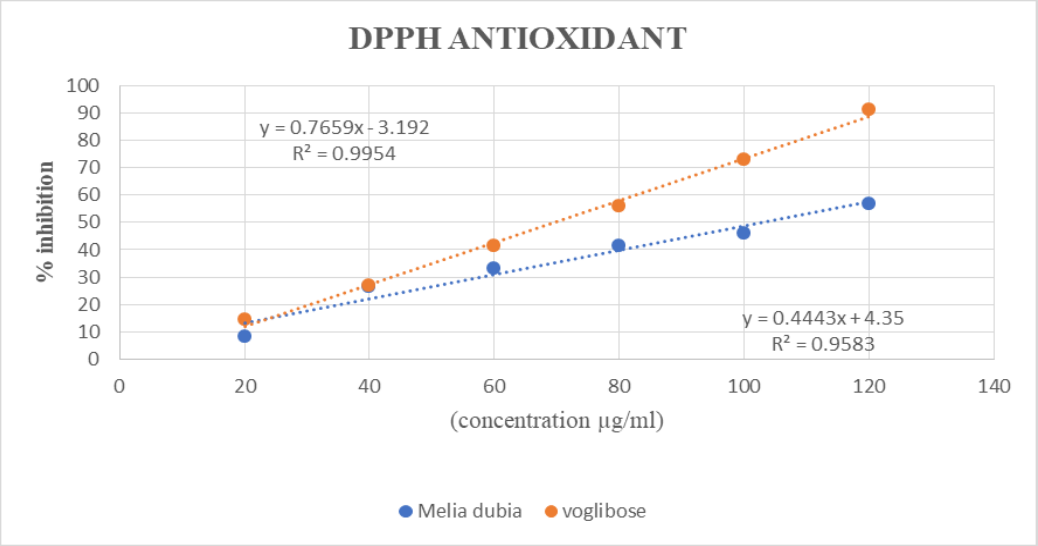

Fig. 1: DPPH inhibition by *Melia Dubia* ethanol extract by graph.

Phosphomolybdenum assay of *Melia dubia* extract.[9]
The antioxidant capacity of the samples was evaluated based on the formation of a green Phosphomolybdenum complex. Leaf extract (10 mg) was dissolved in 10 ml of DMSO, and aliquots of 40–200 µl of this solution were mixed with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in test tubes. The vials were sealed and incubated in a water bath at 95 °C for 90 minutes. After cooling to room temperature, the absorbance of the reaction mixture was measured at 695 nm against a blank. Ascorbic acid was used as the positive control.

Table 4: Concentration and absorbance value of standard ascorbic acid and extract.

Sample	Concentration (µg/ml)	Absorbance (765 nm)
Standard (Ascorbic acid)	6	0.489
	12	0.595
	18	0.669
	24	0.758
	30	0.880
Ethanolic extract	6	0.367
	12	0.463
	18	0.598
	24	0.689
	30	0.799

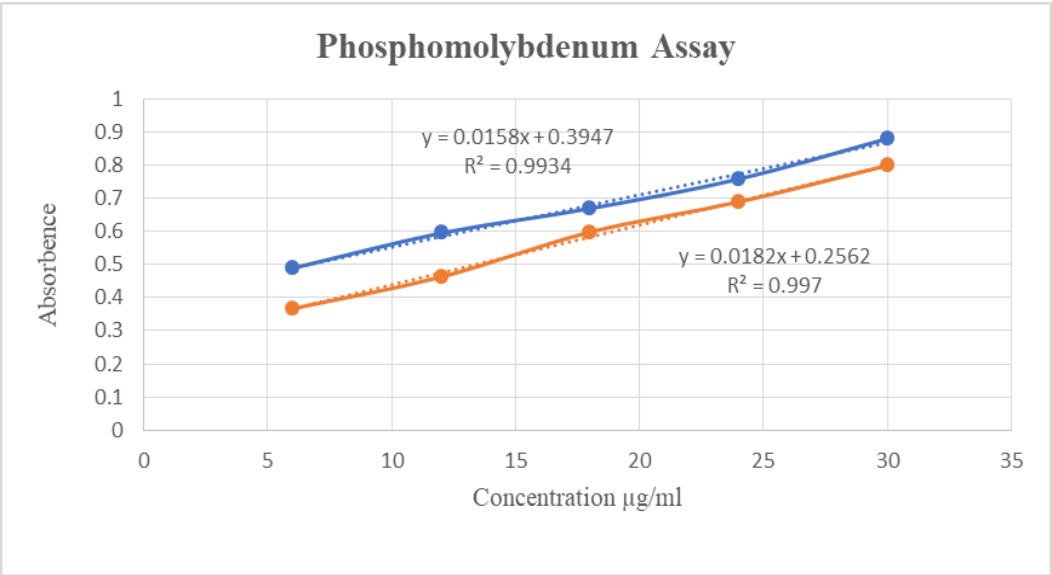

Fig. 2: Phosphomolybdenum inhibition by *Melia dubia* ethanol extract by graph.

Reducing power of *Melia dubia* leaf extract[10]

Principle

The total antioxidant activity can be measured by the ferric reducing antioxidant power assay (FRAP). The flavonoids and phenolic acids are present in the medicinal plant exhibit strong antioxidant activity which is depending on their potential to form the complex with metal atoms, particularly iron and copper. This method is based on the principle of increase in the absorbance of the reaction mixtures; the absorbance increases the antioxidant activity increases. The antioxidant compound present in the samples forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm by UV-Spectrophotometer.

Procedure

Different concentrations of the ethanolic extract of *Melia dubia* and its various fractions (40-200 µg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K3Fe(CN)6] solution. The reaction mixture was vortexed well and then incubated at 50°C for 20 min using vortex shaker. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min.The supernatant (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The colored solution was read at 700 nm against the blank with reference to standard using UV Spectrophotometer. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard.

Table 5: Concentration and absorbance value of standard ascorbic acid and extract.

Sample	Concentration (µg/ml)	Absorbance (700 nm)
Standard (Ascorbic acid)	6	0.058
	12	0.066
	18	0.075
	24	0.086
	30	0.097
Sample	6	0.048
	12	0.059
	18	0.069
	24	0.078
	30	0.087

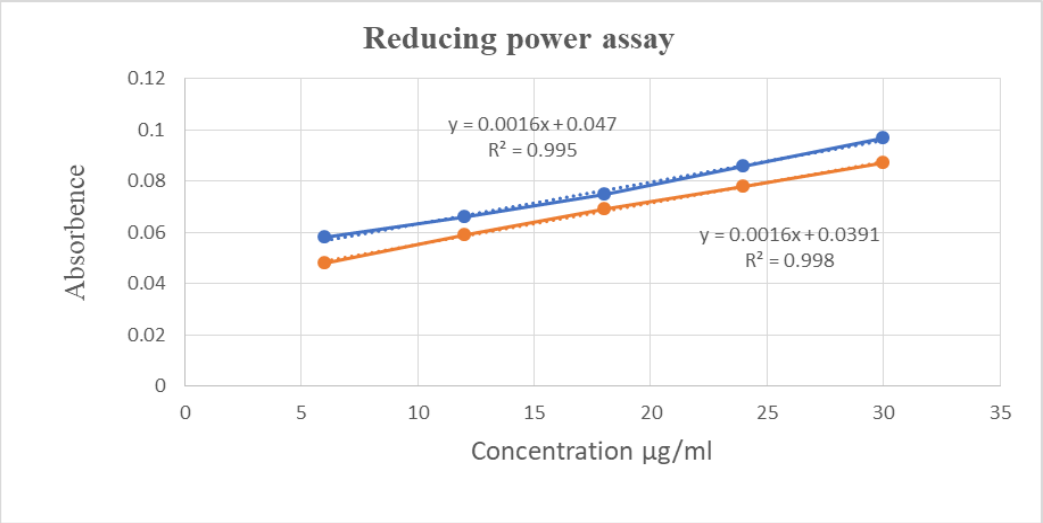

Fig. 3: Reducing power assay inhibition by *Melia dubia* ethanol extract by graph.

***IN-VITRO* ANTIDIABETIC ACTIVITY**

Alpha amylase inhibition assay[11]

The α-amylase inhibition assay was carried out using the 3,5-dinitrosalicylic acid (DNSA) method. The ethanolic leaf extract of *Melia dubia* was initially dissolved in a minimal amount of 10% DMSO and further diluted with buffer (0.02 M Na_2HPO_4/NaH_2PO_4, 0.006 M NaCl, pH 6.9) to obtain concentrations ranging from 50 to 300 µg/ml. Different volumes of the extract (50, 100, 150, 200, 250, and 300 µl) were mixed with 200 µl of α-amylase solution and incubated at 30 °C for 10 minutes. Subsequently, 200 µl of starch solution (1% w/v in water) was added and incubated for 3 minutes. The reaction was terminated by adding 200 µl of DNSA reagent (prepared by dissolving 12 g of sodium potassium tartrate tetrahydrate in 8 ml of 2 M NaOH and 20 ml of 96 mM DNSA solution) followed by heating in a boiling water bath at 85–90°C for 10 minutes. The mixtures were cooled to room temperature, diluted with 5 ml of distilled water, and the absorbance was measured at 540 nm using

a UV–Visible spectrophotometer. A blank representing 100% enzyme activity was prepared by replacing the extract with 200 µl of buffer, while another blank was prepared using the extract (at each concentration) without the enzyme. Acarbose (50–300 µg/ml) served as the positive control under the same assay conditions. The α-amylase inhibitory activity was expressed as percentage inhibition using the following formula. The percentage inhibition was plotted against extract concentration, and IC_{50} values were determined from the graph

$$\alpha\ glucocidase\ inihibition = \text{Abs(Control)} - \frac{\text{Abs(Extract)}}{\text{Abs(Control)}} \times 100$$

Table 6: α amylase inhibition concentration, %inhibition EC_{50} value of Voglibose and plant extract.

Sample	Concentration (µg/ml)	% Inhibition	EC50
Melia dubia	50	9.12 ± 0.68	**220.84 ± 5.18**
	100	17.58 ± 0.79	
	150	35.21 ± 0.37	
	200	47.05 ± 0.69	
	250	58.76 ± 0.61	
	300	66.92 ± 0.72	
Voglibose (Standard)	50	15.84 ± 3.42	**158.67 ± 10.94**
	100	31.08 ± 4.28	
	150	48.15 ± 4.81	
	200	63.18 ± 5.17	
	250	79.06 ± 3.25	
	300	92.34 ± 3.54	

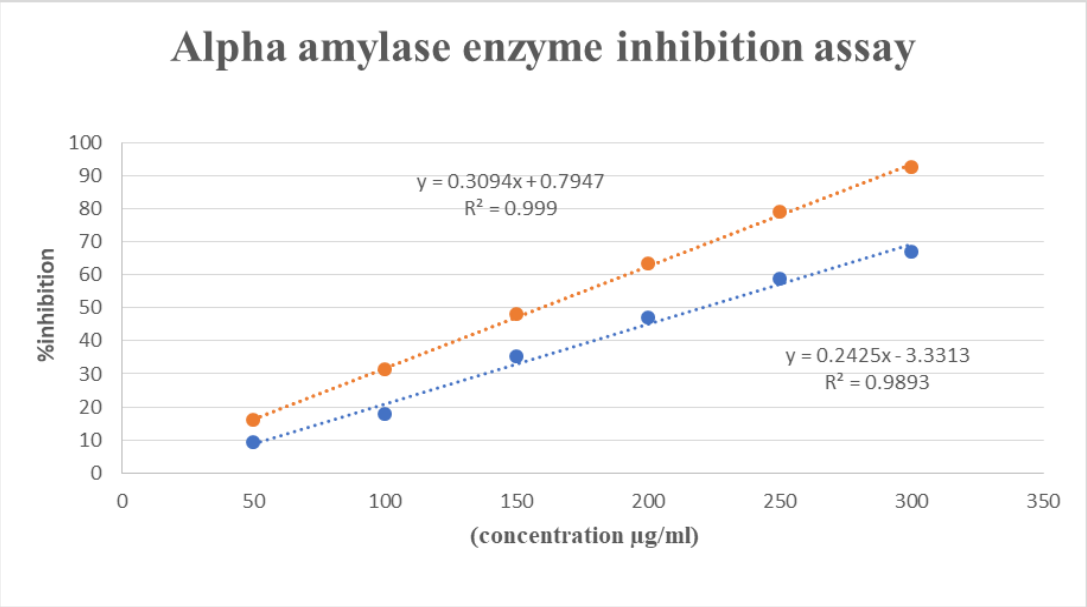

Fig. 4: α amylase enzyme inhibition assay both plant and standard extract.

Alpha Glucosidase Inhibitory Activity[12,13]

The ethanolic leaf extract of *Melia dubia* was dissolved in of 10% DMSO to give concentrations ranging from 20 to 120µg/ml., The alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8. A volume of 10µL alpha glucosidase (1 U/mL) was mixed with concentration range from 20, 40, 60, 80, 100, 120µL of the extract and was pre incubated at 37 °C for 15 min. Then, 20 µL P-NPG (5 mM) was added as a substrate and further incubated at 37 °C for 20 min. Add 3 ml of 50 mM sodium hydroxide was added to the mixture and the absorbance was measured at 410 nm using a UV-Visible spectrophotometer. The blank was prepared by replacing the leaf extract with 50µl of buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using Acarbose (20µg/ml–120µg/ml) and the reaction was performed similarly to the reaction with leaf extract as mentioned above and each experiment was performed in triplicate. The α-glucosidase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below: The % α-glucosidase inhibition was plotted against the extract concentration and the IC50values were obtained from the graph.

$$\%\ \alpha\ \text{glucosidase inhibition} = \text{Abs(Control)} - \frac{\text{Abs(Extract)}}{\text{Abs(Control)}} \times 100$$

Table 7: α glucosidase inhibition concentration, %inhibition ic_{50} value of Voglibose and plant extract.

Sample	Concentration (µg/mL)	% Inhibition (Mean ± SD)	IC_{50} (µg/mL)
Melia dubia	20	9.12 ± 1.35	101.84 ± 4.56
	40	25.64 ± 1.18	
	60	34.58 ± 1.12	
	80	42.31 ± 1.22	
	100	47.43 ± 0.91	
	120	57.82 ± 1.06	
Voglibose (Standard)	20	15.08 ± 1.95	68.42 ± 3.36
	40	27.84 ± 3.92	
	60	42.36 ± 3.88	
	80	56.94 ± 4.67	
	100	73.62 ± 4.39	
	120	90.35 ± 3.28	

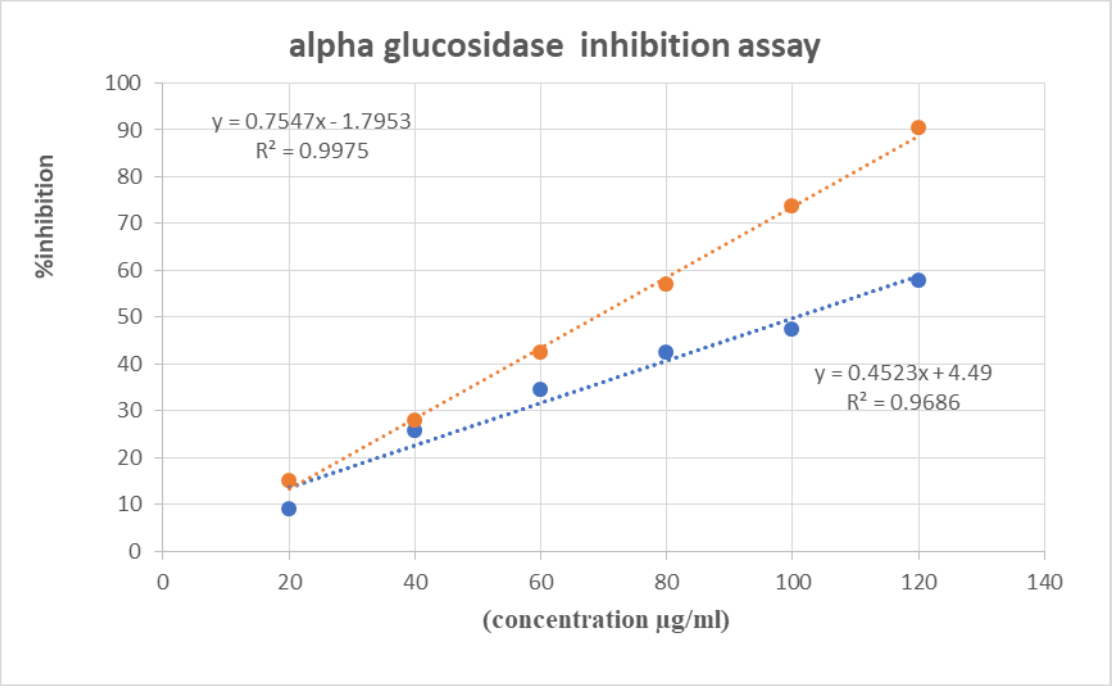

Fig. 5: α glucosidase enzyme inhibition assay both plant and standard extract.

CONCLUSION

The present study demonstrated that the ethanolic leaf extract of *Melia dubia* possesses significant antioxidant and *in-vitro* antidiabetic activities, which may be attributed to the presence of bioactive phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids, proteins, amino acids and carbohydrates. The extract exhibited concentration-dependent free radical scavenging activity in the DPPH assay and showed appreciable antioxidant potential in both the phosphomolybdenum and reducing power assays, indicating its ability to neutralize oxidative stress. Furthermore, the extract effectively inhibited the carbohydrate-hydrolyzing enzymes α-amylase and α-glucosidase in a dose-dependent manner. Although its inhibitory activity was lower than that of the standard drugs, the results suggest that *Melia dubia* leaf extract has promising potential to reduce postprandial hyperglycemia and may serve as a natural therapeutic agent for the management of diabetes mellitus. Overall, the findings provide scientific evidence supporting the traditional medicinal use of *Melia dubia* and highlight its potential as a source of natural antioxidant and antidiabetic compounds. However, further studies involving the isolation and characterization of the active phytoconstituents, elucidation of their mechanisms of action, toxicity evaluation, and comprehensive in vivo and clinical investigations are required to establish the safety, efficacy, and therapeutic applicability of *Melia dubia* in diabetes management.

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CONFLICT OF INTEREST

No conflict of interest.

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