VOLUME 11 ISSUE 2 2025

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 11, No. 2, 501-507 (2025)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

In Vitro Evaluation of Anti-Diabetic and Antioxidant Properties of *Bruguiera cylindrica* Ethanolic Leaf Extract

Prabhash T.[1], Geegi P.G.[2]*, Baskar A.[2] and Asha Monica A.[3]

[1]Department of Biochemistry, Bharathidasan University, Tiruchirappalli 620024, India
[2]Department of Biochemistry, St Joseph's College (Autonomous) (affiliated to Bharathidasan University), Tiruchirappalli 620002, India
[3]Department of Biotechnology, St Joseph's College (Autonomous) (affiliated to Bharathidasan University), Tiruchirappalli 620002, India

**Corresponding Author*

Received: 24th October, 2024; **Accepted:** 2nd February, 2025; **Published online:** 22nd September, 2025

https://doi.org/10.33745/ijzi.2025.v11i02.046

Abstract: In recent decades, the field of natural medicine has witnessed exponential growth due to the increasing demand for remedies derived from plant sources, known for their natural origin and minimal side effects. Natural drugs form a significant part of various officially recognized healthcare systems in India, such as Ayurveda, Yoga, Unani, Siddha, Homeopathy, and Naturopathy. Over 70% of India's population continues to rely on these non-allopathic systems of medicine for treatment. Natural remedies are not only considered safer but are also more accessible to the masses. The present study focuses on evaluating the *in vitro* anti-diabetic and antioxidant properties of the ethanolic leaf extract of *Bruguiera cylindrica*, a mangrove species. Through the extraction process using the Soxhlet apparatus, the research aims to investigate the potential of this plant extract in mitigating oxidative stress and managing diabetes through various biochemical assays. The results of this study may contribute to the ongoing efforts to explore plant-based solutions for chronic diseases such as diabetes.

Keywords: *Bruguiera cylindrica,* Soxhlet apparatus, Diabetes, Ethanolic leaf extract, Hyperglycemia, DPPH method

Citation: Prabhash T., Geegi P.G., Baskar A. and Asha Monica A.: *In vitro* evaluation of anti-diabetic and antioxidant properties of *Bruguiera cylindrica* ethanolic leaf extract. Intern. J. Zool. Invest. 11(2): 501-507, 2025.

https://doi.org/10.33745/ijzi.2025.v11i02.046

Introduction

Diabetes has become a major global health issue, with the number of people diagnosed with the condition rising from 108 million in 1980 to 422 million in 2014. The prevalence of diabetes is growing more rapidly in low- and middle-income countries compared to high-income countries. Diabetes is not only a metabolic disorder but also a leading cause of several life-threatening complications, including blindness, kidney failure, heart attacks, strokes, and lower-limb amputations (Abeysinghe, 2010). Between 2000 and 2019, there was a 3% increase in global diabetes-

related mortality, and in 2019, an estimated 2 million deaths were caused by diabetes and its complications, particularly kidney disease. Effective management of diabetes involves lifestyle interventions, such as a healthy diet, regular physical activity, maintaining a normal body weight, and avoiding tobacco use. While type 2 diabetes can often be prevented or delayed with these measures, many patients require medications and regular screening to avoid or reduce complications. Diabetes occurs when the body either does not produce enough insulin or fails to use it effectively, leading to elevated blood glucose levels (hyperglycemia). Uncontrolled hyperglycemia can cause damage to the body's systems, especially the nerves and blood vessels. In 2019, diabetes was responsible for 1.5 million deaths, with almost half of these occurring before the age of 70. In addition, it contributed to nearly 20% of cardiovascular deaths and a significant number of deaths related to kidney disease (Acharya *et al.*, 2020).

Diabetes mellitus is classified into two main types: Type 1 diabetes, which is insulin-dependent and results from the autoimmune destruction of insulin-producing pancreatic β-cells, and Type 2 diabetes, which is often lifestyle-related and characterized by insulin resistance. The increasing prevalence of Type 2 diabetes is linked to the modern lifestyle, which includes poor diet and lack of physical activity, contributing to obesity and other external risk factors. Insulin, a hormone produced by the beta cells in the pancreas, plays a critical role in regulating blood sugar levels. When food is consumed, the pancreas releases insulin into the bloodstream. This hormone allows glucose to enter cells, where it is used as a source of energy for muscle and other tissues (Alex *et al.*, 2024) Insulin reduces blood sugar levels by facilitating this process and also signals the liver to store excess glucose as glycogen.

When blood sugar levels drop, such as between meals, the pancreas reduces insulin secretion, and the liver breaks down glycogen to release glucose into the bloodstream, ensuring that glucose levels remain stable. In both Type 1 and Type 2 diabetes, this delicate balance is disrupted due to insufficient insulin production or improper insulin utilization. While genetic and environmental factors contribute to both types of diabetes, the exact causes remain unclear. Given the critical role of insulin in managing blood glucose levels and the widespread health impact of diabetes, there is a growing interest in alternative treatments that can complement or enhance current therapies. One promising area of research involves the use of plant-based compounds to provide natural antioxidant and anti-diabetic properties (Asha Monica *et al.*, 2024). This study focuses on evaluating the *in vitro* anti-diabetic and antioxidant activity of the ethanolic leaf extract of *Bruguiera cylindrica*, a mangrove plant, which may offer potential therapeutic benefits in the management of diabetes.

The present study aims to explore the anti-diabetic and antioxidant activity of the ethanolic leaf extract of *Bruguiera cylindrical* using *in vitro* models. The findings could offer insights into developing plant-based therapeutic interventions for managing diabetes and related oxidative stress. This study involves the extraction of plant compounds using a Soxhlet apparatus and the assessment of their bioactivity through biochemical assays. By doing so, it seeks to validate the potential of *Bruguiera cylindrica* in contributing to alternative treatments for diabetes.

Materials and Methods

Collection of Plant Material:

The leaves of *Bruguiera cylindrica* were collected from the Pichavaram mangrove forest, located near Chidambaram in the Cuddalore district of Tamil Nadu, India. The collection was carried out post-monsoon, after obtaining the necessary permissions from the forest office.

Extract Preparation:

The leaves of *Bruguiera cylindrica* were thoroughly cleaned and dried in a hot air oven at 50°C. Once dried, they were ground into a fine

powder. A Soxhlet apparatus was used for sequential extraction, employing 50 g of the powdered leaves and 150 ml of ethanol. This process was conducted from low to high polarity. Upon completion, the ethanolic extract demonstrated superior antioxidant and anti-diabetic activity, and thus, it was selected for further *in vitro* analysis (Asha Monica and Senthilkumar, 2020).

Antioxidant Activity:

DPPH Radical-Scavenging Activity: The radical-scavenging activity of the extract was assessed using the DPPH method. A total of 1,000 µl of the extract was mixed with 5,000 µl of a 0.1 mM DPPH solution in methanol. The mixture was incubated in the dark for 50 min at room temperature, and absorbance was measured at 517 nm. The DPPH radical-scavenging activity was calculated using the following formula (Asha Monica and Senthilkumar, 2020):

DPPH radical scavenging activity (%) = (($Abs_{control}$ – Abs_{sample})/ $Abs_{control}$)*100

Reducing Antioxidant Power: Reducing antioxidant power was measured by mixing different concentrations of the plant extract (200 ppm) with phosphate buffer and potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After centrifugation, the absorbance was measured at 700 nm. An increase in absorbance indicated enhanced reducing power (Banerjee *et al*., 2008).

Determination of Total Antioxidant Capacity: The total antioxidant capacity was determined by the phosphomolybdenum method. A 2 ml sample of the extract was mixed with 1 ml of reagent solution, incubated at 50°C for 90 min, and then measured at 695 nm. Results are expressed as ascorbic acid equivalents (AAE) in milligrams per gram of dry material (Karim *et al*., 2020).

In Vitro Anti-Diabetic Activity:

Alpha-Amylase Inhibition Assay: The alpha-amylase inhibition activity was assessed using a 0.02 M phosphate buffer (pH 6.9) and starch solution. Test samples and standard drugs (100-1,000 µg/ml) were incubated with alpha-amylase and starch (Ravikumar *et al*., 2011). After stopping the reaction with dinitrosalicylic acid, absorbance was measured at 540 nm. The percentage inhibition of alpha-amylase activity was calculated as:

Inhibition % = (Abs control-Abs sample)/Abs control x100

Glucose Adsorption Assay: The glucose adsorption capacity was determined by incubating the extract with glucose solutions of varying concentrations (5-30 mM) at 37°C for 6 h. The amount of bound glucose was measured by using a glucose oxidase peroxidase diagnostic kit.

Glucose Bound = Abs G0 – Abs G6 x Sample volume/ Sample weight

Where Abs GO =Absorbance of glucose at zero hour; Abs 6= Absorbance of glucose at six hour.

Glucose Diffusion Assay: The glucose diffusion retardation index (GDRI) was determined by dialyzing a glucose solution (20 mM) and extract sample. Glucose content in the dialysate was measured at different time intervals, and GDRI was calculated (Ravikumar *et al*., 2011).

GDRI=100 - Glucose content with the addition of fiber/Glucose content of the control X 100

Sucrase Inhibitory Activity: The sucrase inhibitory activity was evaluated by incubating different concentrations of the extract with sucrase enzyme and sucrose solution. After stopping the reaction, absorbance was measured at 540 nm. The percentage inhibition of sucrase activity was calculated as:

% inhibition = Abs control – Abs sample/Abs control x 100

In Vitro Glucose Uptake in Yeast Cells: The ability of the extract to enhance glucose uptake in yeast cells was tested by incubating the extract with glucose solutions and yeast suspension at 37°C. After incubation, glucose concentration was measured in the supernatant, and the percentage inhibition was calculated:

% inhibition =Abs control –Abs sample/Abs control x 100

Results

Antioxidant Activity:

In the DPPH radical-scavenging activity assay, the plant extract demonstrated a concentration-dependent increase in activity. At 100 µg concentration, the radical-scavenging activity was 75.5%, which increased to 86.9% at 500 µg. Comparatively, the standard ascorbic acid at 50 µg and 100 µg concentrations showed radical-scavenging activities of 0.07% and 0.08%, respectively (Fig. 1).

In the FRAP (Ferric Reducing Antioxidant Power) assay, the plant extract showed increasing antioxidant power as concentration increased. At 100 µg, it exhibited 54.6% inhibition, which increased to 68.2% at 500 µg. In contrast, ascorbic acid at 150 µg and 250 µg demonstrated much lower inhibition rates of 0.54% and 0.58%, respectively (Fig. 2).

For the Total Antioxidant Capacity assay, the plant extract at 50 µg demonstrated 31.3% inhibition, which increased to 73.2% at 200 µg. The ascorbic acid standard, at 50 µg and 100 µg, exhibited inhibition rates of 0.18% and 0.22%, respectively.

Anti-Diabetic Activity:

The plant extract's ability to inhibit alpha-amylase was concentration-dependent. At 0.05 ml, the inhibition was 10.11%, which increased to 64% at 0.20 ml (Fig. 3).

For glucose adsorption, the plant extract showed increasing glucose binding capacity with higher glucose concentrations. At 5 mM, the glucose binding capacity was 13.16%, which rose to 51.78% at 100 mM.

In the glucose diffusion retardation assay, the plant extract demonstrated time-dependent retardation of glucose diffusion. At 30 min, the GDRI was 14.28%, which increased to 58.33% at 120 min (Fig.4).

The plant extract exhibited dose-dependent inhibition of sucrase activity. At 0.05 ml concentration, the inhibition was 20.63%, which increased to 61.90% at 0.25 ml.

In the glucose uptake by yeast cells assay, the plant extract enhanced glucose uptake, with inhibition rates of 9.25% at 0.10 ml and 53.70% at 0.80 ml.

Discussion

Antioxidant Activity:

The antioxidant activity of the *Bruguiera cylindrica* extract was evaluated using three different assays. In the DPPH radical-scavenging assay, the plant extract exhibited significant radical scavenging activity, which increased with concentration (Tung and Thuy, 2022). This suggests the presence of potent antioxidants capable of neutralizing free radicals, potentially preventing oxidative damage. The comparison with ascorbic acid highlights the plant extract's superior antioxidant potential. In the FRAP assay, the plant extract demonstrated strong reducing power, again showing a concentration-dependent increase. This result confirms the extract’s ability to donate electrons and neutralize reactive species, important in mitigating oxidative stress (Tung and Thuy, 2022).

The Total Antioxidant Capacity assay further confirmed the extract's antioxidant potential, with a maximum inhibition of 73.2% at 200 µg (Vinoth *et al.*, 2019). This broad-spectrum antioxidant activity suggests that the plant extract contains various phytochemicals, such as phenolics and flavonoids, contributing to its protective effects against oxidative stress.

Anti-Diabetic Activity:

The anti-diabetic activity of the *Bruguiera cylindrica* extract was demonstrated through multiple mechanisms. The extract showed a concentration-dependent inhibition of alpha-amylase, an enzyme involved in the breakdown of starch into glucose. By inhibiting alpha-amylase, the extract may help slow glucose release into the

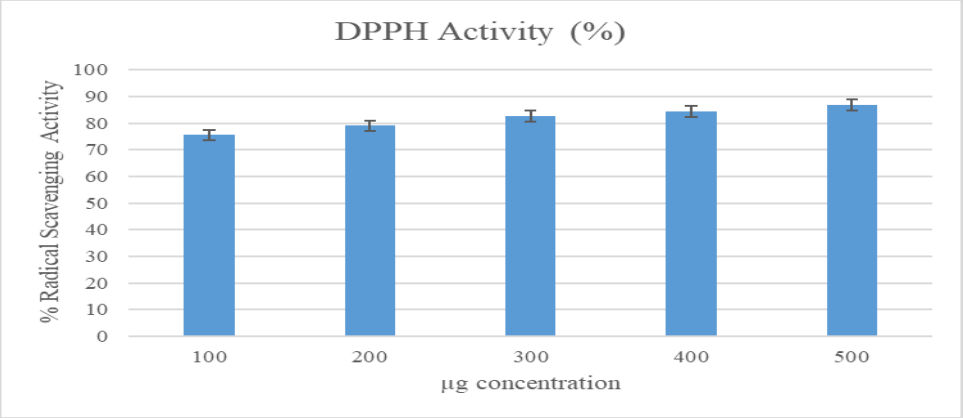

Fig. 1: DPPH radical-scavenging activity.

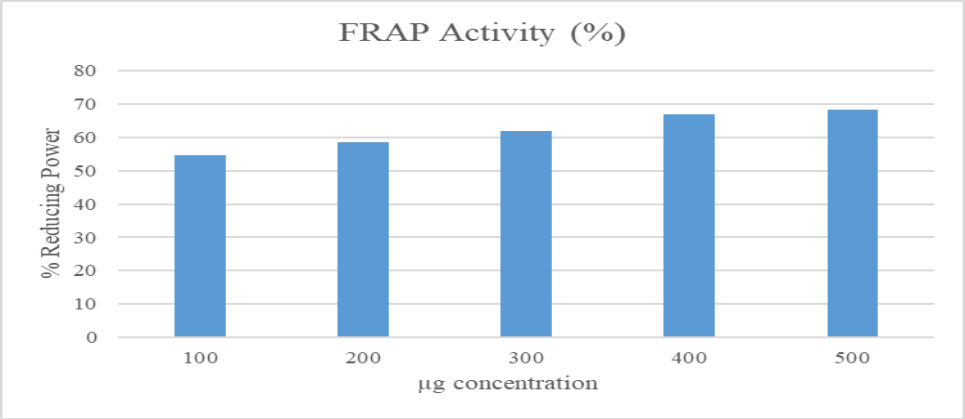

Fig. 2: Reducing antioxidant power.

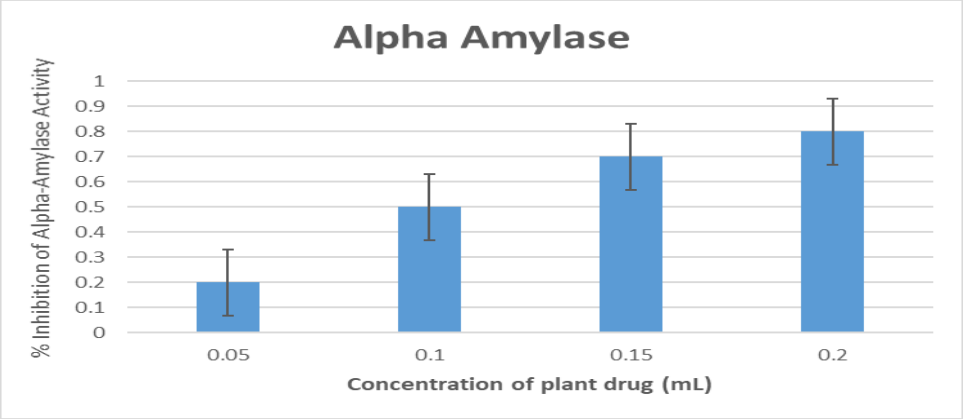

Fig. 3: Effect of plant drug on inhibitory activity of alpha-amylase.

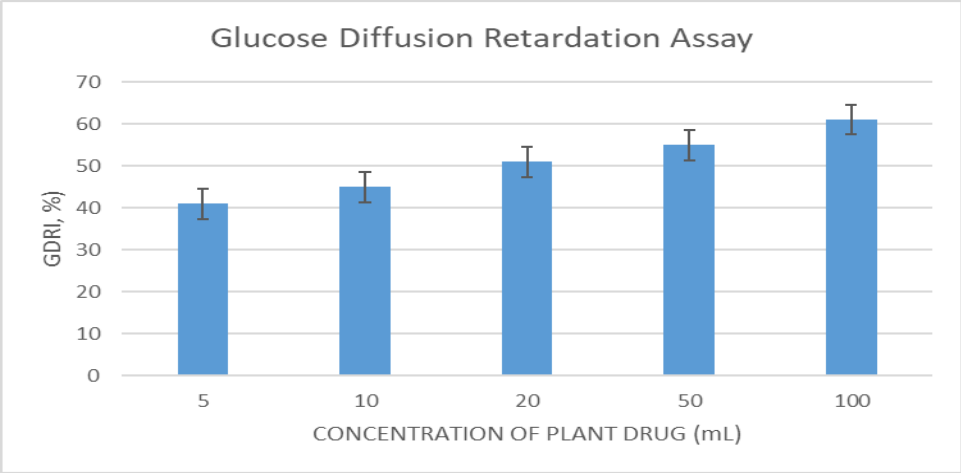

Fig. 4: Glucose diffusion retardation assay.

Blood stream, aiding in the control of postprandial glucose levels.

The glucose adsorption capacity of the extract was directly proportional to glucose concentration, with higher glucose concentrations resulting in greater glucose binding. This suggests that the plant extract may reduce glucose absorption in the gastrointestinal tract, thereby lowering post-prandial glucose levels (Zhuang *et al.*, 2012).

In the glucose diffusion retardation assay, the plant extract significantly slowed glucose diffusion over time. The retardation of glucose diffusion suggests that the plant extract may help slow glucose absorption, reducing post-meal glucose spikes. This effect may be due to the plant's fiber content, which acts as a physical barrier to glucose diffusion.

The extract also exhibited significant sucrase inhibitory activity, with a dose-dependent reduction in sucrase function. This inhibition may reduce the rate at which sucrose is broken down into glucose and fructose, further contributing to better glucose control.

In the glucose uptake by yeast cells assay, the plant extract enhanced glucose uptake, suggesting it may promote glucose utilization and reduce circulating glucose levels. This property supports the plant's potential as a natural remedy for managing diabetes (Zhuang *et al.*, 2012).

Conclusion

The *Bruguiera cylindrica* extract demonstrated strong antioxidant and anti-diabetic properties in the conducted assays. The extract exhibited potent radical-scavenging, reducing, and total antioxidant capacities, indicating its potential to protect against oxidative stress. Furthermore, the extract showed promising anti-diabetic activity by inhibiting carbohydrate-metabolizing enzymes, reducing glucose absorption, slowing glucose diffusion, and enhancing glucose uptake. These findings suggest that *Bruguiera cylindrica* could be a valuable natural source for managing oxidative stress and hyperglycemia associated with diabetes.

Ethical Statement

No animal has been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors thank DST-FIST, Government of India, for funding for infrastructure and instrumentation at ACIC, St. Joseph's College (Autonomous), Tiruchirappalli, India.

Conflict of Interest

The authors declare no conflicts of interest.

References

Abeysinghe PD. (2010) Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. Indian J Pharmaceut Sci. 72(2): 167.

Acharya S, Patra DK, Pradhan C and Mohapatra PK. (2020) Anti-bacterial, anti-fungal and anti-oxidative properties of different extracts of *Bruguiera gymnorrhiza* L. (Mangrove). European J Integrat Med. 36: 101140.

Alex AM, Subburaman S, Chauhan S, Ahuja V, Abdi G. and Tarighat MA. (2024) Green synthesis of silver nanoparticle prepared with Ocimum species and assessment of anticancer potential. Sci Rep. 14(1): 11707.

Asha Monica AR, Kumar S, Swabna V and Arockiasamy E. (2024) Green synthesis and characterization of silver nanoparticles from *Santalum album* and their antimicrobial antioxidant and anticancer activity. Intern J Zool Invest. 10(1): 355-363.

Asha Monica A and Senthilkumar RS. (2020) Green synthesis and characterization of silver nanoparticles from *Ocimum basilicum* and their antimicrobial antioxidant and anticancer activity. Res J Pharm Technol. 13(12): 5711-5715.

Banerjee D, Chakrabarti S, Hazra AK, Banerjee S, Ray J and Mukherjee B. (2008) Antioxidant activity and total phenolics of some mangroves in Sundarbans. African J Biotechnol. 7(6): 805-810.

Karim MA, Islam MA, Islam MM, Rahman MS, Sultana S, Biswas S, Mohammad JS, Kishor MM, Rahman MM and Hasan MN. (2020) Evaluation of antioxidant, anti-hemolytic, cytotoxic effects and anti-bacterial activity of selected mangrove plants (*Bruguiera gymnorrhiza* and *Heritiera littoralis*) in Bangladesh. Clin Phytosci. 6: 1-12.

Ravikumar, S., Ali, M. S., Ramu, A., &Ferosekhan, M. (2011). Antibacterial activity of chosen mangrove plants against bacterial specified pathogens. *World Applied Sciences Journal*, *14*(8), 1198-1202.

Tung BT and Le Thuy NT. (2022) Antioxidant activity of isolated compounds from ethyl acetate extract of *Bruguiera parviflora*. Ho Chi Minh City Open Univ J Sci Engineer Technol. 12(2): 42-49.

Vinoth R, Kumaravel S and Ranganathan R. (2019) Therapeutic and traditional uses of mangrove plants. J Drug Deliv Therapeut. 9(4-s): 849-854.

Zhuang Y, Zhang Y and Sun L. (2012) Characteristics of fibre-rich powder and antioxidant activity of pitaya (*Hylocereus undatus*) peels. Int J Food Sci Technol. 47(6): 1279-1285.