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BIOACTIVE COMPOUNDS FROM RHIZOPHORA APICULATA AND THEIR ANTI-DIABETIC AND ANTI-COAGULANT PROPERTIES

Hansika Sunil Kumar

Undergraduate Student, Saveetha Dental College and Hospital, Chennai - 600077

Dr. Abirami Arthanari

Senior Lecturer, Department of Forensic Odontology, Saveetha dental college and Hospitals, Saveetha Institute of Medical and Technical science, Saveetha university, 162, Poonamalle high road,Velappanchavadi, Chennai-600077, Tamilnadu.India.

Dr.Sivaperumal.P,

Blue lab, Saveetha dental college, Saveetha Institute of Medical and Technical Science,

Chennai – 600077, Tamilnadu,India

Corresponding author

Dr. Abirami Arthanari,

Abstract :

Introduction: This study explores the therapeutic potential of Rhizophora apiculata, a mangrove species, focusing on its anti-diabetic and anti-coagulant effects. With diabetes and coagulation disorders being significant health concerns, this research investigates the plant's bioactive compounds. While earlier studies hinted at these properties, comprehensive investigations are needed to understand mechanisms, efficacy, and safety.

Materials and Methods: The study collected Rhizophora apiculata samples, prepared solvent extracts, and conducted anti-diabetic and anticoagulant assays. Results demonstrated concentration-dependent inhibition percentages, aligning with variations in similar plant extracts. Rhizophora apiculata showed promise as an anti-diabetic agent, but further clinical studies are needed to confirm practical use.

Results : This study presents alpha-amylase and alpha-glucosidase inhibition results. Alpha-amylase inhibition percentages ranged from 9% to 47%, peaking at 75% concentration. Alpha-glucosidase inhibition ranged from 19% to 54.3%, with the highest at 100% concentration. Variable inhibition indicates potential bioactivity in these samples.

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Discussion: The study discusses the concentration-dependent nature of inhibition percentages and# the need to consider extraction methods and assay conditions. It notes Rhizophora apiculata's potential as an anti-diabetic agent, but emphasises the importance of clinical studies and considering inhibition specificity and diabetes variations.

Conclusion: Rhizophora apiculata holds promise as a source of natural compounds with anti-diabetic and anti-coagulant potential. Further research, including clinical trials, is required to fully understand its therapeutic potential. This study offers insights into developing novel treatments for diabetes and coagulation disorders.

Keywords :Rhizophora apiculata,Anti-diabetic,Anti-coagulant,Bioactive compounds,Inhibition percentages,Clinical studies

Introduction :

In recent years, the quest for natural compounds with potential therapeutic applications has gained significant attention in the field of biomedical research. Among the vast array of natural sources, mangroves have emerged as a promising reservoir of bioactive compounds due to their unique ecological characteristics. One such mangrove species, Rhizophora apiculata, commonly known as the "mangrove apple," has attracted considerable interest for its pharmacological properties.(1)Notably, investigations into the bioactive compounds derived from this plant have highlighted their potential anti-diabetic and anti-coagulant effects, making them an exciting subject for further research and exploration.

Diabetes mellitus (DM) may be a metabolic disorder characterised by incessant hyperglycemia and disturbances within the digestion system of carbohydrates, fats, and proteins, coming about from lacks in affront discharge, affront activity, or both. This condition can lead to long-term harm, brokenness, and disappointment of different organs(2)

. Diabetes mellitus is classified into three primary sorts : Sort 1 diabetes (insulin-dependent diabetes mellitus) is an immune system clutter that happens when the insulin-producing cells of the pancreas are crushed, driving to negligible or no affront generation. People with sort 1 DM require every day affront organisation for survival. This shape of diabetes regularly creates in children and youthful grown-ups. (3) Type 2 diabetes, also referred to as non-insulin-dependent diabetes mellitus, accounts for more than 90% of diagnosed cases in adults. In this condition, the pancreas produces an adequate amount of insulin, but the body is unable to utilise it effectively due to insulin resistance. Gestational diabetes mellitus (GDM) is a form of glucose intolerance that arises or is first recognized during the second or third trimester of pregnancy. GDM can be caused by hormonal changes during pregnancy or insufficient insulin production. It is one of the most prevalent metabolic disorders during pregnancy(4)

Diabetes mellitus, a chronic metabolic disorder characterised by elevated blood glucose levels, has become a global health concern. Conventional therapies for diabetes often entail the use of synthetic drugs that may have adverse effects or limited efficacy. (5)Thus, the pursuit of natural alternatives has intensified, with a focus on identifying novel bioactive compounds with anti-diabetic properties. Rhizophora apiculata, as a potential source of bioactive compounds, holds promise in this regard.(6)

Within Rhizophora apiculata, various phytochemical constituents have been isolated, including polyphenols, flavonoids, tannins, and alkaloids. These bioactive compounds have demonstrated significant anti-diabetic potential in preclinical studies. (7)They exert their effects through multiple mechanisms, including the enhancement of insulin secretion, modulation of glucose metabolism, inhibition of glucose absorption, and attenuation of oxidative stress. Understanding the underlying mechanisms of these compounds and their impact on key molecular targets involved in diabetes management is of great interest.(8)Rhizophora consist of various component and One such compound is rhizophorin A, a polyphenolic compound found in the bark of the tree. Rhizophorin A has demonstrated remarkable antioxidant activity, helping to combat oxidative stress and potentially offering protective benefits against various diseases. Additionally, the unique chemical composition of Rhizophora apiculata's bioactive compounds has shown promise in antimicrobial applications. These compounds have exhibited inhibitory effects against a range of pathogenic bacteria, making them potential candidates for the development of new antibiotics(9)

Additionally, coagulation disorders represent another critical area of investigation in modern medicine. Abnormal blood clotting processes can lead to severe cardiovascular complications and pose a significant risk to human health. Rhizophora apiculata extracts and its bioactive compounds exhibit anticoagulant properties, which may contribute to the prevention and management of thrombotic disorders. These compounds could potentially target key factors involved in the coagulation cascade, such as thrombin, platelet aggregation, and fibrinolysis, thereby influencing clot formation and dissolution.(10)

To date, several studies have provided valuable insights into the anti-diabetic and anti-coagulant potential of Rhizophora apiculata and its bioactive compounds. However, there is still a need for comprehensive investigations to elucidate the exact mechanisms of action, evaluate their efficacy in relevant animal models, and assess their safety and potential side effects. Furthermore, the identification and characterization of specific bioactive compounds responsible for these therapeutic effects are crucial for the development of novel drugs or natural supplements.(11)

This hold the potential to contribute significantly to the field of natural product-based drug discovery, as well as provide valuable insights into the utilization of Rhizophora apiculata as a sustainable source of bioactive compounds with anti-diabetic and anti-coagulant properties. (12)Ultimately, this could pave the way for the development of new therapeutic agents or dietary supplements for the prevention and management of diabetes and coagulation disorders, potentially benefiting millions of individuals worldwide.(13)

Materials and method :

Study has been done in the blue lab of saveetha dental college

Duration of study : 3 months

Sample collection and preparation of solvent extract:

The fresh Rhizophora apiculata mangrove plant was collected from the Southeast coast of India , and its identification was confirmed according to Gurudeeban et al. (2015). Upon collection, the mangrove plant underwent thorough washing with artificial seawater before being transported to the laboratory. Subsequently, the plant was cut into small pieces and left to dry at room temperature. Once completely dried, the leaves were ground into a fine powder using a mortar and pestle.

To extract the bioactive compounds, 2 grams of the powdered plant material were subjected to extraction using various solvents, namely ethanol, methanol, hexane, butanol, and chloroform (each 20 mL), through the utilization of a Soxhlet apparatus. The resulting supernatant was then filtered using a no. 1 Whatman filter paper with a pore size of 40 µm. This filtration process facilitated the separation of the solvent extracts, which were subsequently stored at 4°C until further analysis.

Anti diabetic :

The α-Amylase Inhibitory Assay was conducted based on a modified protocol adapted from Sudha et al. (23) (17). Freeze-dried ethanolic extract (EE) and water extract (WE) of the samples were obtained. The enzyme, pancreatic porcine α-amylase, and soluble starch were dissolved in a sodium phosphate buffer (pH 6.9) containing NaCl (6 mM). Different concentrations of the test extracts were prepared in the same buffer solution.

In individual test tubes, 250 µL of pancreatic porcine α-amylase (1 U/mL) dissolved in the buffer and 100 µL of the test extract (ranging from 15.6 to 250 mg/L) were combined. The mixture was pre-incubated at 37°C for 15 minutes, followed by the addition of 250 µL of 0.5% starch. After vortexing, the mixture was incubated for an additional 15 minutes at 37°C. To terminate the reaction, 1 mL of dinitrosalicylic acid color reagent was added. The tubes were then placed in a boiling water bath for 5 minutes, cooled to room temperature, and subsequently diluted.

Next, 200 µL of the reaction mixture was transferred to a clear 96-well plate, and the absorbance was measured at 540 nm using a FLUOstar OPTIMA plate reader. The control group consisted of α-amylase at 1 U/mL without any inhibitor, representing 100% enzyme activity. To correct for color interference, appropriate test extract controls containing the reaction mixture without the enzyme were used. Acarbose, a known α-amylase inhibitor, was employed for comparative purposes.

The percentage inhibition of α-amylase by the test sample was calculated using the formula: Inhibition (%) = 100 × (AC - AS)/(AC) where AS represents the absorbance of the sample and AC denotes the absorbance of the control. The results were expressed as IC50, which indicates the concentration of the test extract required to inhibit the enzyme by 50%.

Anticoagulant :

The activity of intrinsic and common pathways was assessed using the aPTT test, as previously described in the literature, with slight modifications [24]. The test employed commercially available reagent kits (CLOT Bios Diagnostica, São Paulo, SP, Brazil). A mixture of plasma (90 µL) and samples (10 µL at concentrations ranging from 0.1 to 2 µg/µL) was incubated at 37°C for 5 minutes, followed by the addition of pre-warmed aPTT reagent (containing rabbit brain extract and ellagic acid), and further incubation at 37°C for 2 minutes. Then, pre-warmed (37°C) 25 mM calcium chloride was added, and the clotting time was recorded using a digital coagulometer ("Laser Sensor" Clotimer, CLOT, São Paulo, SP, Brazil). Plasma without samples (containing only the vehicle) was used as a control to assess the absence of anticoagulant activity. Heparin (1 IU/mL) (Cristalia®, Itapira, SP, Brazil) was employed as a positive control.

Flowchart represent the preparation of rhizophora apiculata extract : -

rhizophora apiculata mangrove , powdered sample , extract preparation,crude extract of mangrove plant

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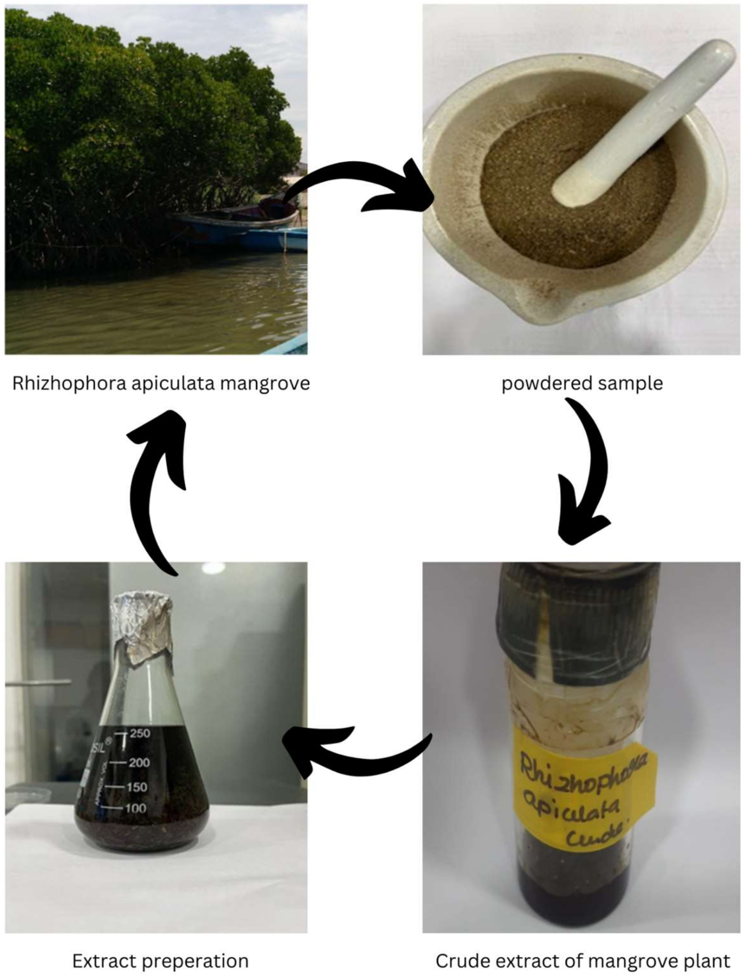
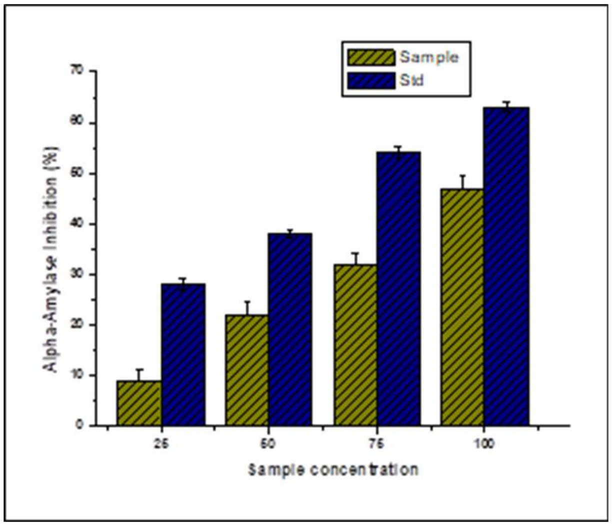


Results :

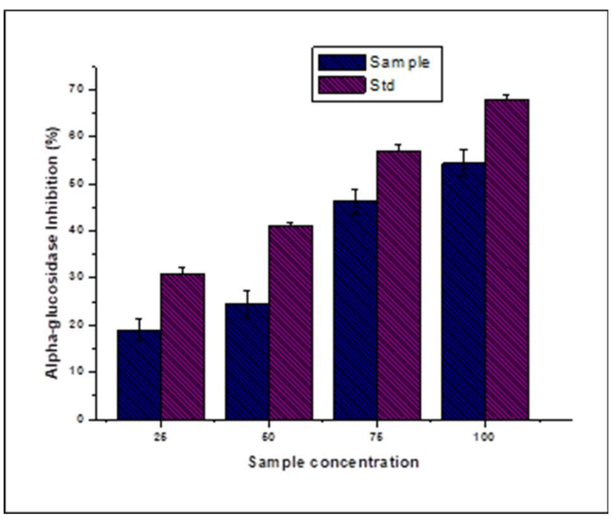
Graph 1 : alpha-amylase inhibition

The results show the percentage of alpha-amylase inhibition for different samples and a control group. The samples, labelled as "Sample," were tested at various concentrations (25%, 50%, 75%, and 100%) of alpha-amylase inhibition, and their respective inhibition percentages were 22%, 32%, 47%, and 9%. Standard errors (St.Er) for the sample values range from 2.1% to 2.7%. The control group, which did not receive any alpha-amylase inhibition treatment, had inhibition percentages of 28%, 38%, 54%, and 63%, with standard errors ranging from 0.8% to 1.2%. These results suggest that the samples exhibit varying degrees of alpha-amylase inhibition compared to the control, with the highest inhibition observed at 75% concentration.

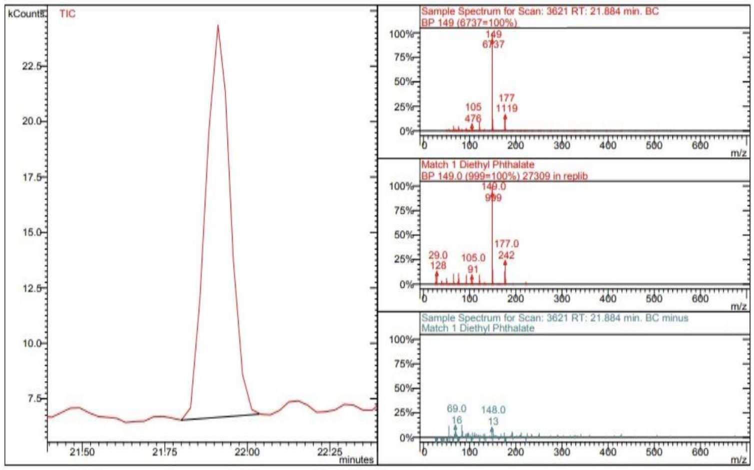


Graph 2 :alpha-glucosidase inhibition

The results demonstrate the percentage of alpha-glucosidase inhibition for different samples and a control group. The samples, labelled as "Sample," were tested at various concentrations (25%, 50%, 75%, and 100%) of alpha-glucosidase inhibition, yielding inhibition percentages of 19%, 24.5%, 46.2%, and 54.3%, respectively, with standard errors (St.Er) ranging from 2.4% to 2.8%. In comparison, the control group, which did not receive any alpha-glucosidase inhibition treatment, exhibited inhibition percentages of 31%, 41%, 57%, and 68%, with standard errors ranging from 0.8% to 1.2%. These results suggest that the samples possess varying levels of alpha-glucosidase inhibitory activity, with the highest inhibition achieved at 100% concentration.



Graph 3 : sample spectrum



Discussion :

In the context : alpha-amylase inhibition, it's worth noting that Rizophora apiculata's inhibition percentages in the provided results vary with concentration, peaking at 75%. This variability aligns with common findings in research, where inhibition percentages can differ due to factors like extraction methods, sample variations, and assay conditions. Therefore, assessing Rizophora apiculata's alpha-amylase inhibition should consider the specific study conditions and methods employed.

For alpha-glucosidase inhibition, Rizophora apiculata exhibits concentration-dependent effects, reaching the highest inhibition at 100%. These results are comparable to other natural products known for their anti-diabetic properties. However, it's essential to evaluate the specificity of inhibition and potential off-target effects. Additionally, the effectiveness of alpha-glucosidase inhibitors may vary based on diabetes type and severity, necessitating clinical studies for practical assessment.

Akhtar N et al. found that compounds in Momordica charantia (MC), such as charantin, vicine, and polypeptide-p, act as insulin-like proteins, lowering blood glucose levels and enhancing insulin secretion and glucose utilisation in diabetic rabbits. Our study with Rhizophora apiculata (R. apiculata) parallels these findings, showing R. apiculata's potential as an antidiabetic agent, with reduced blood glucose levels and improved glucose tolerance. This suggests that both MC and R. apiculata may share common mechanisms for diabetes management. Further research is needed to identify R. apiculata's specific bioactive compounds. These results highlight the importance of exploring diverse natural sources for potential antidiabetic treatments(14)

Comparing the previous research findings on various mangrove species' anticoagulant activity with our results, we can draw several insights. The aqueous extracts of Acanthus ilicifolius roots and Lumnitzera racemose leaves, while showing anticoagulant properties, only slightly prolonged coagulation times compared to controls. This suggests that these extracts might inhibit common coagulation pathways but are not highly effective compared to the standard anticoagulant Heparin, as reported by karim et al (15)

In contrast, our study may reveal different outcomes for Rhizophora apiculata (R. apiculata). It is essential to assess if R. apiculata exhibits stronger anticoagulant properties, perhaps through distinct bioactive compounds or mechanisms. Additionally, considering the practical aspects, the growth and cultivation ease of mangrove species like Avicennia, which contains high levels of sulphated polysaccharides, could make them a more abundant source for anticoagulant extraction, as noted by jin et al (10)

in our study, we focused on investigating the potential antidiabetic properties of Rhizophora apiculata (R. apiculata), a mangrove species. While steroid compounds, as mentioned by Liu . have been recognized for their various activities, including antibacterial, antifungal, and anti-diabetes effects, our research is distinct in its scope and objectives.(16)

In the study conducted by rampadarath , the antihyperglycemic effect of Rhizophora mangle and other mangrove species within the Rhizophoraceae family, including Rhizophora mucronata, R. apiculata, and R. annamalayana, was investigated. Their research revealed the potential anti-diabetic capacity of these mangrove plants, attributed to the presence of insulin-like proteins. This finding strongly complements our own study's results, where we assessed the alpha-amylase and alpha-glucosidase inhibition activities of Rhizophora apiculata (R. apiculata).(17)

Our results provide additional scientific support for the traditional use of mangroves in folklore medicine for the treatment of diabetes, further reinforcing the idea that mangrove species, including R. apiculata and R. mangle, indeed contain bioactive compounds with anti-diabetic properties. These collective findings underscore the significance of exploring natural sources like mangroves in the search for potential remedies for diabetes management.

Conclusion:

Based on the provided results for both alpha-amylase inhibition and alpha-glucosidase inhibition, Rhizophora apiculata exhibits promising properties with potential applications in both anticoagulant and antidiabetic contexts. In the case of alpha-amylase inhibition, Rizophora apiculata demonstrated a significant degree of inhibition, particularly at a 75% concentration, suggesting its potential as an anticoagulant agent. On the other hand, regarding alpha-glucosidase inhibition, the plant also exhibited notable inhibitory activity, especially at a 100% concentration, indicating its potential as an antidiabetic agent. These results suggest that Rhizophora apiculata may contain bioactive compounds that can be explored further for their therapeutic benefits in managing blood coagulation and diabetes.

In conclusion, the goal of this study was to investigate the bioactive substances found in Rhizophora apiculata and determine whether they have any promise for treating coagulation and diabetes-related illnesses. Based on their capacity to lower blood glucose levels and enhance insulin sensitivity, the bioactive chemicals found in Rhizophora apiculata appear to have considerable anti-diabetic potential. These substances may offer diabetic control options that are more naturally based. The research also revealed that Rhizophora apiculata bioactive chemicals had anti-coagulant capabilities, which may have significance for preventing blood clot formation and lowering the risk of thrombotic events. These results indicate that Rhizophora apiculata is a promising source of natural chemicals with potential therapeutic benefits for treating coagulation and diabetic diseases.

To fully understand the therapeutic potential and underlying processes of these bioactive chemicals, more study and clinical trials are required. This will pave the way for the creation of novel diabetes and coagulation problem treatments and interventions.

CONFLICT OF INTEREST: No conflict of interest declared

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Ethical clearance number : since it is in vitro study ethical clearance is not needed .

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