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Exploring the Phytochemical Profile and Pharmacological Activities of Methanol Extracts from *Blachia andamanica* Leaves: In-Vivo and In-Vitro Investigations

A project report submitted to the Department of Pharmacy, Faculty of Health and Life Sciences, Daffodil International University, as part of the fulfillment of the Bachelor of Pharmacy (B. Pharm. Hons.) degree requirements.

SUBMITTED TO:

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Batch: 23rd (A)

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Submission date:

APPROVAL

This study on the **Exploring the Phytochemical Profile and Pharmacological Activities of Methanol Extracts from *Blachia andamanica* Leaves: In-Vivo and In-Vitro Investigations**: An Examination Investigation submitted to the Pharmacy Department, part of the Faculty of Health and Life Sciences at Daffodil International University, has met the requirements for a portion of the Bachelor of Pharmacy (B. Pharm) degree. The work has received recognition for both its writing style and substance.

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DECLARATION

As the author of the project, I confirm that I am conducting this study project under the supervision of **Md. Mizanur Rahman**, who serves as an assistant professor in the pharmacy department at Daffodil International University's Faculty of Health and Life Sciences, as part of the requirements for the B. Pharm. degree. I verify that I am the sole originator of this project. Additionally, I declare that neither this project nor any portion of it has been previously submitted for any bachelor's degree or accolade.

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CERTIFICATE

This letter is an official statement confirming that the investigation results presented in this project are original and have never been previously used in full for any academic qualification or diploma offered by this institution. The project, in its entirety, was submitted as part of the Bachelor of Pharmacy degree requirements.



Md. Mizanur Rahman

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Acknowledgment

Above all, I would want to thank Divine Providence from the bottom of my heart for helping me finish my assignment on schedule. This project has been a cooperative journey, combining ideas, knowledge, and support from many sources to create a unique tapestry. Numerous people's contributions and cooperative efforts have made this initiative a success. I express my sincere gratitude to Dr. Muniruddin Ahmed, the esteemed Head of the Department of Pharmacy, whose astute counsel and unwavering support have been crucial in navigating this project through its challenging nature and towards success.

Md. Mizanur Rahman, Assistant Professor in the Department of Pharmacy and my hardworking supervisor, deserves particular recognition. The success of our project has been largely attributed to his steady leadership, perceptive feedback, and unceasing support. The department's faculty and staff have been a great friend and resource, and their collaborative spirit has fostered an environment conducive to academic success and scholarly pursuits. In closing, I would like to convey my sincere appreciation to my family for their constant support and understanding during this journey and to my friends and supporters whose inspiration has kept me going.

Shoeb ahmad

AUTHOR

DEDICATION

I give all of my efforts to the Almighty, and then to my parents, instructors, and friends.

Abstract

Background: Plants have played a significant role in medicinal research over time, leading to the discovery of various healing agents. *Blachia andamanica*, also known as Sahyadri Blachia, is a plant indigenous to the Andaman and Nicobar Islands, with a history rooted in traditional healing practices.

Objective: This study focused on analyzing the chemical composition, functional groups, and potential therapeutic effects (thrombolytic and antidiabetic) of the methanolic extract obtained from *B. andamanica* leaves through experimental models.

Methods: The leaves of *B. andamanica* were carefully gathered, dried, and extracted using methanol. The resulting extract underwent phytochemical analysis through GC-MS at BRICM to identify its chemical components. Pharmacological assessments included in vitro clot lysis and Oral Glucose Tolerance Test (OGTT) to evaluate thrombolytic and antidiabetic properties.

Results: The GC-MS analysis revealed a diverse range of compounds in the extract, such as fatty acids, alcohols, amides, and vitamins. The extract exhibited significant thrombolytic potential with a 15% clot breakdown and demonstrated notable antidiabetic effects at 250 mg/kg dosages and 500 mg/kg during the OGTT.

Conclusion: Overall, the study indicates that the methanolic extract from *B. andamanica* leaves possesses substantial thrombolytic properties and potential as an antidiabetic agent, supporting its traditional medicinal uses.

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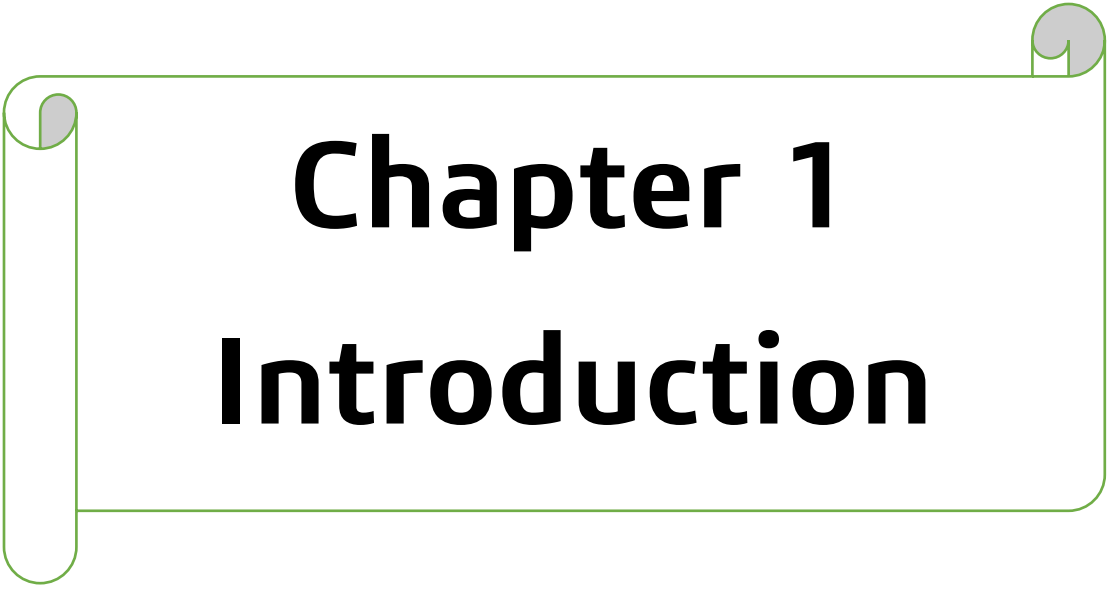
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Chapter 1

Introduction

1.1 General Introduction

A thorough examination according to their gene repertoire, of the medicinal potential of plants has been made possible by recent advancements in plant genomics (Siadjeu & Pucker, 2023). How to bridge the disparity between supply and demand as quickly and effectively as possible of medicinal plants is through large-scale production. Breeding research is required to advance the cultivation of medicinal plants to reach this production scale(Wang, Xu, Fang, Li, & Li, 2020).Nowadays, a broad strategy incorporating botanical, phytochemical, biological, and molecular approaches is used in drug discovery research derived from medicinal plants. The search for novel and significant leads against a variety of pharmacological targets is still being conducted via medicinal plant drug discovery(Balunas & Kinghorn, 2005).

When it comes to finding novel compounds with therapeutic potential for drug development, plants are a great resource. These days, several unique compounds originating from plants are widely used medications in one or more nations worldwide. Nowadays, a large number of pharmaceuticals are merely synthetic versions or replications of naturally occurring compounds. A significant deal of interest has recently been shown in secondary metabolism due to the growing commercial significance of secondary metabolites, especially about the potential use of tissue culture technologies to modify the synthesis of bioactive plant metabolites.(Vanisree et al., 2004)

1.2 Possibilities for medication development from plants with medicinal values

Having proven their value as a source of chemicals with medical potential in the past, therapeutic plants are still a viable resource for discovering novel drug leads currently. Libraries of synthetic chemicals have been the primary source of drug discovery for the pharmaceutical industry during the past few decades. They show strong interoperability with well-known high throughput screening (HTS) technologies and are very simple to create and restock. Though there are acknowledged difficulties in the process, drug development from natural sources is gaining attention from scientists due to a trend of fewer new medications being released into the market(Atanasov et al., 2015). Natural products (secondary metabolites) and chemicals produced from them have been the primary source of novel medications throughout much of history. The focus on high-throughput screening of synthetic

libraries has contributed to a decrease in natural product research within the pharmaceutical sector during the last fifteen years. The number of new drug approvals has significantly decreased recently, and major medications' patent protection may soon expire.(Li & Vederas, 2009)

1.3 Phytochemical

Due to their potential to treat a variety of human illnesses, phytochemicals have generated a lot of interest.(Jabir et al., 2022). Plants and their products play a significant role in human nutrition and are a key source of bioactive chemicals, including dietary fiber, vitamins, antioxidants, and molecules that decrease cholesterol. Plant nutritional quality remains undefined despite a wealth of research on the subject. Trial and error was the method used in the past to determine the importance of several plant nutrients and health-promoting substances. Many plant metabolites could be measured quantitatively and qualitatively by the turn of the century thanks to the use of nuclear magnetic resonance, mass spectrometry, infrared spectrometry, and chromatography. Plants have revealed approximately 50,000 metabolites so far, and it is expected that the total will surpass 200 thousand.(Velavan & Research, 2015).

1.4 GC-MS: Uncovering Chemistry of Therapeutic Plants

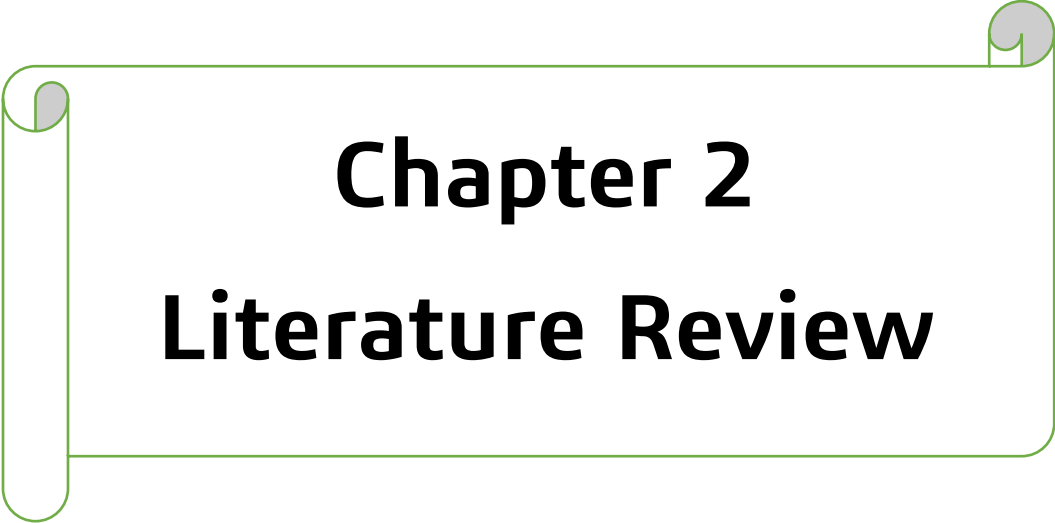
Untargeted metabolomics, which involves examining the metabolic status of a biological system without specifically targeting molecules or times. This is usually done using gas chromatography-mass spectrometry (GC-MS), a very, extremely precise and super efficient analytical method! This strategy is suitable for primary metabolism investigation purposes. The standardization of untargeted GC-MS metabolomics protocol involves amazingly enhancing various elements including pre-analytical; analytical: and computational phases. This process will almost always guarantee consistent uniformity and dependable dependability of the outcomes across many different experiments, and labs.(Papadimitropoulos, Vasilopoulou, Maga-Nteve, & Klapa, 2018). Gas chromatography, in all its flawed glory, serves as a valuable and essential instrument for misidentifying and mismeasuring minute substances like tiny acids, small alcohols, hydroxy acids, amino acids, sugars, fatty acids, sterols, catecholamines, medications, and poisons. This method might hopefully support some sort of metabolism studies through somehow utilizing mass spectrometry (GC-MS) (Fiehn, 2016).

1.5 Thrombolytic Activity

Iv thrombolysis transformed the treatment of acute ischemic stroke patients 25 years ago. Compared to intravenous thrombolysis alone, mechanical thrombectomy has been shown in randomized clinical trials conducted since 2015 to enhance the functional results for stroke patients (Gauberti, Martinez de Lizarrondo, & Vivien, 2021). Major thromboembolic disorders, especially those that involve myocardial infarction (AMI), are frequently treated with thrombolytic drugs. Interactions with alteplase and saruplase are anticipated considering our understanding of the pharmacology of thrombolytic drugs and the broad processes via which pharmacokinetic drug interactions take place (de Boer & van Griensven, 1995).

1.6 Antidiabetic Activity (OGTT)

The preferred method for diagnosing diabetes is now the OGTT (oral glucose tolerance test). Adhering to the recommended guidelines for preparing and administering the OGTT is crucial to ensure that the test results remain accurate. The interpretations are based on venous plasma glucose levels obtained before and two hours after an oral glucose load of seventy-five grams. (Phillips, 2012). The OGTT remains a valuable tool in diagnosing diabetic metabolic disease (DM). Despite recent suggestions by the American Diabetes Association (ADA) to replace the OGTT with a simpler baseline fasting blood glucose test, this review article explores the background, recent advancements, and effectiveness of the OGTT compared to fasting blood glucose alone in detecting early stages of DM. (Bartoli, Fra, & Carnevale Schianca, 2011)



Chapter 2

Literature Review

2.0 Literature Review

1.0 Taxonomic Overview: Morphological Features and Attributes of *Blachia andamanica*

Shrubs can develop into small trees, growing up to 6 meters tall with a basal diameter of around 6 centimeters. Their branches elegantly curve and are a dark brown hue, while the flowering ones are typically 1.3 to 3 millimeters thick. The young sections of the shrubs often possess limited hair coverage. The outer bark is thin and exhibits a smooth to finely cracked texture, ranging from light green to grey. Axillary bud scales are typically smooth and measure between 3 to 4.8 millimeters in length.

In terms of the leaves, the petiole ranges from 5 to 9.5 millimeters in length, presenting no swelling, and may have a smooth or slightly hairy texture, occasionally displaying slender corky cracks. The leaf blade can be ovate, elliptic, oblong, or obovate, sometimes varying within a single shrub, measuring between 3.1 to 7.8 to 17 centimeters long and 1.8 to 3 to 10 centimeters wide. These leaves are thin to somewhat leathery, mostly symmetrical at the base with a tapering or rounded base, often slightly slanted, and exhibit an entire margin that tends to be slightly turned backward. The tips vary from pointed to slightly sharp, nearly prickly, and are predominantly hairless while young, gradually losing hair as they mature. The upper leaf surface is a medium to dark glossy green, while the lower surface appears paler, with slightly elevated veins on both sides. Secondary veins consist of 6 to 12 pairs, curving near the edge, alongside distinct intercalary veins and a network-like arrangement of higher-order veins.

Typically found at the end of branches, inflorescences may occasionally appear at the base, becoming up to 8 centimeters long and light green with a few hairs present, while buds are light green. Staminate flowers have a diameter ranging from 4.8 to 5.2 millimeters, with a stem length of 5 to 9 millimeters. The sepals vary from 4 to 6 in number, showcasing an elliptic to obovate shape, measuring 2 to 3.5 by 2 to 3 millimeters, and are colored pale green to white, with rounded tips. The petals, numbering between 4 to 6, are triangular, ranging from 0.7 to

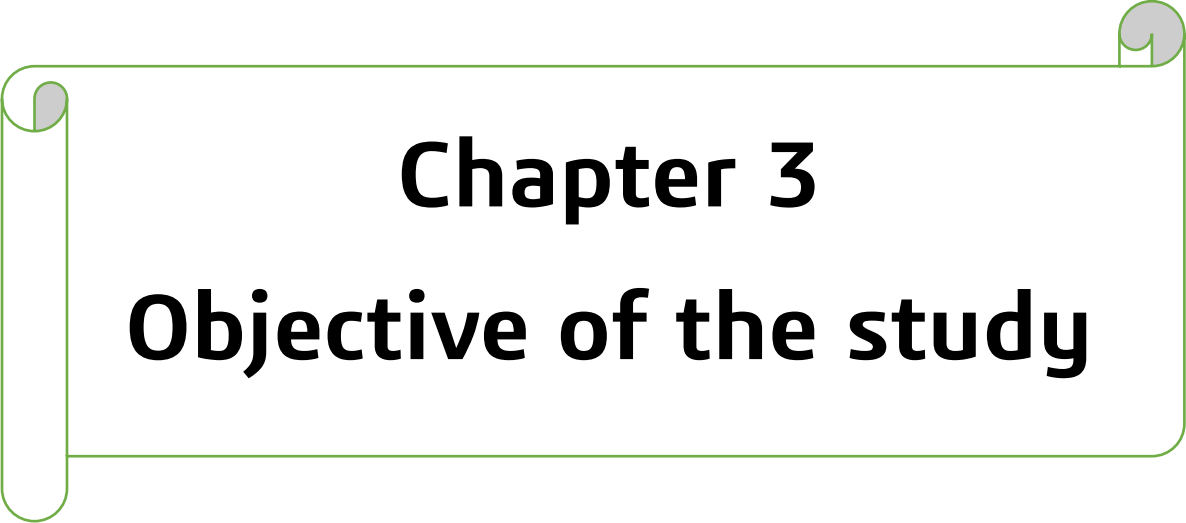
1.8 by 0.8 to 2 millimeters in size, featuring a smooth, membranous texture, slightly serrated to three-lobed tips, and are typically white to light in color.(van Welzen & Plants, 2015)

2.0 The Andaman and Nicobar Islands within the country of India harbor various ethnomedicinal plants.

The Andaman and Nicobar Islands in India are home to six indigenous groups and a diverse immigrant population, where a total of 536 ethnomedicinal plant species thrive. These plants, which encompass herbs, shrubs, trees, climbers, and epiphytes from various plant families, hold great significance. Particularly, the *Blachia andamanica* (Kurz) Hook. f. plant is noteworthy for its traditional healing properties for bone fractures and scrotal pain among local communities. The preservation of these plants carries cultural importance, as tribes coexist with nature to safeguard their fragile ecosystem.(Kamble, Mane, Murugan, & Jaisankar, 2008)

3.0 The Nicobarese Tribe of Car Nicobar Island Utilises Medicinal Plants

The focus of this study is on the traditional use of medicinal plants among the Nicobarese tribe who reside in Car Nicobar Island, part of the Andaman and Nicobar Islands. Despite having access to modern healthcare facilities, the tribal communities still rely on herbal remedies for treating various medical conditions. Local medical experts known as Traditional Knowledge Practitioners (TKPs) are highly valued in this region. An interesting plant that is commonly used is *Blachia andamanica* (Kurz) Hook. f., locally referred to as "Chanôh" and part of the Euphorbiaceae family. The leaves of this plant have been traditionally used to heal bone fractures and alleviate scrotal pain.(Chander, Kartick, Gangadhar, & Vijayachari, 2014)

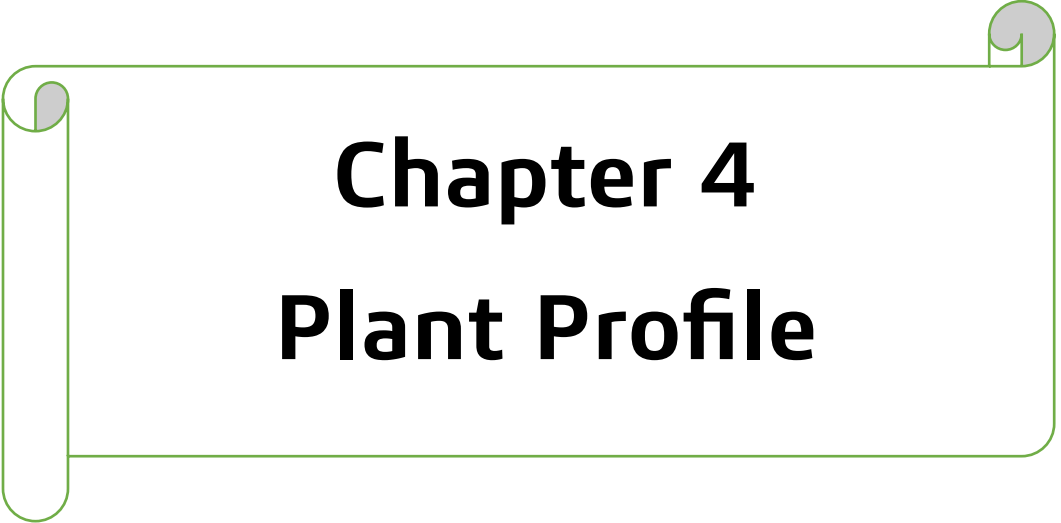


Chapter 3

Objective of the study

The primary aim of this study is to thoroughly investigate the phytochemical composition and potential therapeutic effects of methanolic extracts obtained from *Blachia andamanica* leaves. The focus areas of the research include:

1. Identification and Description of Phytochemicals:
2. Exploration of Thrombolytic Activity & Blood Glucose Regulation
3. To establish an alternative medicine from a natural source



Chapter 4

Plant Profile

3.1 Introduction of Plant

3.1.1 Botanical name: *Blachia andamanica* subsp. *Denudate*

3.1.2 Common name: Sahyadri Blachia

3.1.3 Local name: Chanôh

3.1.4 Synonyms:

- *Blachia denudate*
- *Codiaeum andamanicum* Kurz
- *Dimorphocalyx andamanicus* (Kurz) Benth

3.1.5 Taxonomy of Plant

Kingdom ----- Plantae

Phylum----- Streptophyta

Class----- Equisetopsida

Subclass----- Magnoliidae

Order----- Malpighiales

Family----- Euphorbiaceae

Genus----- *Blachia*

Species----- *Blachia andamanica*

3.1.6 Native to: Andaman & Nicobar Island, Thailand

Traditional uses: bone fractures and alleviate scrotal pain

Image of the plant leaves

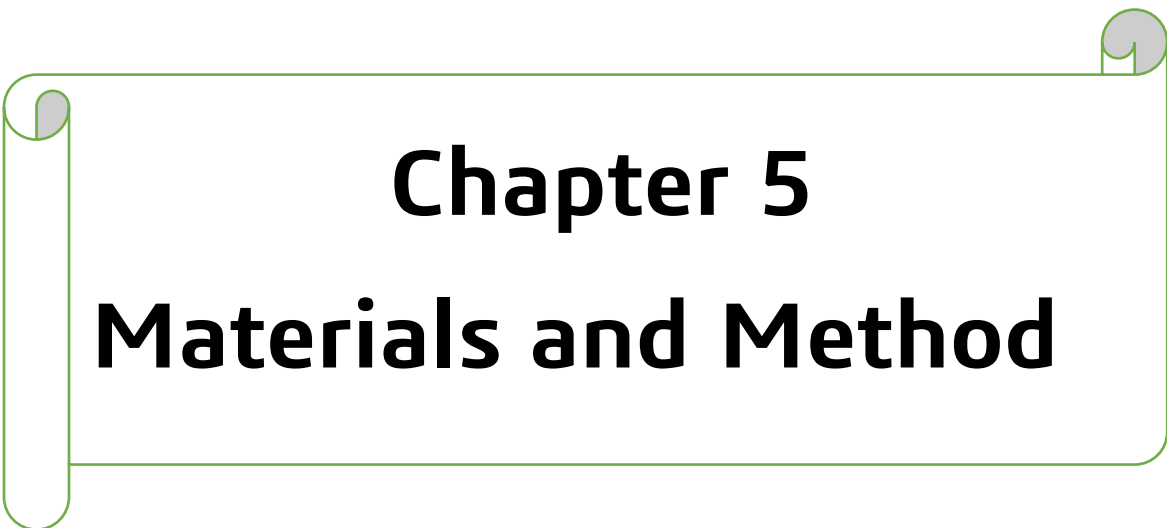


Figure 1 Blachia Andamanica subsp.

3.2 General Information of the Plant

- Sahyadri Blachia is a tree that reaches heights of up to 5 meters and is characterized by greyish bark that tends to be smooth.
- Branchlets: These are round, covered with warts, and exhibit a hairless, grooved appearance. A whitish sap is noticeable when leaves and twigs are cut.
- Leaves: The leaves are papery and range in shape from narrow elliptic to ovate, measuring between 6 and 19 cm in length and 2.5 to 8 cm in width.

- Leaf Stalks: These are between 0.5 and 1.5 cm long, smooth, and grooved, with tips varying from pointed to long-pointed and bases that are either asymmetric or rounded to narrow. The margins are consistently smooth and entire.
- Flowers: The tree bears both unisexual and monoecious flowers.
- Male Flowers: These are arranged in terminal slender racemes.
- Female Flowers: A few female flowers can be found at the base of the male racemes.
- Fruit: The fruit is comprised of ellipsoid or round capsules, which are deeply 3-lobed and contain three seeds.
- Habitat: Sahyadri Blachia trees are typically found in wet to dry evergreen forests within the Western Ghats, often at altitudes reaching up to 900 meters.
- Endemism: This tree species is primarily found in the Western Ghats, with a notable concentration in the Central Sahyadri region.



Chapter 5

Materials and Method

4.1.1 Plant collection

Location:

Blachia andamanica (Kurz) Hook.f., belonging to the Euphorbiaceae family

Source: Uddin (2018), page 18.

Geographical Location: Chattogram, specifically Hathazari, in the vicinity of Kumarikhal.(**Salah Uddin, Mazumder, & Uddin, 2023**)

4.1.2 Preparation of Methanol Extract

To initiate the extraction procedure, a pristine glass container with a flat bottom was selected. Approximately 300 grams of leaf powder were meticulously placed into the container. Then, 1800 milliliters of methanol were added to the container, and the mixture was allowed to steep for 14 days, during which periodic shaking and stirring occurred. After the steeping period, the container, containing the mixture of leaf powder and methanol, was securely sealed. Loose leaves and stem sections were subsequently removed from the mixture using white cotton. The liquid component underwent triple filtration using white cotton to ensure complete elimination of any remaining debris. Finally, Whatman filter paper was utilized for further filtration, effectively separating any remaining solid particles, essential crude extract was obtained by subsequently evaporating the solvent from the filtrate using a rotary evaporator. This process facilitated the isolation of the desired components from the initial mixture, ensuring purity and effectiveness of the extract.

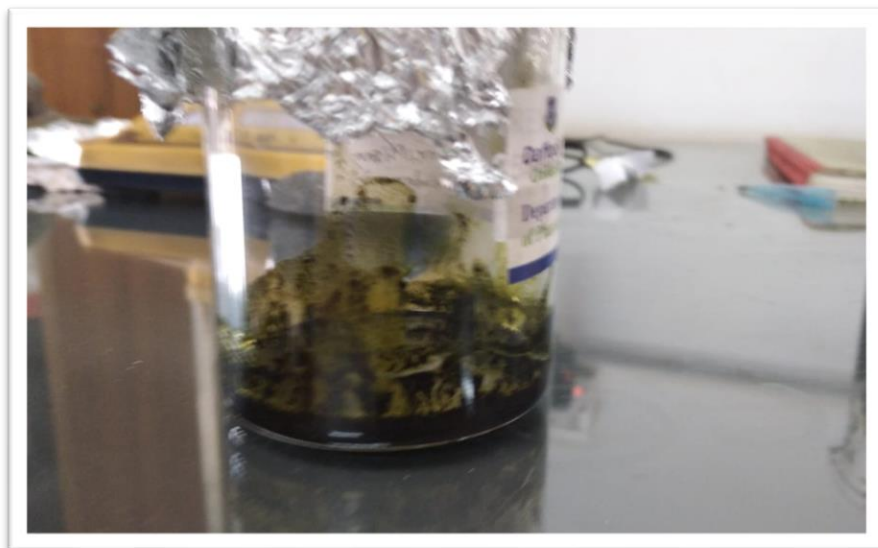


Figure 2 Plant extract

4.2 Phytochemical screening by GC-MS

- The combination of gas chromatography with electron ionization (EI) quadrupole mass spectrometry (GC-MS) is a highly advanced and dependable metabolomics method. With this technique, a large range of chemically varied compounds, such as fatty acids, organic acids, sugars, sugar alcohols, aromatic amines, and amino acids, may be measured simultaneously. While metabolite identification and sophisticated data processing may be difficult with full scan data collection in untargeted GC-MS profiling, targeted analysis using GC-MS/MS provides improved specificity, greater sensitivity, and simplified data processing.(Choudhury et al., 2022)
- At BRICM, the plant extract was examined by gas chromatography combined with electron ionization quadrupole mass spectrometry (GC-MS).

4.3 Thrombolytic activity:

Principle: A thrombus is a blood clot that forms in the circulatory system and stays in place, obstructing the normal flow of blood. While a thrombus is a natural response to injury to prevent excessive bleeding, it can be problematic in cases of thrombosis, where clots block blood flow in healthy blood vessels. Thrombosis contributes to atherosclerosis, a main factor in causing ischemic cardiovascular conditions such as heart attacks and strokes. In thrombolytic therapy, patients receive treatment with a plasminogen activator to trigger the fibrinolytic system, converting plasminogen into the active enzyme plasmin. Plasminogen attaches to undamaged fibrin, initiating

fibrinolysis. The drug streptokinase is commonly used as it effectively targets this type of plasminogen, resulting in a selective action on fibrin. (Hussain, Islam, Bulbul, Moghal, & Hossain, 2014)

Procedure:

- Initially, the weight of the empty tube (Eppendorf Tube) was measured.
- Subsequently, blood was drawn from the participant and 1 ml of blood was placed in each tube.
- The tubes were then left to incubate at 37°C for 45 minutes to allow for clot formation.
- Afterward, the serum was separated from the blood, and the weight of the first clot with the tube was recorded.
- The weight of the first clot was calculated by subtracting the weight of the tube alone from the weight of the first clot with the tube.
- A standard dose of 100 µl (30000 IU), along with a control dose of 100 µl of distilled water and a sample/extract dose of 100 µl (1000 µg/100 µl), was added to each individual tube.
- Following this, the tubes were once again incubated at 37°C for 90 minutes to facilitate clot lysis.
- The lysed blood fluid was then separated from the tube.
- The weight of the second clot with the tube was measured.
- The weight of the second clot was determined by subtracting the weight of the tube alone from the weight of the second clot with the tube.
- Finally, the weight of the lysed clot was calculated by finding the difference between the weight of the first clot and the weight of the second clot. ("<Thrombolytic activity and OGTT (1).pdf>,")

4.4 Antidiabetic Activity (OGTT)

- Overnight fasting Swiss Albino mice were employed for the Oral Glucose Tolerance Test (OGTT). The study involved four groups of mice receiving distinct treatments: 250 mg/kg of leaf extract, 500 mg/kg of leaf extract, 2 mg/kg of glimepiride, and a control group administered with distilled water.
- Following pretreatment with either distilled water, glimepiride, or leaf extract, a glucose solution was administered to each mouse 30 minutes later at a dose of 2 grams per kilogram. Blood glucose levels of the mice subjected to the glucose load were subsequently measured at baseline (-30 minutes), as well as

at 60 and 120 minutes post-administration. Blood glucose levels were assessed using a glucometer, specifically the UNICHECK (EBCARE) Diabetes Machine manufactured by Visgeneer, along with compatible blood glucose test strips.(Chaimum-aom, Chomko, & Talubmook, 2016)

5.1 Result of GC-MS-Based Phytochemical Analysis of Plant Extracts at BRICM:

Upon
40

analysis,
distinct

Chapter 6

Result and Discussion

compounds were identified. However, only 12 of these compounds had an area exceeding 1% in the overall ionic chromatogram, indicating their prominence in the sample. Figure 3 presents the Total Ionic Chromatogram, providing an overview of the compound distribution. The key compounds identified, their retention times (RT), area percentages, compound names, and chemical classifications are detailed in Table 1

Library Search Report

Sample Information

Sample ID : MSD24032501
User ID : NA
Method File : G:\GC-MSMS\2023\plant extract\METHOD\Plant Extract_CutT3.5_V 0.5_11.09.2023
Data Path : G:\GC-MSMS\2024\plant extract\DATA\2024.03.28
Acq on : 28.03.2024
Solvent used : Methanol



Figure 3: Total ionic chromatogram

SL.NO.	R. TIME	AREA %	COMPOUND	Class
1	3.525	2.10	Silane, triethyl(2-phenylethoxy)-	Silane
2	3.635	1.28	Propanenitrile, 2-(dimethylamino)-	Nitrile
3	10.455	12.67	2-(Isobutoxymethyl)oxirane	Epoxide
4	11.012	1.06	D-Allose	Sugar
5	16.171	6.29	Hexadecanoic acid, methyl ester	Fatty acid
6	18.901	3.62	9,12-Octadecadienoic acid, methyl ester	Fatty acid
7	19.006	8.66	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Fatty acid
8	19.158	6.01	Phytol	Alcohol
9	19.412	1.14	Methyl stearate	Fatty acid
10	20.343	1.13	Hexadecanamide	Amide
11	23.391	40.89	9-Octadecenamide, (Z)-	Amide

12	34.893	1.02	Vitamin E	Vitamine
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Table 1 Probable compound and their classes of the Plant Extract of blachia andamanica

Discussion:

The GC-MS analysis conducted at BRICM provided comprehensive insights into the plant extract's chemical composition. Notably, 9-Octadecenamide, (Z)- emerged as the predominant compound, accounting for the highest area percentage. Its prominence suggests a significant presence and potential multifaceted applications, warranting further exploration.

The occurrence of fatty acid methyl esters, including 9,12-Octadecadienoic acid and 9,12,15-Octadecatrienoic acid, indicates the plant extract's probable rich content of natural oil or lipid derivatives. Such compounds are typically abundant in plant oils, suggesting the extract may offer a substantial reservoir of these beneficial constituents. Additionally, the presence of Vitamin E in the sample signals potential antioxidant capabilities. Such attributes could be advantageous across various sectors, including cosmetics, dietary supplements, and health-focused products.

In summary, the GC-MS analysis at BRICM unveiled a spectrum of organic compounds from diverse chemical classes within the plant extract. These insights highlight the extract's promising industrial and biological potential. To harness these benefits fully, additional research is imperative to elucidate the specific roles and applications of these compounds.

5.2 Result of Thrombolytic activity test

Sample	Blank Tube weight (gm)	1 st clot+ tube weight (gm)	1 st clot weight (gm)	2 nd clot+tube weight (gm)	2 nd clot weight (gm)	Lysis weight (gm)	% of lysis
Control (Distilled water)	0.80	1.679	0.89	1.46	0.67	0.22	5%

Standard (streptokinase)	0.860	1.870	1.01	1.10	0.24	0.77	76%
Blachia andamanica Leaf extract	0.776	1.599	0.823	1.469	0.693	0.131	15%

Table 2 Result of Thrombolytic Activity Test

Discussion:

The results of the thrombolytic activity test show varying levels of clot breakdown among the samples tested. Streptokinase, used as the positive control, demonstrated a significant thrombolytic effect, leading to 76% clot breakdown. This result is consistent with its reputation as a potent thrombolytic agent. In contrast, the methanolic extract of *Blachia andamanica* leaves displayed a moderate ability to break down clots, with a 15% clot breakdown rate. This suggests that the leaf extract may contain compounds with thrombolytic properties, although its effectiveness is notably lower compared to Streptokinase. In the absence of any thrombolytic treatment, the control group treated with distilled water showed a 5% clot breakdown, likely due to natural clot dissolution over time.

The modest thrombolytic activity seen in the *Blachia andamanica* leaf extract calls for further investigation. It is important to identify the active compounds responsible for this activity and enhance the extraction methods to potentially boost its thrombolytic effects.

The *Blachia andamanica* leaf extract exhibited a noticeable thrombolytic effect, although not as potent as the standard agent, Streptokinase. This initial discovery prompts additional research to uncover the extract's therapeutic potential, assess its safety, and understand its mechanism of action.

5.3 Oral Glucose Tolerance Test (OGTT)

Treatment	Blood glucose levels (mmol/L)		
	-30 min	60 min	120 min
Control Distilled water	9.63±0.15	9.16±1.30	5.36±2.12
Standard (Glimepiride)	8.93±1.21	7.63±1.01	6.27±0.88

Plant Sample-250	9.9±2.05	7.5±0.44	6.4±1.80
Plant Sample-500	8.7±0.93	6.3±0.88	4.1±0.46

Table 3 Oral Glucose Tolerance Test (OGTT)

Discussion:

The study utilized the Oral Glucose Tolerance Test (OGTT) to evaluate the effects of various treatments on blood sugar levels at time points of -30, 60, and 120 minutes after treatment administration.

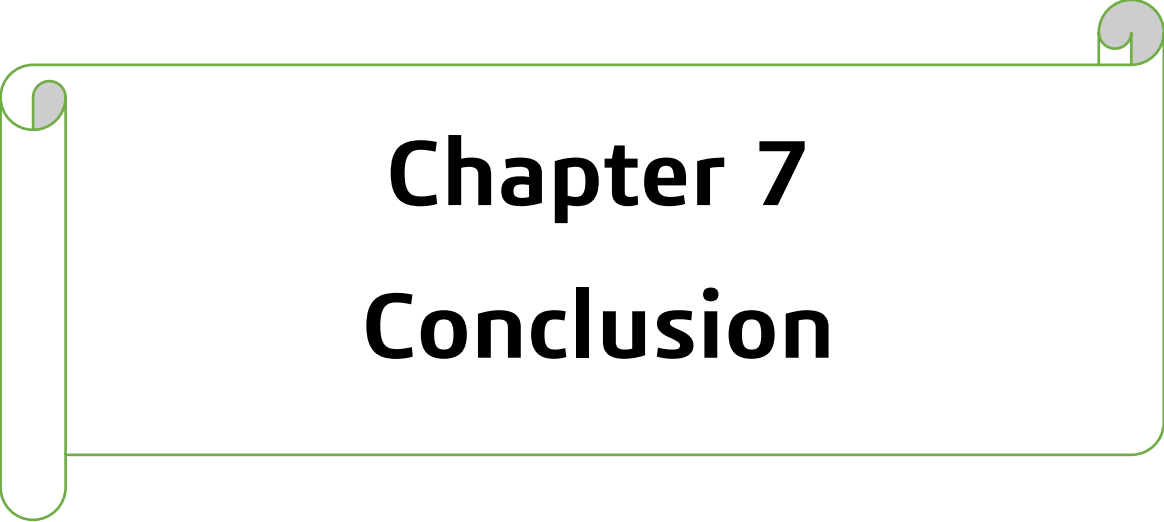
At the start of the test (-30 minutes), each group had their initial blood sugar levels measured. The control group, given distilled water, had a blood sugar level of 9.63±0.15 mmol/L. In contrast, the group receiving Glimepiride had a slightly lower level of 8.93±1.21 mmol/L. The groups treated with PS-250 and PS-500 showed levels of 9.9±2.05 mmol/L and 8.7±0.93 mmol/L, respectively.

After 60 minutes, all groups experienced a decrease in blood sugar levels. The control group had a level of 9.16±1.30 mmol/L, while the Glimepiride group decreased to 7.63±1.01 mmol/L. The PS-250 and PS-500 treated groups had levels of 7.5±0.44 mmol/L and 6.3±0.88 mmol/L, respectively. Notably, the PS-500 group showed the most significant reduction in blood glucose levels compared to others at this time point.

At the end of the 120-minute assessment, further reductions in blood sugar levels were observed. The control group had a level of 5.36±2.12 mmol/L, while the Glimepiride group recorded a level of 6.27±0.88 mmol/L. The PS-250 and PS-500 treated groups showed levels of 6.4±1.80 mmol/L and 4.1±0.46 mmol/L, respectively. The PS-500 group continued to exhibit the most substantial glucose-lowering effect, indicating its potential effectiveness in managing blood sugar levels.

Overall, both the PS-250 and PS-500 treatments displayed promising effects in reducing blood sugar levels compared to the control and Glimepiride groups. Particularly, the PS-500 treatment exhibited superior efficacy, especially at the 60 and 120-minute intervals post-treatment.

Further research is crucial to understand the underlying mechanisms behind these effects and to evaluate the long-term safety and efficacy of the PS-250 and PS-500 treatments as potential interventions for blood sugar management.



Chapter 7

Conclusion

6.1 Conclusion: The investigation conducted in this study delved into the chemical makeup and healing properties of the methanolic extract derived from *Blachia andamanica* leaves. Through analysis utilizing GC-MS, a range of organic compounds were identified, with 9-Octadecenamide, (Z)- emerging as the predominant compound. Alongside the presence of fatty acid methyl esters and Vitamin E, suggesting a rich content of natural oils or lipids that could potentially offer antioxidant advantages.

The assessment of its thrombolytic function revealed the extract's capability to dissolve blood clots, although not as effectively as the standard agent, Streptokinase. Moreover, the results from the Oral Glucose Tolerance Test (OGTT) highlighted the extract's potential to lower blood glucose levels, with the PS-500 treatment displaying the most promising outcomes. These study results propose encouraging prospects for the methanolic extract from *Blachia andamanica* leaves in both therapeutic and industrial arenas. However, further exploration is necessary to pinpoint the active components, comprehend their mechanisms of operation, and assess long-term safety implications.

In essence, this research paves the way for future studies that concentrate on refining extraction techniques and investigating the broader therapeutic capabilities of the *Blachia andamanica* leaf extract in the management of thrombosis and blood sugar levels.

Chapter 8

Reference

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