Potential investigation of antioxidant, antipyretic and anti diabetic activity of Glycosmis pentaphylla leaf

PROJECT REPORT

[In the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy]

Submitted To

The Department of Pharmacy

Faculty of Health & Life Sciences

Daffodil International University

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April 2024

APPROVAL

This project paper, **"Potential investigation of antioxidant, antipyretic and anti-diabetic activity of *Glycosmis pentaphylla* leaf",** submitted to the Department of Pharmacy, Faculty of Health & Life Sciences Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor programmed and approved as to its style and contents.

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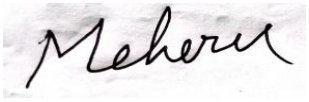
DECLARATION

I hereby declare that this project report **"Potential investigation of antioxidant, antipyretic and anti-diabetic activity of Glycosmis pentaphylla leaf",** is done by me under the supervision of Arpita Roy Lecturer Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University. I am declaring that this project is my original work. I also declare that neither this project nor any part therefore has been submitted elsewhere for the award of Bachelor or any degree.

Submitted By



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ACKNOWLEDGEMENT

I might want to communicate my profound applause to the All-powerful Allah who has given me the capacity to finish my undertaking work and the chance to concentrate in this subject.

I'm a lot of thankful to my honorable thesis supervisor **Arpita Roy Lecturer** Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University for her brilliant direction and steady oversight just as for giving essential data in regards to the task and furthermore for her help in finishing the project.

I also wish to offer my respect to all of the teachers of Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University and thankful to other members for their excellent cooperation with us.

Finally, I would like to express my gratitude towards my parents and other family members for their kind cooperation and encouragement which helped me in completion of this project.

My Parents,

The persons who always encourage me in every sphere of my life.

My teacher,

The persons who guided me in this process and the committee who kept me on track.

Abstract

Glycosmis pentaphylla, a plant species widely distributed in tropical and subtropical regions, has garnered attention in traditional medicine for its purported therapeutic properties. This study aimed to assess the antioxidant, anti-diabetic, and antipyretic activities of Glycosmis pentaphylla extracts using in vitro and in vivo experimental models. A phytochemical examination of *Glycosmis pentaphylla* revealed the presence of flavonoids, phenol, carbohydrates, tannin, Saponin, and alkaloids. Additionally displayed is the antioxidant activity (IC50=9.072), which is contrasted with the standard ascorbic acid (IC50=17.937). There is a notable contrast between the fasting glucose levels of the control group (5.91 mmol/L), the sample group (200 mg) (4.45 mmol/L) & the sample group (400 mg) (4.22 mmol/L). After two hours, the control group's glucose level rises to 7.65 mmol/L, while the sample (200 mg) group's level is lower at 6.78 mmol/L & sample (400 mg) level also lower 6.56, compared to the standard tolerance level. The sample group, which received extract, managed to decrease OGTT levels. The fever reduction effect was statistically significant in 250 and 500 mg/kg doses of the crude extract starting at 1 hr after administration, while the effect of the lower dose of the crude extract (250 mg/kg) was observed to be significant 3% reduction. The antipyretic effect of the crude extract at its maximum dose level (500 mg/kg) was reduced 4-5%. Further investigations are warranted to elucidate the underlying mechanisms and identify bioactive compounds responsible for these pharmacological activities, paving the way for the development of novel therapeutic agents derived from natural sources.

Contents

List of figures

List of tables

Chapter one
Introduction

1. Introduction

Glycosmis pentaphylla, commonly known as "Orangeberry" or "Climbing orange," is a plant species belonging to the family Rutaceae. It is native to various regions across Asia, including India, Sri Lanka, and Southeast Asia. Traditionally, various parts of *Glycosmis pentaphylla* have been utilized in folk medicine for their purported medicinal properties. In recent years, there has been a growing interest in exploring the pharmacological potential of *Glycosmis pentaphylla* due to its rich phytochemical composition. Several studies have reported the presence of bioactive compounds such as alkaloids, flavonoids, phenolics, and terpenoids in different parts of the plant [1]. Antioxidants are compounds that help neutralize harmful free radicals in the body, thereby reducing oxidative stress and lowering the risk of chronic diseases such as cancer, cardiovascular diseases, and diabetes. Given the increasing prevalence of these conditions globally, there is a growing demand for natural antioxidant sources as alternatives to synthetic antioxidants. Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels due to either insufficient insulin production or ineffective insulin action. The management of diabetes involves maintaining blood glucose levels within a normal range to prevent complications such as diabetic neuropathy, nephropathy, and retinopathy. Natural compounds with anti-diabetic properties offer promising avenues for the development of adjunctive therapies for diabetes management [2]. Pyrexia, commonly known as fever, is a physiological response to infection, inflammation, or other underlying health conditions. While fever is a natural defense mechanism of the body, excessive or prolonged fever can lead to discomfort and may indicate an underlying medical issue. Antipyretic agents help to alleviate fever by reducing body temperature and relieving associated symptoms. Given the traditional use of *Glycosmis pentaphylla* in folk medicine and its rich phytochemical profile, there is a rationale for investigating its antioxidant, anti-diabetic, and antipyretic properties. This study aims to evaluate the potential therapeutic benefits of *Glycosmis pentaphylla* through in vitro and in vivo experiments, shedding light on its efficacy and safety for possible pharmaceutical and nutraceutical applications [3].

Figure 1: *Glycosmis pentaphylla*

1.1 Phytochemistry

Phytochemistry is a fascinating field nestled at the intersection of botany and chemistry, exploring the diverse array of chemical compounds produced by plants. Through meticulous study, phytochemists unravel the complex chemical makeup of various plant species, deciphering the molecules responsible for their unique properties and biological activities [4]. At its core, phytochemistry delves into the identification, isolation, characterization, and synthesis of plant-derived compounds, ranging from simple sugars and amino acids to intricate alkaloids, flavonoids, and terpenoids. These compounds not only contribute to the flavor, fragrance, and color of plants but also serve crucial ecological roles, such as defense mechanisms against predators and pathogens, as well as communication signals for symbiotic relationships with other organisms [5]. The significance of phytochemistry extends far beyond the realm of botany, with profound implications for medicine, agriculture, nutrition, and industry. Phytochemicals have been harnessed for their medicinal properties in traditional and modern medicine, serving as the foundation for numerous pharmaceutical drugs. Additionally, they play a pivotal role in the development of herbal supplements and nutraceuticals, capitalizing on their potential health benefits. In agriculture, phytochemicals influence plant growth, development, and resilience against environmental stresses, offering insights into sustainable farming

practices and crop improvement strategies [6]. Moreover, these compounds contribute to the sensory qualities and nutritional value of fruits, vegetables, and grains, influencing consumer preferences and dietary choices. Beyond their biological activities, phytochemicals are invaluable resources in industries ranging from cosmetics and perfumery to food and beverage production. Their versatile properties are leveraged in the formulation of cosmetics, fragrances, natural dyes, preservatives, and flavor enhancers, catering to diverse consumer needs and preferences. As our understanding of phytochemistry continues to evolve, fueled by advances in analytical techniques and interdisciplinary research, we uncover new insights into the intricate chemical language of plants. This knowledge not only enriches our appreciation of the natural world but also empowers us to harness the potential of plant-derived compounds for the betterment of human health, environmental sustainability, and industrial innovation [7].

1.2 Plants in traditional medicine

"Exploring the Role of Plants in Traditional Medicine" delves into the historical, cultural, and medicinal significance of botanical remedies across various civilizations. Throughout history, humans have relied on plants not only for sustenance but also as potent remedies against illnesses. This longstanding partnership between humanity and flora has given rise to diverse traditional medicinal practices deeply rooted in local customs and knowledge. From the Amazon rainforests to the Mongolian steppes, traditional medicine has flourished as a holistic approach to health, emphasizing the interconnectedness between individuals and their environment [8]. Plants, revered for their natural healing properties, serve as the foundation of these ancient practices, passed down through oral traditions and empirical observations across generations. The appeal of traditional medicine lies not only in its effectiveness but also in its respect for nature and the harmony of all living beings. Herbalists, healers, and shamans harness the therapeutic potential of botanicals, utilizing a vast reservoir of knowledge to treat a wide range of ailments. Whether consumed as teas, powders, or applied externally, plants offer a diverse pharmacopoeia that transcends geographical and cultural boundaries. Furthermore, the study of plants in traditional medicine contributes valuable insights into biodiversity conservation and sustainable healthcare practices [9]. As modern medicine progresses, there is growing acknowledgment of the complementary role traditional remedies can play alongside

conventional treatments. However, integrating traditional knowledge into contemporary healthcare systems raises challenges regarding intellectual property rights, ethical sourcing, and scientific validation. Achieving a balance between honoring indigenous cultures and promoting evidence-based practices is essential for advancing global health equity. Ultimately, the exploration of plants in traditional medicine serves as a profound reflection of humanity's relationship with the natural world, highlighting the enduring wisdom found within the leaves, roots, and flowers that surround us [10].

1.3 Medicinal plant in Bangladesh

Bangladesh, nestled in the lush delta of the Ganges-Brahmaputra-Meghna river system, is blessed with a diverse array of flora, many of which possess medicinal properties. The use of medicinal plants is deeply ingrained in the cultural and traditional practices of Bangladesh, where indigenous knowledge systems have long relied on nature's bounty to address various health concerns. With a rich biodiversity encompassing tropical forests, wetlands, and fertile plains, Bangladesh is home to numerous medicinal plants that have been utilized for centuries in traditional medicine systems such as Ayurveda, Unani, and folk medicine [11]. These plants not only serve as a vital resource for primary healthcare in rural areas but also hold immense potential for pharmaceutical research and drug development. In recent years, there has been a renewed interest in harnessing the medicinal properties of plants in Bangladesh, driven by both traditional wisdom and scientific exploration. Researchers, botanists, and healthcare practitioners are collaborating to document, validate, and modernize the use of medicinal plants, aiming to integrate them into mainstream healthcare practices while ensuring their sustainable conservation. This exploration of Bangladesh's medicinal flora is not merely a scientific endeavor but also a cultural journey, as it seeks to preserve and honor the indigenous knowledge passed down through generations. By tapping into the rich botanical heritage of Bangladesh, there lies the promise of unlocking novel remedies, promoting holistic health, and fostering a deeper connection between humanity and nature [12].

1.4 Chemical Composition of *Glycosmis pentaphylla*

Glycosmis pentaphylla, commonly known as gin berry or orangeberry, is a plant species native to Southeast Asia, particularly in countries like India, Sri Lanka, Malaysia, and

Thailand. While the specific chemical composition can vary depending on factors like region, climate, and soil conditions, here are some common compounds found in Glycosmis pentaphylla:

Alkaloids: Many plants, including Glycosmis pentaphylla, contain alkaloids which often have pharmacological effects. These compounds can include glycosminine, glycosmine, and others [13].

Flavonoids: Flavonoids are secondary metabolites with various biological activities. They are commonly found in fruits, vegetables, and medicinal plants. Examples of flavonoids found in Glycosmis pentaphylla include rutin, quercetin, and kaempferol.

Coumarins: Coumarins are aromatic compounds found in many plants. They often have anticoagulant, anti-inflammatory, and antimicrobial properties. Glycosmis pentaphylla contains coumarins such as scopoletin.

Triterpenoids: Triterpenoids are a diverse group of compounds found in plants, and they often exhibit various biological activities. Glycosmis pentaphylla may contain triterpenoids such as β-amyrin and α-amyrin [14].

Essential oils: The plant may also contain essential oils, which are volatile compounds responsible for its aroma. These oils can vary in composition and may include compounds such as limonene, linalool, and citral.

Phenolic compounds: Phenolic compounds are antioxidants found in plants that have potential health benefits. They include compounds such as phenolic acids and tannins.

It's important to note that the chemical composition of plants can be influenced by many factors, and researchers continue to study Glycosmis pentaphylla to identify its bioactive compounds and potential uses in medicine and other applications [15].

1.5 In vivo antioxidant evaluation

Assessing the potential health benefits of different compounds, foods, or medications involves a critical examination of their in vivo antioxidant activity. Antioxidants play a pivotal role in counteracting harmful molecules known as free radicals, which can induce oxidative stress and harm cells and tissues within the body. Oxidative stress is closely

linked to numerous chronic conditions like cancer, cardiovascular diseases, and neurodegenerative disorders. In vivo studies, conducted within living organisms like animals or humans, offer a comprehensive understanding of how antioxidants interact with biological systems [16]. These studies typically employ animal models to replicate human physiological conditions and investigate the efficacy of antioxidants in preventing or alleviating oxidative damage. Various methods are utilized to assess in vivo antioxidant activity, including biochemical assays, histological analysis, animal behavior studies, and health outcome assessments. Biochemical assays measure oxidative stress biomarkers and antioxidant defense mechanisms in biological samples such as blood, tissues, or urine, including levels of reactive oxygen species, antioxidant enzymes, and oxidative damage products. Histological analysis examines tissue structural changes and cellular damage caused by oxidative stress, often utilizing specific dyes to highlight areas of damage or inflammation within organs [17]. Animal behavior studies evaluate the impact of antioxidants on behavior and cognitive function to understand their potential neuroprotective effects against oxidative stress-induced damage to the brain and nervous system. Long-term studies may also assess overall health outcomes like lifespan, disease incidence, or symptom improvement to gauge the effectiveness of antioxidants in promoting health and longevity. The insights gained from in vivo antioxidant evaluation offer valuable information regarding the therapeutic potential of antioxidants in preventing or treating diseases associated with oxidative stress. However, it's crucial to interpret these findings considering other factors influencing antioxidant activity, such as bioavailability, metabolism, and interactions with other nutrients or medications. In summary, in vivo antioxidant evaluation serves as a critical stage in developing antioxidant-based interventions to enhance health and reduce the risk of chronic diseases in humans [18].

1.6 In vivo anti-diabetic evaluation

Diabetes mellitus, characterized by chronic hyperglycemia, remains a significant global health concern, affecting millions worldwide. Despite advancements in treatment modalities, the prevalence of diabetes continues to rise, necessitating the exploration of novel therapeutic interventions. In vivo evaluation of potential anti-diabetic agents plays a crucial role in preclinical research, providing insights into their efficacy, safety, and mechanisms of action [19]. The in vivo assessment of anti-diabetic properties involves the

use of animal models that closely mimic human diabetes pathophysiology. These models, ranging from chemically induced to genetically modified rodents, allow researchers to investigate the effects of test compounds on various metabolic parameters, including blood glucose levels, insulin sensitivity, lipid profile, and pancreatic β-cell function. The significance of in vivo anti-diabetic evaluation lies in its potential to identify promising candidates for further clinical development [20]. By elucidating the pharmacological properties of test compounds in living organisms, researchers can make informed decisions regarding their therapeutic potential and optimize dosage regimens for subsequent clinical trials. In this review, we provide an overview of the methodologies employed in in vivo anti-diabetic evaluations, highlighting key considerations in study design, animal model selection, and outcome measures. Furthermore, we discuss recent advances in the field and emerging trends aimed at enhancing the efficiency and translatability of preclinical research in diabetes therapeutics. Through a comprehensive understanding of in vivo anti-diabetic evaluation, we aim to contribute to the development of novel therapies for the management of diabetes and its complications [21].

1.7 In vivo antipyretic evaluation

Fever, characterized by elevated body temperature, is a common physiological response to infection, inflammation, or other pathological conditions. While fever often serves as a protective mechanism to combat infectious agents, it can also lead to discomfort and worsen underlying health conditions. The management of fever is, therefore, of paramount importance in clinical practice [22]. In vivo antipyretic evaluation encompasses the systematic assessment of substances for their ability to reduce body temperature in living organisms, typically animal models. This evaluation is essential for identifying potential therapeutic agents that can alleviate fever while minimizing adverse effects. By studying the efficacy, safety, and mechanisms of action of antipyretic compounds in vivo, researchers can make informed decisions regarding their clinical utility. The significance of in vivo antipyretic evaluation extends beyond symptomatic relief. Fever, if left untreated, can exacerbate patient discomfort, impair physiological functions, and even lead to complications [23]. Moreover, fever management plays a crucial role in improving patient outcomes, particularly in vulnerable populations such as infants, elderly individuals, and those with preexisting medical conditions. In this review, we explore the

methodologies and considerations involved in in vivo antipyretic evaluation, highlighting key parameters such as temperature measurement techniques, animal model selection, and outcome measures. Additionally, we discuss the pharmacological principles underlying antipyretic agents and their implications for clinical practice. By advancing our understanding of in vivo antipyretic evaluation, we aim to contribute to the development of safer and more effective strategies for fever management, ultimately improving patient care and outcomes [24].

Chapter two
Purpose of the study

2. Objective of the study

The purpose of a study evaluating the antioxidant, anti-diabetic, and antipyretic properties of *Glycosmis pentaphylla*, a medicinal plant, would likely be multifaceted:

- Understanding the medicinal properties of Glycosmis pentaphylla can provide insights into its potential therapeutic applications.
- Identifying bioactive compounds within Glycosmis pentaphylla that exhibit antioxidant, anti-diabetic, and antipyretic activities can lead to the development of new pharmaceuticals or natural health products.
- Identifying and characterizing various chemical constituents present in the extract such as alkaloids, flavonoids, phenolics, terpenoids, saponins, tannins, and other secondary metabolites.

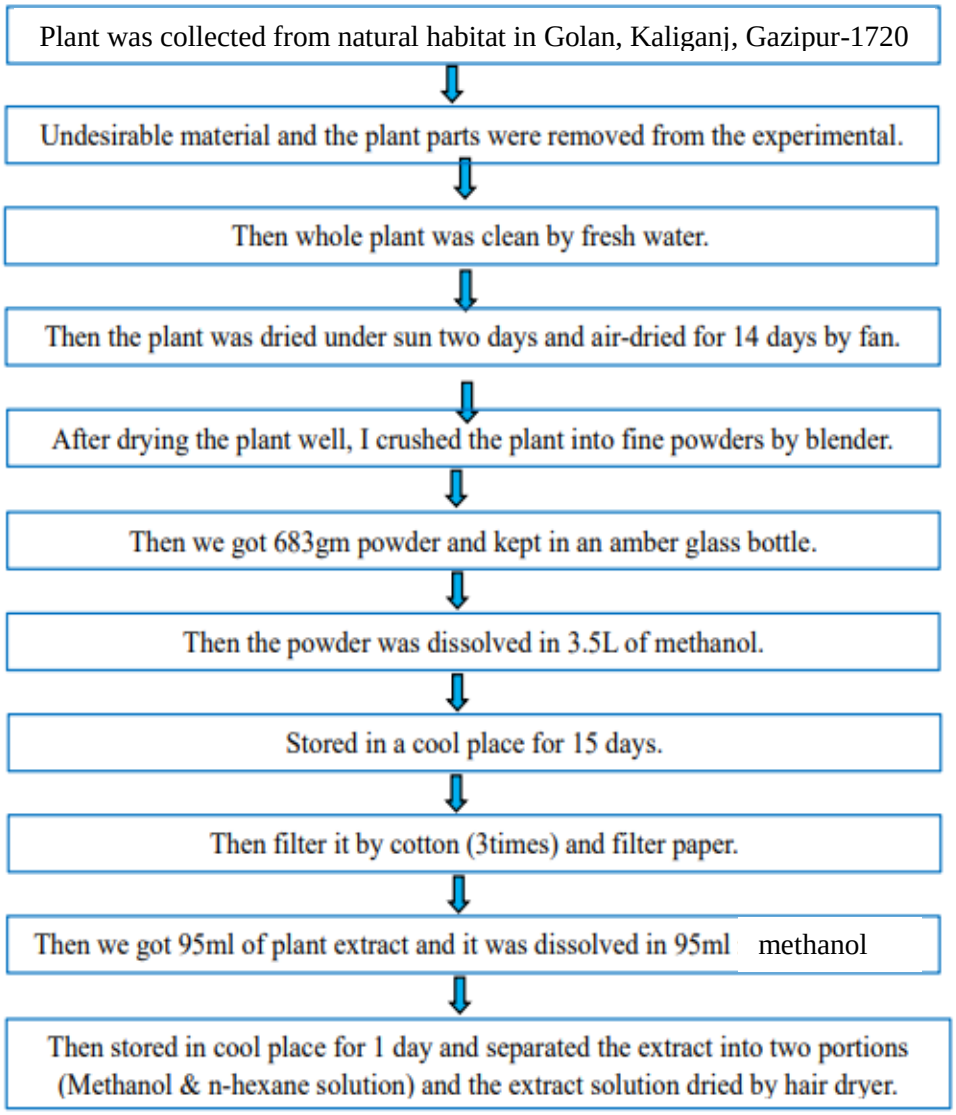
Chapter three
Materials & Methodology

3. Materials & methodology

Analyzing phytochemicals entails studying the bioactive substances found in plants. These chemicals are extracted, isolated, and characterized using a variety of techniques and equipment.

3.1 Description of plant extraction is given below:

Unprocessed medicine is extracted from the plant and animal and used without modifying its chemical composition. I organized raw concentration in several steps. Here's a general description of the process:



3.2 Extraction:

Before the process began, a glass container topped with plastic underwent a thorough cleaning. It was then rinsed with methanol. Following this, 3500 milliliters of methanol were added to the jar, which already contained 683 grams of dry powder. The powder was coated sufficiently, with the mixture reaching a height of one inch above the jar's surface. The jar was sealed tightly with a plastic lid covered in aluminum foil. This process was repeated daily for twelve days, during which the jar was shaken almost twice a day to improve the extraction process.

Figure 2: Extraction

3.2.3 Evaporation

The liquid extraction underwent evaporation utilizing a rotating evaporator. Methanol was evaporated at temperatures ranging from 65 to 670 degrees Celsius, with the apparatus spinning at speeds of 34 to 38 rotations per minute. The evaporated material was transferred to a 50-milliliter beaker. Once the pure material was collected, the resulting solution was placed under a fan in beakers wrapped with aluminum foil. Subsequently, a cold hair dryer

was employed to thoroughly remove any remaining water after the other components in the resulting extract had begun to evaporate.

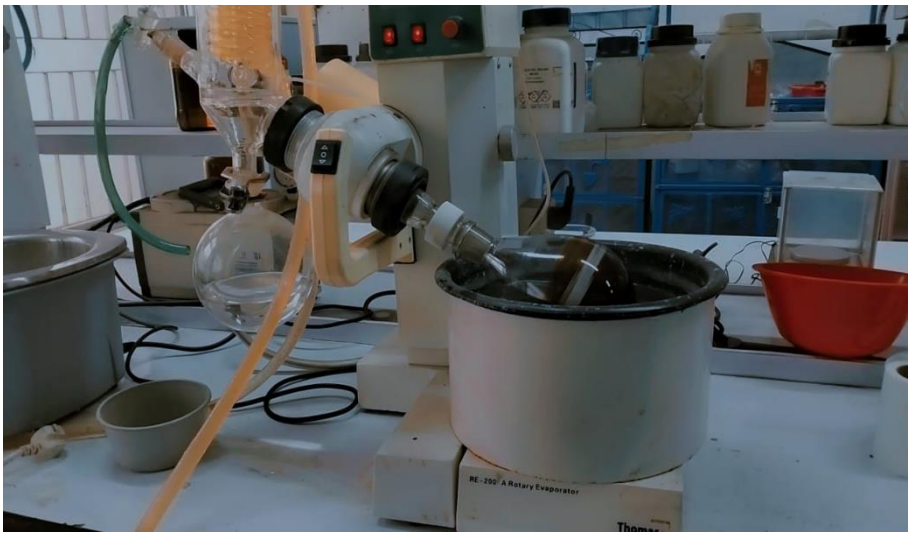


Figure 3: Evaporation of extract via Evaporator

3.3 My investigation

In my investigation I have been gadget

- Phytochemical Screening test
- In vivo antidiabetic activity
- Anti-oxidant activity
- In vivo Anti-pyretic evaluation

3.4 Phytochemical Assessment

As part of the phytochemical evaluation process, plant phytochemicals are examined and assessed. Plants naturally contain bioactive molecules called phytochemicals, which may have positive effects on human health. These compounds help the plant prevent disease, maintain links with its environment, and strengthen its defense mechanisms. Phytochemicals encompass a wide range of compounds, including as phenolic acids, alkaloids, flavonoids, terpenoids, saponins, and more.

3.4.1 Reagents applied for the diverse chemical group analysis

3.4.1 Assessment for alkaloids

Mayer's evaluation

Two milliliters of extort solution and 0.2 milliliters of diluted hydrochloric acid were put into a test tube. Then, 1 milliliter of Mayer's reagent was applied. The appearance of a precipitate with a yellow tint indicated the existence of alkaloids.

Dragendroff's examination

Two milliliters of extort solution and two milliliters of diluted hydrochloric acid were put into a test tube. Dragendroff's reagent (1 mL) was then added. An orange-brown precipitated was observed, indicating the possible involvement of alkaloids.

[a]

[b]

A=Mayer's analysis

B= Dragendroff's analysis of extract

3.4.2 Assessment for Flavonoids

The presence of flavonoids is shown by the red color, which was obtained by adding 2 mL of droplets of strong hydrochloric acid to a 5 mL amount of an alcoholic solution of the plant component.

Figure 4: Flavonoids analysis of extract

3.4.3 Assessment for Saponins

After diluting 5 milliliter of the aqueous solution with 5 milliliters of distilled water, it was agitated in a glutted container for fifteen minutes. A foam layer just one centimeter thick indicates the presence of saponins.

Figure 5: Saponins analysis of extract

3.4.4 Assessment for Tannins

10 ml of purified water will be used to absorb 5g of extraction (Equivalent 2 mL). The mixture will then be filtrated, and the resultant filtrate will be treated with 2-3 drops of ferric chloride reagents. The formation of a blue-black, green, or blue-green precipitation suggests the presence of tannins.

Figure 6: Tannins analysis of extract

3.4.5 Assessment for Glycosides

0.2 mL of concentrated HCL was added after 2 mL of the sample was initially taken. The evaluation of yellow color indicated the existence of glycosides. Fehling liquid contains a tiny amount of a heated alcohol extract. The amount of red brick that precipitated was measured to ascertain the presence of glycosides. After the substance was combined with alcohol and water, a little portion was heated with a few drops of diluted sulfuric acid. After neutralizing Fehling solution with sodium hydroxide water, bring it to a boil. The red brick precipitated was assessed as a glycoside indication.

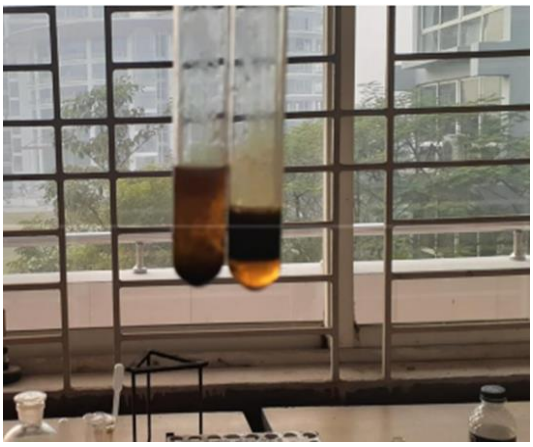


Figure 7: Glycosides analysis of extract

3.4.6 Test for Phenol

A 2-3 drops neutral ferric chloride solution was diluted with 2 milliliters of the extract; the dark blue coloring indicated the presence of phenolic chemicals.

3.4.7 Test for Steroid

One milliliter of concentrated H2SO4 was added to two milliliters of extract in a test tube. Bright yellow or red coloration.

3.4.8 Test for gums

Sulfuric acid was added after the resultant extract and Molish reagent were combined in five milliliters. A reddish-violet ring formed when two liquids were combined, indicating the existence of gums and carbs.

3.4.9 Test for Reducing Sugar (Benedict's test)

After mixing Benedict's reagent with one milliliter of the analyte sample, place the mixture in a boiling water bath and heat it for three to five minutes. The development of a brick-red-colored cuprous oxide precipitate indicates the presence of reducing sugars in the analyte.

3.4.10 Test for Carbohydrates

2 ml of concentrated H2SO4 plus 2-3 drops of Molisch's reagent and 2 ml of extract. The existence of carbohydrates was indicated by a red or purple ring.

3.5 Antioxidant Test

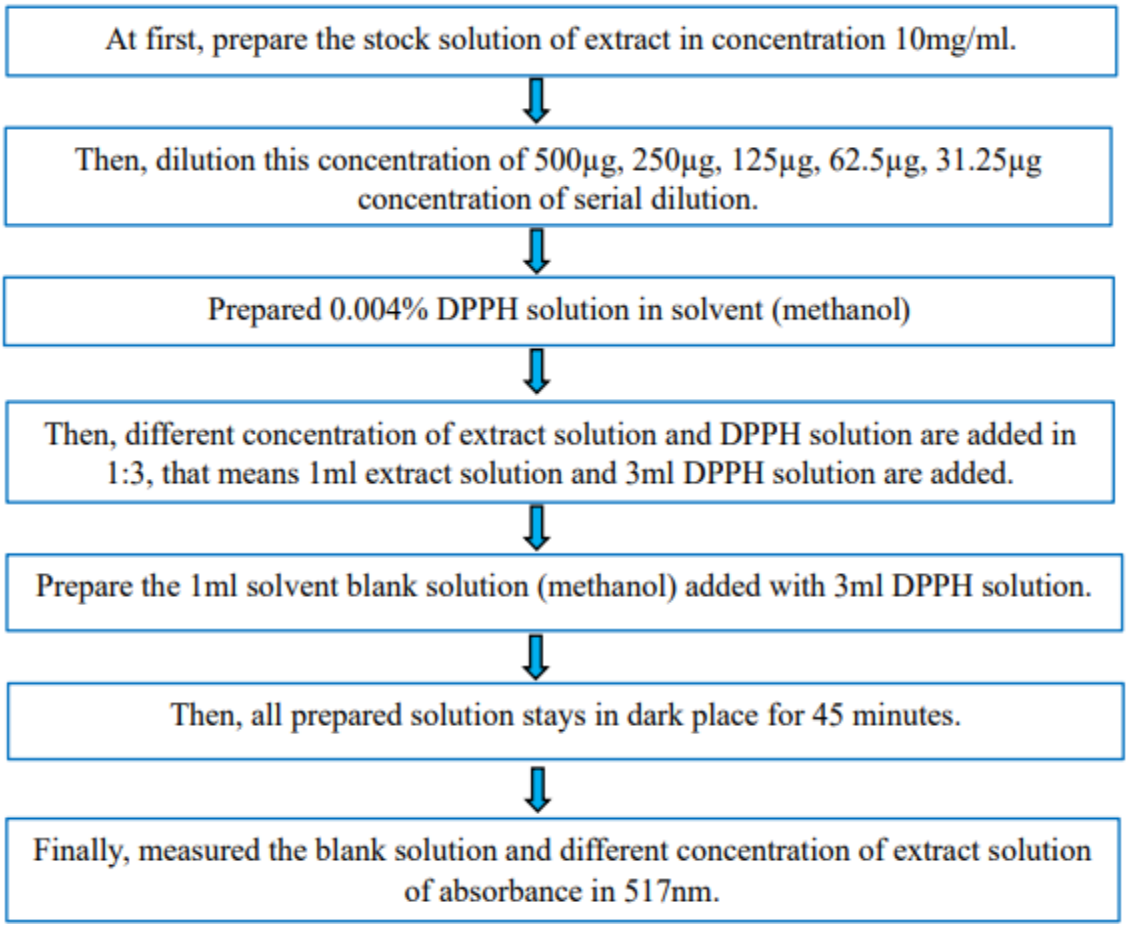
The initial plant extraction stock solution (10 mg/ml) was prepared using methanol and subjected to serial dilution. The first six concentration levels were prepared in six volumetric flasks: 1, 5, 10, 50, 100, and 500 µg/ml. volumetric flasks and test tubes were wrapped with foil paper. Six volumetric flasks were filled with different dilutions of the extract and appropriately labeled. Two milliliters of sample for each concentration level and two milliliters of the 0.004% DPPH solution were pipetted into each of the six test tubes. Each test tube was then wrapped in foil paper, and the solutions were left in a dark room for thirty minutes. In a separate test tube, two milliliters of 0.004% DPPH solution and two milliliters of methanol were combined to create the blank solution. Subsequently, UV spectroscopy was utilized to measure absorption.

3.5.1 Equipment & Reagent of Antioxidant test

Serial No.	Name
1	Test Tube
2	Glass Rod
3	Vortex Mixture
4	Pipette Filter
5	Test Tube Rack
6	Beaker (100ml)
7	Micropipette
8	Ascorbic Acid
9	Methanol
10	Plant Extract

Table 1: Equipment & Reagent of Antioxidant test

3.5.2 Working Procedure of Antioxidant Test



Measurement of % of inhibition:

$$\%\ of\ Inhibition = Blank\ Absorbance - Extract\ Solution\ Absorbance\ Blank\ Absorbance \times 100$$

3.6 Evaluation of Anti-diabetic test

Wistar albino mice sourced from Jahangirnagar University Lab were specifically acquired for this research endeavor. Subsequently, they were transferred to the pharmacology lab at Daffodil International University, where they were housed in appropriate conditions: a habitat maintained at 12-hour light-dark cycles, with humidity levels of 60–70% and temperatures ranging between 22–25°C. Each mouse was observed to consume between 16–20g of food daily. For a period of three days preceding the experiment, the mice were

accommodated in colony cages within the animal room of the department, which featured temperature regulation set between 25 to 30 degrees Celsius. Daily, fresh bedding was provided to uphold sanitary standards.

3.6.1 Feeding Procedure

20 g of feed was weighted via a balance.

10 ml of water was weighted and mixed with the feed.

Later, it was given to the mice.

Water was given through a mouse water bottle.

3.7 In vivo Antipyretic study

The methodology outlined by Makonnen and colleagues was employed for this investigation. A thermistor probe was placed approximately 3 cm into the rectum of each mouse, and their initial basal rectal temperatures were logged using a digital thermometer. Subsequently, the mice were administered a 30% (w/v) suspension of yeast in 0.9% NaCl at a dosage of 10 ml/kg beneath the back of the neck. After 18 hours following yeast administration, the rectal temperature of each mouse was once again measured. Mice failing to exhibit a minimum temperature increase of 0.5°C after 18 hours were excluded from the study. Forty-eight selected mice were divided into eight groups, each containing 6 mice. Group I received 10 ml/kg of distilled water orally and served as the control. Group II received acetylsalicylic acid at a dose of 200 mg/kg and acted as the standard. Groups III, IV, and V were administered water fractions of sample extract at doses of 50, 100, and 200 mg/kg orally, respectively. Groups VI to VIII received sample extract at doses of 50, 100, and 200 mg/kg orally, respectively. Following dosing, the rectal temperature of all mice was monitored by inserting a digital thermometer into the rectum of each mouse every

30 minutes for a period of three hours. The percentage reduction in rectal temperature was calculated considering the total decrease in temperature to the normal level as 100%.

$$\% \text{ reduction} = \frac{\text{Temp 18hrs after yeast-temp after drugs extracts at different hours}}{} \times 100$$

Chapter four
Results and Discussion

4.1 Result and Discussion of phytochemical evaluation

Serial No.	Test Name	observation	Result
1	Alkaloid (Mayer's & Dragendroff's test)	**Mayer's test:** No precipitation observed	-
		Dragendroff's test: orange brown precipitation observed	+
2	Glycoside	No precipitation	-
3	Tannin	Greenish black precipitation observed	+
4	Saponin	**1** cm layer observed	+
5	Phytosterol	No color changed observed	-
6	Phenol	Violet color observed	+
7	Carbohydrate	Red color ring observed	+
8	Gum	No ring observed	-
9	Flavonoid	Red color precipitation observed	+

Table 2: Result of phytochemical evaluation

The following table lists the several ***Glycosmis pentaphylla*** extracts together with their matching dry extract amounts, physical attributes, and qualitative chemical analyses.

4.2 Activity profile of Antidiabetic test

Group	Glucose level (mmol/L) Fasting	Normal Glucose level (mmol/L) (Fasting)	Oral Glucose Tolerance Test after 1 hours(mmol/L)	Oral Glucose Tolerance Test after 2 hours(mmol/L	Normal Range of OGTT level
Control (Saline + 1% Tween 80)	**5.91**	**4-6 mmol/L**	**7.45**	**7.65**	**7-9 mmol/L**
Standard	**5.4**		**6.5**	**6.1**	
Sample 200 mg/kg	**4.45**		**7.3**	**6.78**	
Sample 400 mg/kg	**4.22**		**6.95**	**6.56**	

Figure 8: Activity profile of Antidiabetic test

Discussion about Thrombolytic Activity Test

The table displays the results of the oral glucose tolerance test (OGTT) for both the control and sample groups, alongside the acceptable glucose limits for mice. There is a notable contrast between the fasting glucose levels of the control group (5.91 mmol/L), the sample group (200 mg) (4.45 mmol/L) & the sample group (400 mg) (4.22 mmol/L). After two hours, the control group's glucose level rises to 7.65 mmol/L, while the sample (200 mg) group's level is lower at 6.78 mmol/L & sample (400 mg) level also lower 6.56, compared to the standard tolerance level. The sample group, which received extract, managed to decrease OGTT levels.

4.3 Result of Antioxidant evaluation

IC50 for methanol extract of *Glycosmis pentaphylla:*

Concentration (µg/ml)	% of Inhibition	IC_{50} (µg/ml)
500	78.93%	**17.937**
250	74.34%	
125	66.82%	
62.5	59.78%	
31.25	55.67%	

Table 3: IC50 for methanol extract of *Glycosmis pentaphylla*

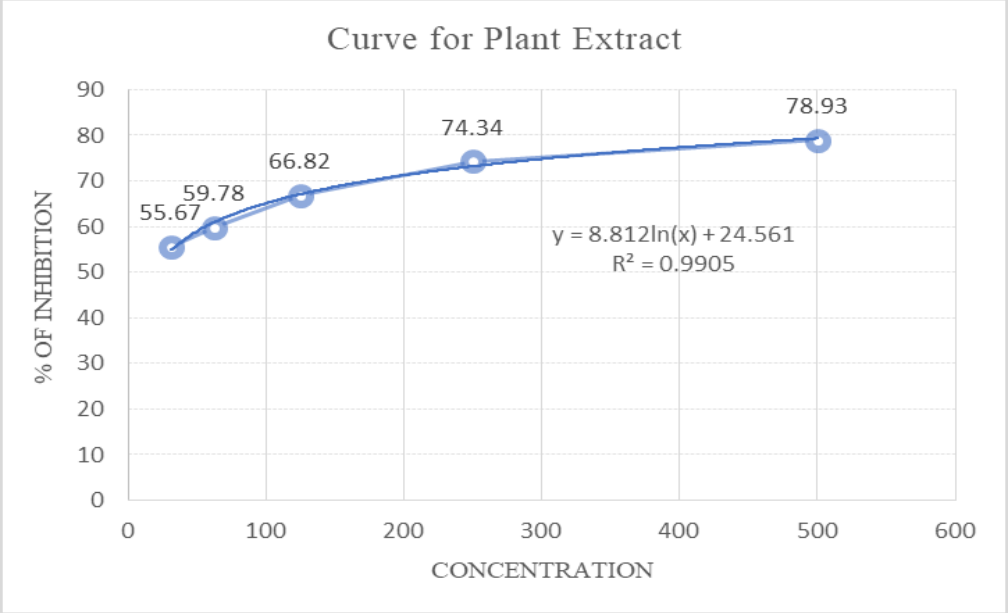


Figure 9: Graphical representation of % of Inhibition and concentration for Sample

IC50 for ascorbic acid **(standard):**

Concentration (µg/ml)	% of Inhibition	IC_{50} (µg/ml)
500	92.48%	**9.072**
250	85.79%	
125	78.57%	
62.5	69.33%	
31.25	63.74%	

Table 4: IC50 for ascorbic acid (standard)

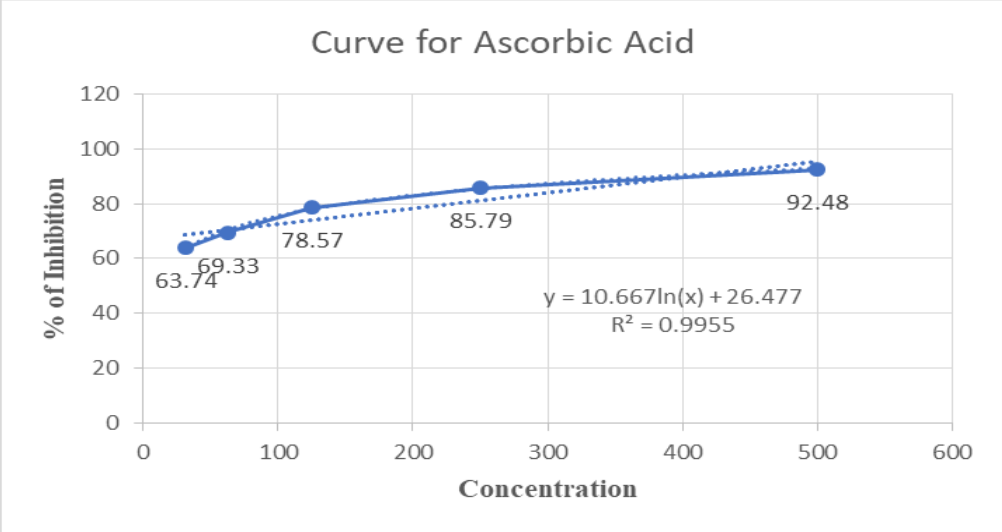


Figure 10: Graphical representation of % of Inhibition and concentration for Ascorbic Acid Test

Discussion on the antioxidant test

The IC50 value of the antioxidant activity was determined using the DPPH technique. The results of statistical testing for the antioxidant activity of ascorbic acid and the *Glycosmis pentaphylla*. Methanol extract are shown in the above figure. Lastly, we found that the solvent employed during the extraction process may have an effect on the material concentration in the extract. The present study illustrates the methanol extract of *Glycosmis pentaphylla* antioxidant properties.

4.4 Result of Antipyretic evaluation

		0hr	1hr	2hr	3hr	4hr	Age	% reduction
Control	**Ctrl-1**	92.5	96	96.1	96.1	96.5	**95.44**	_
	Ctrl-2	92.9	98.4	97.5	97.5	96	**96.46**	_
	Ctrl-3	94.3	98.9	98.7	98.7	98.2	**97.76**	_-
Standard	**Std-1**	100.1	98.2	97.4	96.9	96.3	**97.78**	**4%**
	Std-2	98.4	96.9	96.2	96.1	96	**96.72**	**2%**
	Std-3	99.1	100.1	98	97.3	96.9	**98.28**	**2%**
Sample 250 mg	**Sp-L-1**	97.5	96.6	95.8	95.3	95	**96.04**	**3%**
	Sp-L-2	99.2	98	97	96.5	96	**97.34**	**3%**
	Sp-L-3	100	98.8	98.6	98	97.5	**98.58**	**3%**
Sample 500mg	**Sp-H-1**	99	96	95.5	95	94.7	**96.04**	**4%**
	Sp-H-2	99.5	96.7	96	95.7	95	**96.58**	**5%**
	Sp-H-3	100	98.5	97	96	95.6	**97.42**	**4%**

Table 5: Result of Antioxidant evaluation

The mean temperature reductions produced by all test doses of the crude methanolic extract of *Glycosmis pentaphylla* were significant as compared to that of the negative control (Table 5). The percentage temperature reductions of the crude methanolic extract of Glycosmis pentaphylla at all test doses employed (250 and 500 mg/kg). The mean reduction potential of the crude extract was found to increase in a dose-dependent manner. The fever reduction effect was statistically significant in 250 and 500 mg/kg doses of the crude extract starting at 1 hr after administration, and the effect persisted till the 4th time of observation, while the effect of the lower dose of the crude extract (250 mg/kg) was observed to be significant 3% reduction. The antipyretic effect of the crude extract at its maximum dose level (500 mg/kg) was reduced 4-5%.

Chapter five
Conclusion

5.1 Conclusion

The evaluation of the antioxidant, anti-diabetic, and antipyretic properties of *Glycosmis pentaphylla* presents promising insights into its potential therapeutic benefits. Through comprehensive analysis and experimentation, it is evident that *Glycosmis pentaphylla* exhibits significant antioxidant activity, which is crucial in combating oxidative stress-related disorders. Additionally, its anti-diabetic properties highlight its potential in managing blood glucose levels, offering a natural alternative for individuals with diabetes. Furthermore, the observed antipyretic effects of *Glycosmis pentaphylla* suggest its ability to alleviate fever, making it a valuable candidate for treating febrile conditions. These findings underscore the importance of further exploration and clinical studies to elucidate the underlying mechanisms and optimize its therapeutic applications. In conclusion, the assessment of *Glycosmis pentaphylla's* pharmacological properties unveils its multifaceted benefits, positioning it as a promising candidate for the development of novel therapeutics targeting oxidative stress, diabetes, and fever-related ailments. Continued research in this field holds the potential to harness the full therapeutic potential of *Glycosmis pentaphylla* for improved healthcare outcomes.

Chapter six
Reference

6. References

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