Phytochemical and Hypoglycemic Screening of *Heritiera fomes*

By
Farjeen Binte Feda
ID: 18146079

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Farjeen Binte Feda

18146079

Approval

"Phytochemical and Hypoglycemic Screening of *Heritiera fomes*" submitted by Farjeen Binte Feda of spring 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on March, 2022.

Examining Committee:

Supervisor:
(Member)

Dr.Farhana Alam Ripa
Assistant Professor
School of Pharmacy
Brac University

Program Coordinator:
(Member)

Namara Marium Chowdhury
Lecturer, School of Pharmacy
Brac University

Deputy Chair:
(Member)

Dr. Hasina Yasmin
Co-chair, School of Pharmacy
Brac University

Departmental Head:
(Chair)

Dr. Eva Rahman Kabir
Dean, School of Pharmacy
Brac University

Ethics Statement

The Swiss albino mice used in this research were collected from International Centre for Diarrhoeal Disease and Research's Animal Research Branch in Bangladesh (ICDDRB) following the research guidelines approved by the institutional animal ethical committee. (MacArthur Clark et al., 2020)

Abstract

Phytotherapy in curing various health complications has shown major prospects in recent times. Therefore, people living in rural areas highly depend on phytotherapy as a potential treatment option for numerous diseases. This study focuses on the phytochemical screening and the assessment of hypoglycemic effect of different parts (root, leaves, and bark) of the ethanol extract of *Heritiera fomes.* The phytochemical screening confirmed the presence of alkaloid, carbohydrate, tannins, flavonoid, saponin, glycoside, steroid, phenol and resin. The therapeutic efficacy was determined by assessing the hypoglycemic effect of this plant extracts in doses of 200 mg/kg and 400 mg/kg body weight by alloxan induced diabetic mice in comparison with the standard drug Metformin hydrochloride of 150mg/kg. The experimental Swiss albino mice were divided into eight different groups. Significant reduction in glucose level was observed in the diabetic mice after treating with EHB, EHR & EHL (Ethanol extracts of bark, root and leaves of *H.fomes*). Results have shown that *H.fomes* can be considered as potential hypoglycemic agent and can be utilized in minimizing associated health complications upon further research.

Keywords: *Heritiera fomes;* Hypoglycemia; Alloxan; Swiss albino mice; Ethanol extract; Phytochemical Screening

Dedication

This research is dedicated to my family, teachers and friends.

Acknowledgement

At first, I would like to thank Almighty Allah who has given me the strength to work hard.

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List of Acronyms

ROS	Reactive Oxygen Species
NOGP	**N**on-oxidative Glucose Pathways
EHL	Ethanol extract of *H.fomes* leaves
EHB	Ethanol extract of *H.fomes* barks
EHR	Ethanol extract of *H.fomes* root

Chapter 1: Introduction

Humankind has always been looking for prospective medication to cure diseases that can have serious consequences in case of long-term exposure. In the verge of looking for promising and sustainable healthcare solutions, researchers have portrayed the importance of phytotherapy over the years. Scientists have shown how medicinal plants can exert possibilities in treating numerous health conditions that can turn fatal if left untreated. One of the most common yet unprecedented health conditions among them is hyperglycemia, and many other health complications can stem from such situations.

1.1 Prevalence of Hyperglycemia around the Globe

Hyperglycemia refers to the condition of elevated blood glucose level of greater than 125 mg/dL (milligrams per deciliter) in fasting condition due to the presence of high blood sugar. Such incidents can occur due to various reasons like lack of the necessary amount of insulin or inadequate response to the existing insulin. Blood glucose level being higher than 180 mg/dL after 1-2 hours of eating is considered hyperglycemic. Impairment in glucose regulation is now considered a global epidemic that has been affecting almost 415 million people around the globe (Xiao et al., 2019).

Hyperglycemia can potentially trigger type 1 and type 2 diabetes and according to a global estimation, Diabetes is responsible for an estimated 4.2 million deaths in individuals aged 20 to 79. Diabetes is thought to be responsible for 11.3 percent of mortality worldwide and it is accountable for over half (46.2%) of all fatalities in people under the age of 60 (Saeedi et al., 2020). In order to carve out preventive strategies for hyperglycemia, we should identify the possible risk factors of elevated plasma glucose level.

1.2 Effects of elevated blood glucose level & Diabetes

Elevated blood glucose level in hyperglycemia can have severe and even life-threatening consequences. High blood sugar can not only result in diabetes but also can cause various associated health complications like impaired blood vessels, damaged tissues, cardiovascular diseases, stroke, damaged nerve and even can cause tumor progression. Acute hyperglycemia can also affect gastric motility in diabetic patient by exerting weakened gastric emptying and overall functionality in the duodenum and the jejunum (Björnsson et al., 2009).

Moreover, hyperglycemia can mediate the activation of non-oxidative glucose pathways (NOGPs) which can give birth to further cardiovascular complications (Mapanga & Essop, 2016). High blood glucose level has also reportedly been contributing to the dysfunction of pancreatic β-cells inducing diabetic condition and attributing to glucotoxicity (Solomon et al., 2012). Along with these, kidney impairment, vascular dysfunction in diabetes as well as high potential of certain malignancies like pancreatic, endometrial and breast cancer can also be the noteworthy concerns of hyperglycemia for which we need prospective options of treatment (Li et al., 2019).

1.3 Phytotherapy as Prospective Treatment option for hyperglycemia

There is immense number of treatment options for hyperglycemia includes different lifestyle interventions and nutritional modifications. But severe conditions must be treated with drug therapy like insulin as well as oral hypoglycemic agents like sulfonylureas and biguanids. Unfortunately, these therapies can be associated with various side effects and can have further drawbacks upon long term exposure (Pareek et al., 2009).

However, the exploration of many clinically beneficial medications has resulted from the hunt for new pharmacologically active molecules discovered by evaluating natural ingredients such as traditional medicines or their derivatives (Pareek et al., 2009). As a result, anti-diabetic agents derived from natural sources have been recognized as potential treatment options because of having fewer side effects and higher therapeutic potential.

1.4 Significance of Phytotherapy

Through research initiatives, surveys, and dissertations, the pharmacological qualities of a variety of medicinal plants, as well as potential in phytotherapy have now been investigated. Researchers show that herbal medicines can be used in novel ways to treat ailments. However, a couple of core aspects play a role in improving phytotherapy in clinical settings (Colalto, 2018).

Current research has verified the significance of these compounds, as well as extracts' potential to function in combination with all other ingredients, with the goal of enhancing effectiveness and tolerability in acute management of various diseases. In addition to this, the identification of novel compounds and plant extracts have enabled us to develop diverse range of therapeutic organic drugs (Colalto, 2018). Since plant-derived medications have fewer adverse effects than synthetic drugs, they are thought to be the least hazardous and much more suitable with biological systems. As a result, scientific research is devoted to the development of novel medications based on the natural substances of herbal extracts for the treatment of various health conditions such as hyperglycemia and diabetes (Kumar et al., 2011).

1.5 Brief history of Herbal medicines

Herbs have been used as the basis for medical treatments for the majority of human civilization, and herbal medicines is still being used today. Many organic chemicals are used as the basis for evidence-based pharmacological medications in modern medicine. The usage of medicinal herbs may be traced back to the Paleolithic period, some 60,000 years ago, according to archeological data. Herbal treatments have been documented in writing since the Sumerians created catalogues of plants around 5,000 years ago. Herbals literatures were written by ancient societies about plants and their medicinal purposes (Matole et al., n.d.). Because of its cultural acceptability, compatibility with the human body, and lack of adverse reactions, herbal medicine continues to be a key health care choice for around 75% of the earth's population, particularly in emerging nations (Sam, 2019).

However, their use in the industrialized world has grown substantially. The first pharmaceutical molecule, morphine, was created 200 years ago using opium derived from poppy flower seeds. Ever since, scientists have studied plants in order to improve medications, and people are starting to give greater attention to herbal medicinal treatment, which has a lower cost than standard medication synthesis. To avoid illness and ailments, herbal vitamins and natural medications are consumed and absorbed. Every drug or supplements aids in the prevention of various diseases and mild illnesses such as headaches, stomach aches, fractures, sprains, and a variety of other ailments (Sam, 2019). Among different kinds of traditional medicines, Ayurveda, Homeopathy, Neuropathy, Acupuncture etc. are the most common forms.

However, India is widely known for its world-famous traditional systems of medicines. Ayurveda is the most ancient, widely acknowledged, practiced, and flourished indigenous system of medicine in India. Unani, Siddha, Homeopathy, Yoga, and Naturopathy are the other allied

medical systems here (Mukherjee, 2001). In recent decades, Ayurveda has undergone a significant radical shift, as well as a significant shift in scientists' attitudes on its implementations. The therapeutic ideas of Ayurveda are based on the concepts of prakriti and tridoshas, which indicate the unique constitution of each person known as prakriti. Prakriti identifies each person's unique sensitivity to drugs, environmental circumstances, and nutritional considerations. Ayurgenomics, a newly developed scientific topic is a prospective theory that binds genomics and Ayurveda together and aids in the assessment of inter-individual variability in therapeutic responsiveness in a variety of disorders (Jaiswal & Williams, 2017).

1.5.1 Use of medicinal plants in Bangladesh

Bangladesh being located in the tropical zones has the ideal climate and geographic proximity for the growth of a diverse range of medicinal plant species. As a result, the use of medicinal plants in the treatment of ailments has a prestigious history in this country. In Bangladesh, ethnomedicinal professionals (Kavirajes) are the main health providers for large sectors of the rural and urban populations. For the treatment of diseases, the Kavirajes depend mostly on basic concoctions of medicinal plants. While the Kavirajes primarily treat basic ailments, they do occasionally treat more serious diseases that are difficult to treat with conventional medical therapy (Mehedi Hasan et al., 2010).

However, approximately 5000 varieties of phanerogams and pteridophytes flourish as indigenous, naturally occurring, or cultivated plants in Bangladesh's forests, lakes, fields, and streets. Many Kavirajes in Bangladesh have suggested that coughs, fevers, and mucus could be treated by drinking juice made from the leaves of *Justicia adhatoda* mixed with sugar. Moreover, the local health care providers have also observed the potential therapeutic effect of medicinal plants using either a single plant component or a mixture of plant parts from different species to

treat various diseases. Coughs and asthma were treated using the leaves of *Acorus calamus* and liquid juices made from a mixture of macerated barks of *Lannea coromandelica*, *Terminalia arjuna* etc was used to treat chronic diarrhea. Numerous plant components from a single plant could also be utilized to cure a disease; for instance, juice from macerated leaves and barks of *Terminalia arjuna* can be used to treat heart ailments (Israt Jahan et al., 2011).

Some other significant medicinal plants available in Bangladesh are- *Clerodendrum viscosum* (Bhant), *Dillenia indica* (Chalta), *Clitoria ternate* (Aparajita), *Diospyros peregria* (Gab), *Dipterocarpus turbinatus* (Garjan), *Ecbolium Viride* (Nilkhanta), *Glinus oppositifolius* (Gima), *Saraca asoca* (Asoca), *Glycosmisper taphylla* (Mehedi Hasan et al., 2010).

1.5.2 Phytotherapy in Drug Discovery for hyperglycemic patients

Phytotherapy is one of the most crucial forms of treatment for hyperglycemic patients and can be beneficial in potential drug discovery due to its anti-hyperglycemic and hypoglycemic effects. The use of conventional medications to treat metabolic abnormalities and the pathological consequences of diabetes increases complications that attributes to adverse reactions and can be costly. These medications can often have uncomfortable route of administration and as a result, switching to herbal treatment could be more effective, cost-efficient, have fewer side effects, and have less cytotoxicity.

For hyperglycemic patients, some prospective plants which can be used as potential anti-diabetic agents are shown below-

Researchers have shown *Azadirachta indica*, to be an effective herbal medicine with anti-diabetic potential. Along with that some other prospective anti-diabetic agents are- *Centella asiatica* L. Urb., *Ficus racemose* L., *Ficus hispida* L.f., *Mangifera indica* L., *Momordica*

charantia L., *Syzygium cumini* L., *Terminalia chebula* Retz., *Coccinia grandis* L. Voigt., *Coccinia cordifolia* L. Cogn., *Aegle marmelos* L. Corrêa, *Tinospora cordifolia* Hook. F. and Thoms., *Trigonella foenumgraecum* L., *Tamarindus indica* L., *Moringa oleifera* Lam., *Kalanchoe pinnata* (Lamk.) Pers., *Bombax ceiba* L., *Cajanus cajan* L. Millsp., *Psidium guajava* L., *Clerodendrum viscosum* Vent., *Scoparia dulcis* L. etc (Rahman et al., 2021).

From a comprehensive review of the ethnomedical value of anti-hyperglycemic plants, it has been shown that various different parts of a medicinal plant can be used for the desired hypoglycemic effect. For example, the leaf is basically the mostly utilized part of a medicinal plant which accounts for almost 32% followed by the fruit 14% other parts of the whole plant 12%, root 11%, seed 11%, bark 9%, stem 6%, flower 3%, rhizome 1%, and others 1% (Rahman et al., 2021).

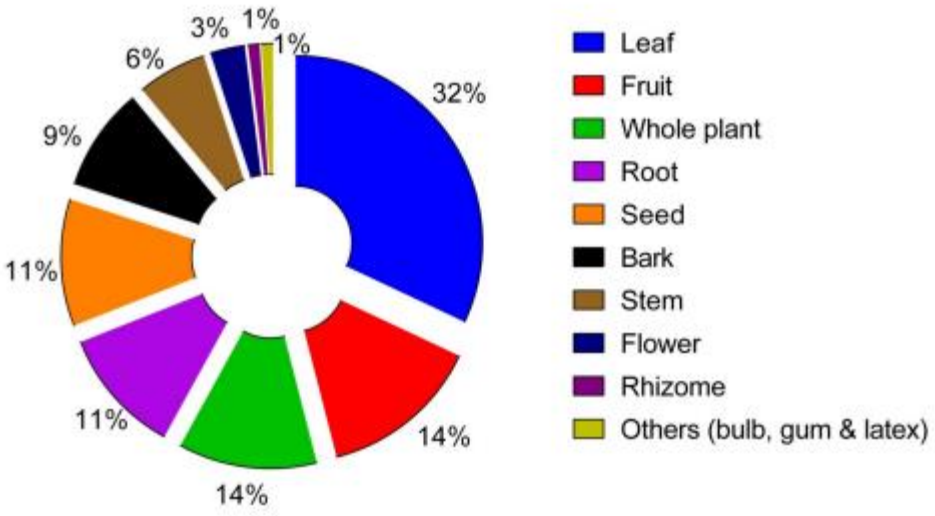

Figure 1: *In Bangladesh, the percentage of antidiabetic plant parts utilized for diabetes therapy (Rahman et al., 2021).*

When compared to the action of a single selective drug, a coordinated regulation of numerous targets can offer a higher therapeutic benefit and a lower adverse effect pattern. As a result, the current therapeutic technique for type II diabetes relies on a combination of an insulin secretagogue and an insulin sensitizer to give a reasonable treatment regimen. Despite the adequate therapy given by these medications, some type II diabetic patients lose tolerance to traditional antidiabetics over time, resulting in poor glycemic control (El-Abhar & Schaalan, 2014).

Herbal remedies may consist of a variety of active ingredients or molecules that can alter different biological processes and treat diabetes symptoms through a variety of mechanisms, delivering complex outcomes. Since current findings reveal that postprandial hyperglycemia might cause non-enzymatic glycosylation of numerous proteins, resulting in the development of chronic problems. This postprandial hyperglycemia plays a significant role in the occurrence of type II diabetes and therefore, maintaining its concentration by reducing the activity of -

glucosidase enzymes is therefore thought to be a key method of controlling this condition. By understanding the key pathophysiology of herbal medicines and how it exerts its effects on patients with hyperglycemia, we can expect to move forward in the drug discovery. Therefore, for the screening of desired therapeutic potential of a medicinal plant, the phytochemical assessment of its crude extracts is necessary (El-Abhar & Schaalan, 2014).

1.6 Phytochemical screening of crude extract of Medicinal Plants

Through phytochemical assessment of the crude extract of medicinal plants, we can avail the secondary metabolites which can then be further examined for the presence of the desired therapeutic effect. The bioactive constituents can indicate potential application of the medicinal plant in therapies. Thus, the qualitative analysis is a crucial indicator of the phytochemicals like steroids, tannins, fatty acids, flavonoids, alkaloids etc. The phytochemical investigation includes the preparation of plant material and compounds that are medicinally active are extracted, identified, and screened. Plants naturally produce a variety of phytochemicals, which have a range of biological implications, including their ability to defend plants from pathogenic microorganisms. These substances are isolated and identified for medicinal plant study using a variety of separation procedures (Solomon et al., 2012).

1.7 Natural Constituents Derived from Medicinal Plants

Tannins, alkaloids, carbohydrates, glycosides and flavonoids are some of the chemical compounds found in medicinal plants that have a definite physiological effect on human health. The primary or secondary metabolism of living organisms produces these chemicals. Secondary metabolites are a group of chemically and taxonomically varied molecules with unknown functions. They're frequently employed in human medicine, veterinary medicine, agricultural, academic research, and a variety of other fields (Nandagoapalan et al., 2016).

1.7.1 Alkaloids

Alkaloids are a class of compounds with a broad variety of biological effects. They are a structurally diverse group of chemicals that include a nitrogen atom in a heterocyclic ring and are synthesized from amino acids. Due to their efficiency in preventing the development of several degenerative illnesses by scavenging of free radicals or binding with oxidative reaction catalysts, such as metal ions, they are significant therapeutic compounds. Microorganisms such as bacteria, fungi, and protozoans are likewise inhibited by these compounds (Mane & Shaikh, 2021).

1.7.2 Flavonoids

Flavonoids are a form of secondary chemical found in plants that has a flavone backbone (2-phenylchromen-4-one). Flavanols, isoflavones, flavanones, flavanonols are significant subcategories that are identified by the molecules connected to the flavonoid molecule (Chen et al., n.d.).

1.7.3 Tannins

Tannins have antioxidant properties. They are cardioprotective, anti-inflammatory, and anti-mutagenic, among other things. Tannins promote uptake of glucose while suppressing adipogenesis. Tannins can help diabetics by managing the unhealthy oxidative condition. Many previous researches have revealed that phenolic chemicals and flavonoids protect against cancer in a variety of ways. They have been found to increase glucose absorption by activating insulin-signaling pathway mediators such as PI3K (Phosphoinositide 3-Kinase) and p38 MAPK (Mitogen-Activated Protein Kinase) and translocating GLUT-4. The decrease in glycemia (blood glucose levels) caused by phenolic compounds has been linked to a number of mechanisms, including reduced nutritional absorption, reduced food intake, stimulation of cell regeneration,

and a direct action on adipose cells that increases insulin function. Tannins have also shown anti-hyperglycemic effect in diabetic mice (Kumari & Jain, 2012).

1.7.4 Terpenoids

Terpenoids have been shown to have anti-inflammatory, antiviral, antimalarial, cholesterol production suppression, and antibacterial properties. These are the most common type of component found in essential oils, and they're also known as isoprenoids (Wawrzyn et al., 2012).

1.8 Analysis of Anti-hyperglycemic activity of Medicinal Plants

In order to analyze the anti-hyperglycemic activity of different medicinal plants, it is important to prepare the plant extracts and examine its therapeutic effects. The analysis can be done using animal models where hyperglycemia is created beforehand with the help of various chemical compounds similar to glucose. These compounds can induce diabetes in experimental animal models by exerting toxic glucose concentration by accumulating in the pancreatic beta cells via GLUT2 glucose transporter. Therefore, investigation of anti-hyperglycemic properties of the hypoglycemic agents present in plant extracts are carried out in various time intervals using these effective diabetic inducing compounds. Alloxan and Streptozotocin are the examples of two vital diabetic inducing components for the analysis of anti-hyperglycemic activity in medicinal plants (Radenković et al., 2016).

1.8.1 Alloxan in producing hyperglycemia

Alloxan is a pyrimidine derivative produced by uric acid oxidation (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil). It's a hydrophilic, fragile molecule with a glucose-like composition.

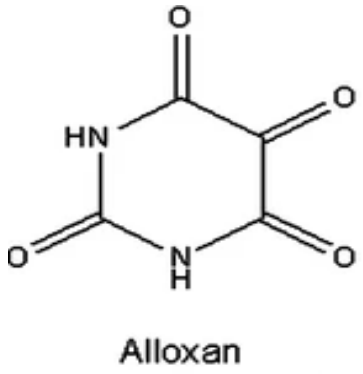

Figure 2: Structure of Alloxan (Furman, 2016)

These alloxan properties are critical for diabetes progression. Its hydrophilicity inhibits alloxan from entering the plasma membrane's lipid bilayer, but its glucose-like structure allows alloxan to enter beta cells (Jö et al., 1997). Alloxan also has a central 5-carbonyl group that interacts with thiol groups in various enzymes, particularly glucokinase (hexokinase IV), which is known to be the most delicate thiol enzyme in beta cells. This is a fundamental structural characteristic for the onset of diabetes (Radenković et al., 2016).

1.8.2 The mechanism of Alloxan induced Diabetes

Given the structural similarity of alloxan and glucose, as well as the fact that both would enter beta cells via GLUT2 transporters, it seems clear that glucose can limit the uptake of alloxan by

B cells. This suggests that glucose can shield B cells from the toxicity of alloxan. It's worth noting that glucose's ability to protect against alloxan toxicity is concentration-dependent (Jö et al., 1997).

Alloxan's hydrophilic character is critical for its usage in diabetic animal models; alternatively, it would be capable of penetrating each type of cell in the animal species and cause severe damage. GLUT2 transporters are expressed in many cells, especially hepatocytes and renal tubular cells, thus explains alloxan-induced liver and kidney damage. Despite the fact that alloxan can penetrate these cell types, it is much more selective for pancreatic B cells because of their faster uptake and the fact that glutathione peroxidase activity and resilience to exogenous peroxide are roughly 20 times higher inside the liver and kidney than in the pancreas (Radenković et al., 2016).

Moreover, the alloxan-induced rise in intracellular calcium is likely one of the plausible causes for this alloxan-induced metabolic impact. Alloxan can depolarize B cells, resulting in the activation of voltage-dependent calcium channels and an elevation in cytosolic calcium levels. The impact of calcium channel blockers on the formation of alloxan-induced diabetes indicates that calcium may play a significant role in this diabetes model (Lin & Accili, 2011).

A temporary decrease in ATP consumption is caused by glucokinase blocking glucose phosphorylation, leading in a transitory increase in ATP in beta cells and the secretion of insulin. Alloxan causes pathology in beta cells via two distinct mechanisms: suppression of glucokinase and formation of the reactive oxygen species (ROS) cycle. Glucokinase is a hexokinase isozyme that is a key glucose phosphorylating enzyme in both the liver and pancreatic B cells (Lin & Accili, 2011).

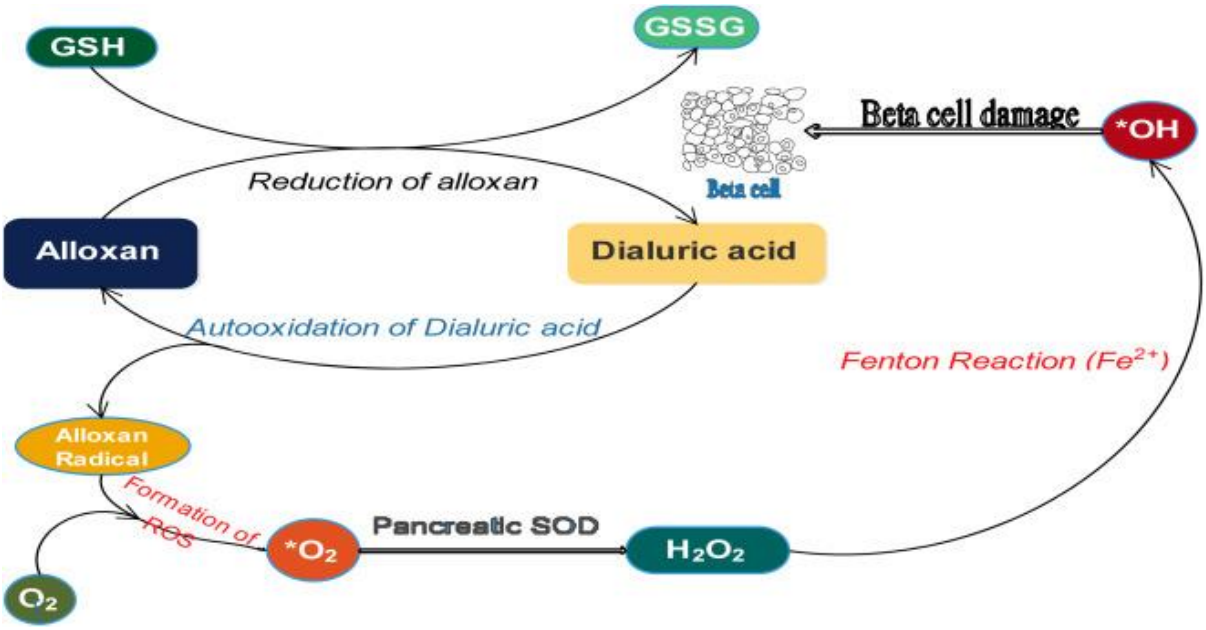

Figure 3: ROS Formation through Redox cycle (Ighodaro et al., 2017).

Only pancreatic beta cells are destroyed once alloxan is administered to experimental mice, leaving alpha and delta cells unharmed. Although alloxan destroys beta cells, the hyperglycemia that results from the therapy is less persistent, and it is a reduction reaction that can sometimes result in blood glucose levels returning to normal after a period of time (S. Kumar et al., 2012).

1.9 Overview of sample plant: *H.fomes*

H.fomes (family: Sterculiaceae), often known as Sundari in Bengali, is a renowned mangrove plant found in Bangladesh's Sundarbans forest in the south in the salt water of Sundarban. Folk medicinal practitioners can employ several components of this plant as herbal remedies. Several studies have found that the plant has potent anti - oxidant, anti - nociceptive, antihyperglycemic,

antibacterial, and anticancer properties. Essential chemical ingredients such as saponins, alkaloids, flavonoids, tannins, steroids etc. have been discovered through phytochemical investigations (Mahmud et al., 2014).

Figure 4: Leaves of H.fomes (Mahmud et al., 2014)

Figure 6: Barks of H.fomes (Mahmud et al., 2014)

Figure 5: pneumatophores of H.fomes (Mahmud et al., 2014)

1.10 Taxonomic classification of *H.fomes*

Kingdom: Plantae

Species: H. fomes

Genus: Heritiera

Family: Malvaceae

Subfamily: Sterculioidea

Order: Malvales

Local name: Sundari (Upadhyay et al., 2008)

1.11 Morphology

It is a medium-sized evergreen tree that can reach a height of 25 meters. The leaves usually dark green in color and have 1 cm long petioles. They're gathered together at the branches' ends. The species starts producing pneumatophores at the age of three years. The pneumatophores stand about 50 cm tall. The wood of this plant is tough, hefty, and long-lasting. Flowers are non-gender specific and clustered in panicles. They are made up of five stamens joined together to produce a cylindrical shape called a pistilloid. The plant usually blooms in March and April. Fruits start as pale green and turn brown as they ripen. With juicy endosperm, these are single seeded. Seeds range in size from 3–5.5 cm in length and 3.5–5 cm in width. During the months of June and July, seeds are shed (Upadhyay et al., 2008).

1.12 Habitat

The plant, *H.fomes* is found in the the world's biggest mangrove forest, Sundarbans, which is situated in Bangladesh's southern region and the Indian state of West Bengal. The forest covers 6017 square kilometers of land, with river regions covering 1874 square kilometers. The forest stretches over 180 miles west and more than 70 miles south to north. With temperatures ranging from 20.4 to 31.5 degrees celsius, the forest has intriguing ecological properties. The annual rainfall varies between 1640 and 2000 millimeters. It takes up 52.7 percent of the land area and 63.8 percent of the total standing capacity. (Mahmud et al., 2014)

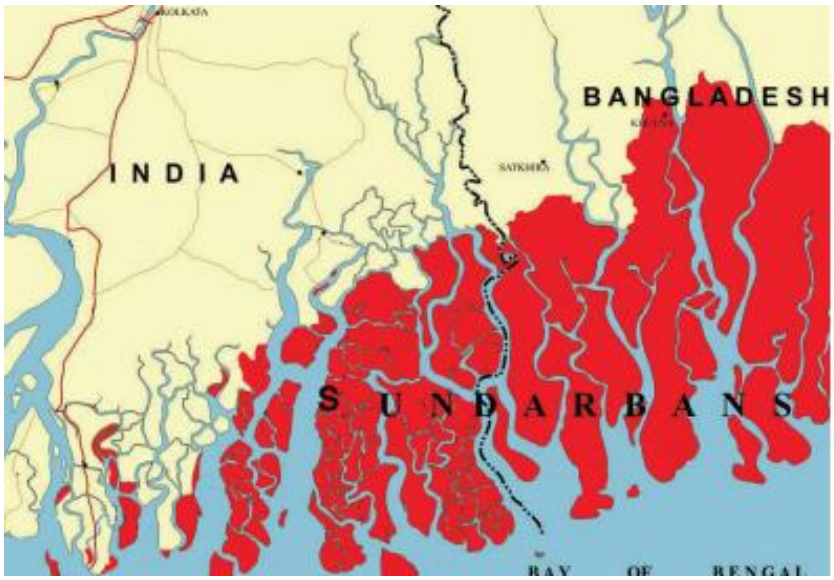

Figure 7: Sundarban Map (Mahmud et al., 2014)

1.13 Literature Review

In previous studies, researchers have shown that *H.fomes* have potential hypoglycemic effect upon various experiments with diabetic animal models. Various phytochemical constituents present in this plant (tannins, alkaloids, flavonoids, glycosides etc.) can exert significant therapeutic effects. The plant has long been used to treat gastrointestinal problems, gout, hyperglycemia and a variety of other ailments (Sarkar et al., 2019).

Moreover, the serum glucose levels were found to be reduced by 19.4%, 35.6%, and 44.7% with 100, 250, and 500 mg extract of the sample plant (*H. fomes*) per kg body weight, respectively which implies the potential anti-hyperglycemic activity of the plant. (Ali et al., n.d.) The amylase enzyme was inhibited to varying degrees by the leaf extracts in α- amylase Inhibition Assay. The extract of this plant has also been known for increasing the glucose adsorption capacity in a dose-dependent manner. According to studies, it can be assumed that the existence of phytochemicals in *H.fomes* may be the core cause of the desired inhibitions which indicates the presence of anti-hyperglycemic agents (Sarkar et al., 2019).

1.14 Rationale of the study

H.fomes is commonly found in Bangladesh's moist and salty regions. It's been used for many years to cure ailments like diabetes, skin diseases, diarrhea etc. The easy availability & therapeutic prospect of this plant makes it an intriguing phytochemical research aspect. The research is aimed to screen the presence of phytochemicals & investigate the plant extract's anti-hyperglycemic efficacy.

1.15 Aim of the study

The aim of this study is to analyze the phytochemical & hypoglycemic screening of *H.fomes* in alloxan induced hyperglycemic mice.

1.16 Objective of the study

The key objective of this study is to assess the therapeutic potential of *H.fomes* as prospective hypoglycemic agent using alloxan induced diabetic mice and screen the phytochemicals present in this plant.

Chapter 2: Methodology

2.1 Collection & Identification of Plant

The leaves, bark, and root of *H.fomes* were collected from Sundarban, Bagerhat district, Bangladesh in December 2019 for the experimental research. A taxonomist from Bangladesh National Herbarium, Mirpur, Dhaka (**DACB Accession No: 50664**) recognized and verified the plant. The plant pieces were washed thoroughly in order to eliminate the dust particles. Separate leaves, stems, and roots were sliced and shade dried for almost two weeks before being processed into a coarse powder using a laboratory electric mixer. For examination, the powdered plant components were preserved separately in sealed jars in a cool, dark, and dry environment.

2.2 Process of Extraction

The extraction process of *H.fomes* is very crucial for the analysis of the anti-hyperglycemic activity in diabetic mice. For proper extraction, preparing the plant parts and drying them prior to the experimentation is necessary.

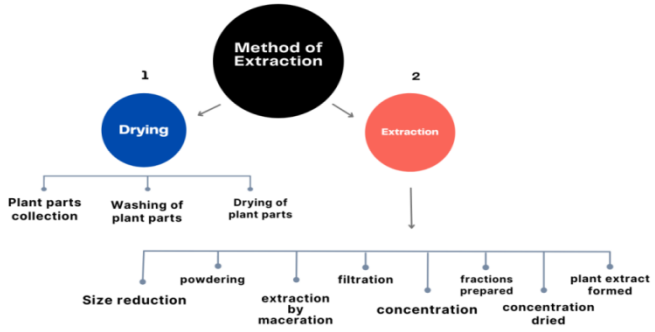

Figure 8: Method of Extraction (Abubakar & Haque, 2020)

2.3 Drying and preparing the parts of *H.fomes*

To eliminate dust or dust mites, leaves, barks, and roots were cleaned thoroughly. After that, the rinsed sections were laid out on fresh sheets to dry completely for 2 weeks in the sunlight.

2.4 Extraction

The extraction process was carried out in some gradual steps for an ideal plant extract formation. At first, the size of the dried parts was reduced and was cut into tiny pieces. Leaves were crushed; the barks and roots were divided into two- to three-inch-long pieces. The finer bits were then crushed into tiny granules and measured separately. Therefore, the crude powders were placed in three clean sealed plastic jars that were labeled with the appropriate information. The size reduction was then followed by maceration process of extraction using ethanol as the solvent. This is an extraction method in which grinded drug material, such as leaves, stem bark, or root bark, is placed in a container and menstruum is poured over it until the drug material is thoroughly coated. After that, the jar is sealed and stored for at least three days.

Filtration separates the micelle from the marc at the end of the extraction process. Evaporation is then used to remove the micelle from the menstruum (Abubakar & Haque, 2020). The powdered root, bark and leaves were soaked in 1 litre of ethanol in individual containers for about 7 days in 25 °C.

After that, the filtration was carried out with the help of clean cotton cloth as well as filter paper followed by a thorough evaporation process in order to obtain concentrated crude extracts by removing the solvent. The concentrated extract was later dried well.

2.5 Phytochemical Screening of *H.fomes*

The phytochemical screening of alkaloid, carbohydrate, tannins, flavonoid, saponin, glycoside, steroid, phenol and resin was done to assess the presence of the phytochemicals in *H.fomes*

2.5.1 Detection of Alkaloid

At first, 3ml of the ethanol leaves, barks, and roots filtrate solution was divided into three test tubes and labeled. The test tubes were then filled with 1 mL of hydrochloric acid and thoroughly shaken. The Wagner's reagent (a mixture of iodine and potassium iodide) was therefore poured drop by drop to each test tube. In each of the test tubes, a reddish brown precipitate appeared, showing that alkaloid was present in the leaves, barks, and roots of *H.fomes* (Nandagoapalan et al., 2016).

2.5.2 Detection of Carbohydrate

Benedict’s test

When the crude extracts of the bark, leaf and root was heated with 2ml Benedict's reagent in three separate test tubes, a reddish brown precipitate appeared which indicated the presence of carbohydrates (Mane & Shaikh, 2021).

2.5.3 Detection of Tannin

In different test tubes, 1 mL of filtrate ethanol solution of the leaves, barks, and roots extract was mixed with 2 mL of distilled water. Following that, 2-3 droplets of Ferric Chloride were added to the mixture, resulting in a green or blue-black tint in the test tube of leaves, showing the presence of Tannin. In the test tubes of barks and roots, a dark blue to bluish black color was detected, confirming that Tannin was found (Mane & Shaikh, 2021).

2.5.4 Detection of Flavonoid

Shinoda test

After taking 3 ml of the extracts in separate test-tubes, 8 - 10 drops of concentrate HCl along with a little amount of magnesium powder was added. Then the mixture was boiled for about 10 to 15 minutes and then cooled. After cooling, a red color was observed which indicated the presence of flavonoids (Nandagoapalan et al., 2016).

2.5.5 Detection of Saponin

1ml of the filtrate ethanol solution of the leaves, barks, and roots extract was poured in test tubes and 1ml of distilled water was added for identifying saponin. The test tubes were then rapidly shaken and left still for 20 minutes to examine the steady persisting forth. When the bark and roots of *H.fomes* were shaken, they had a very foamy appearance, indicating that they contained more saponin than the leaves (Gandhiraja et al., 2009).

2.5.6 Detection of Glycoside

The Keller-Killiani Test was used to detect glycosides, which involved placing 4ml of filtered ethanol extracts of leaves, barks, and roots into three test tubes. The extract-containing test tubes were then given a few drops of Glacial Acetic Acid and a few drops of Ferric Chloride. After that, a few of drops of concentrated Sulphuric Acid were mixed. It resulted in a reddish brown coloration at the intersection of two layers, with the upper layer being bluish-green in tone (Nandagoapalan et al., 2016).

2.5.7 Detection of Steroid

The Libermann-Burchard reagent was made by mixing 5ml of Acetic Anhydride with 5ml of strong Sulphuric Acid, then mixing 50ml of Ethanol and was cooled in ice cold water. The

mixture was then heated for 5 to 10 minutes at 100°C. Following that, 10 mg of the concentrated ethanol extract of leaves, barks, and roots was removed and diluted in 0.5ml of hot Acetic Anhydride in test tubes. After that, 0.5 mL of chloroform filtrate was poured to each of the test tubes. The freshly made Libermann-Burchard reagent was poured drop by drop after the extracts were thoroughly mixed, resulting in the creation of a blue-green ring around the test tubes of bark and root extracts (Nandagoapalan et al., 2016).

2.5.8 Detection of Phenol

Liebermann's test:

In the 3ml of the different extracts placed in three different test-tubes, 1ml of sodium nitrite was added along with few drops of diluted sulphuric acid as well as 2ml of diluted NaOH. As a result, a deep red or blue or green color appeared which indicated the presence of phenol (Nandagoapalan et al., 2016).

2.5.9 Detection of Resin

1ml of the filtrate ethanol solution of the leaves, barks, and roots extract was obtained and several drops of Acetic Anhydride were added to identify resin. 1 mL pure Sulphuric Acid was then poured. All of the test tubes had an orange to yellow coloration, suggesting the presence of resin in the *H.fomes* plant extracts (Nandagoapalan et al., 2016).

2.6 Chemicals & Drugs used for anti-hyperglycemic test

All chemicals in this investigation were of analytical grade. Alloxan (Fluka, Germany), normal saline solution and Metformin (Square Pharmaceuticals Ltd., Bangladesh) were used to analyze the hypoglycemic effect after treatment with EHB, HER and EHL.

2.7 Preparing animal model for hypoglycemic analysis

Young Swiss-albino mice weighing 80-120 grams were utilized in this research. The mice were obtained from the International Centre for Diarrhoeal Disease and Research's Animal Research Branch in Bangladesh (ICDDRB). Mice were kept in individual cages at a temperature of 24 ± 10°C, a relative humidity of 55–65% and 12 hours of light (Okokon et al., 2022).

2.8 Initiation of diabetes in mice

A single subcutaneous injection of freshly made alloxan monohydrate mixed in normal saline was used to induce diabetes in overnight fasting mice. After 72 hours of injecting alloxan, the blood glucose level (BGL) was assessed with a one-touch glucometer, and hyperglycemia was diagnosed. For the study, mice with hyperglycemia (BGL > 10 mml/l) were selected.

2.9 Study on Alloxan induced diabetic mice

The investigational mice were split into eight groups each with six mice. Every mice batch had received a different therapy. The hypoglycemic action of samples given to mice at doses of 200 mg/kg and 400 mg/kg body weight was evaluated. Metformin was given at a dose of 150 mg per kilogram of body weight as standard. Each mouse was correctly weighted prior to the administration of medication and the doses were set accordingly.

The blood glucose levels of the experimental animals were tested at 0 hour using the tail tipping approach to analyze the hypoglycemic impact. (Naik K.H., Patel V.S., Mehta R.D., 2015) The standard, EHL, EHR, and EHB extracts, as well as their various proportions, were then given orally to the mice using a feeding syringe. Prior to medication (day 0), blood samples were taken from the tail vein, and again at periodic intervals on days 1, 2, and 3. Group I, group II, group III, group IV, group V, group VI, group VII, and group VIII were the eight experimental groups.

Chapter 3: Statistical Analysis

All the values in the test are presented as mean $\pm$ STD (n=6). The data were analyzed statistically by using One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. The Differences between the means of the various groups were considered significant at $P<0.05$ compared to control.

Chapter 4: Result

4.1 Phytochemical Screening of Ethanolic Extract of *H.fomes*

Table 1: Screening of Ethanolic Extract of *H.fomes*

Class of Compounds	Name of Test	Present (+)/Absent(-)
Alkaloid	Wagner's Test	+
Carbohydrate	Benedict's Test	+
Tannins	Ferric Chloride Test	+
Flavonoid	Shinoda Test	+
Saponin	Foam Test	+
Glycoside	Keller-Killiani Test	+
Steroid	Libermann-Burchard Test	+
Phenol	Liebermann's Test	+
Resin	Acetic Anhydride Test	+

4.2 Effect on BGL in Alloxan induced diabetic mice

Table 2: Blood glucose level of alloxan induced diabetic mice after treatment with *EHB, EHR, EHL*

Group	Design of Treatment	Dose mg/kg	Blood glucose levels (mml/l)		
			1st day	2nd day	3rd day
I	N.saline		8.07 ± 0.07	7.95 ± 0.04	8.08 ± 0.04
II	Standard	**150**	19.76 ± 0.17*	17.58 ± 0.20*	15.75 ± 0.17
III	EHB-200	**200**	13.47 ± 0.68*	6.53 ± 0.27	4.5 ± 0.35816
IV	EHB-400	**400**	18.67 ± 0.42*	12.95 ± 0.70*	7.08 ± 0.49
V	EHR-200	**200**	16.83 ± 1.19*	11.33 ± 0.76*	7.88 ± 0.27
VI	EHR-400	**400**	17. ± 0.68*	12.50 ± 0.76*	8.17 ± 0.17
VII	EHL-200	**200**	12.5±0.6	10.56±0.58	9.33±0.78
VIII	EHL-400	**400**	17.33±0.57	13.76±0.58	9.50±0.89

All values are expressed as mean ± STD (n=6); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05 significant compared to control.

The effect of varying dosages of ethanol extract of bark, leaves, and root of *H.fomes* on blood glucose levels in hyperglycemic mice can be seen in the table. With 200 and 400mg/kg doses of

EHR, EHL, and EHB extracts, there was a substantial reduction in blood glucose levels on the first, second, and third day ($P<0.05$).

Chapter 5: Discussion

Plants can be used as medicinal sources in a range of methods. In the pharmaceutical industry, many therapeutic herbs are utilized as crude extracts or as raw resources. The portion that bears the pharmacological characteristic can be collected and used as tinctures, fluid extracts, tablets, or capsules. The most common method of extracting and purifying natural resources in order to isolate chosen components that can be used as active pharmaceuticals is to follow the right extraction and purification technique. Therefore, phytochemical screening of medicinal plants is very necessary in order to identify the presence of the core constituents. Through the phytochemical screening of *H.fomes* performed in this study, the presence of alkaloid, carbohydrate, tannins, flavonoid, saponin, glycoside, steroid, phenol and resin was found.

Analyzing the hypoglycemic effect of ethanol extract of *H.fomes* in diabetic Swiss albino mice, reduced blood glucose level was observed upon treatment with EHB, EHR and EHL (Ethanol extracts of bark, root and leaves of *H.fomes*) in a three day trial. As per the findings, high therapeutic efficacy of this plant was observed which indicates that *H.fomes* can be considered as a potential treatment option for hypoglycemia.

Chapter 6: Conclusion

The medicinal use of *H.fomes* can be enhanced due to its efficacy and affirmative responses in various experimental models. Different parts of *H.fomes* are now being considered for the treatment of life-threatening diseases and following the ongoing research, synthesis of new drug substances can be initiated.

To conclude with the findings of this research, various parts of *H.fomes* can be used as hypoglycemic agents for its glucose lowering potential.

Chapter 7: Future Prospects of *H. fomes*

H.fomes contains numerous therapeutic properties due to the presence of significant phytochemicals in the crude extracts. Moreover, the phenolic components found in the plant can be isolated in large quantities and utilized to produce various anti-hyperglycemic, anticancer, antioxidant, as well as antimicrobial agents. Different screening techniques can also be used to determine the chemical constituents present in *H.fomes*.

With thorough phytochemical screening and investigations with the phytochemical compounds, various other pharmacological effects can be measured for several potential effects of *H.fomes* alongside its anti-hyperglycemic activity as illustrated in this study.

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