Combretum indicum

Thesis On

Phytochemical Screening and In Vitro Thrombolytic Potential of the Methanolic Extract of *Combretum indicum*

Submitted To

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APPROVAL

This Thesis Phytochemical Screening and In Vitro Thrombolytic Potential of the Methanolic Extract of *Combretum indicum* , submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Masters of Pharmacy and approved as to its style and contents.

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DECLARATION

I hereby announce that I am carrying out this thesis study under the supervision of Mr. Mohammad Touhidul Islam, Lecturer (Senior Scale) Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Impartial Compliance with the Bachelor of Pharmacy Degree Requirement (M. Pharm). This project, I declare, is my original work. I also state that neither this thesis nor any part thereof has been submitted for the Bachelors award or any degree elsewhere.

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Abstract

According to the results of the most recent study, the leaves of the plant known as *C. indicum* have the potential to be beneficial in the treatment of diabetes as well as the problems that are linked with the condition. These potential advantages might include the preservation of the pancreas via the regeneration of damaged beta islets as well as an improvement in the lipid profile. The thrombolytic processes are not significantly altered as a result of this factor. However, further study is required in order to comprehend the procedure and identify the compounds that are biologically active. This extract of C. indicum was carried out using a solvent containing methalonoic acid. In the presence of in-vitro thrombolytic activity, lysis of 68.81% of the clots was achieved by treating them with 100 ml of streptokinase (30,000 I.U.) and incubating the mixture at 37 degrees Celsius for 90 minutes. The rate of clot lysis was very low (3.44%) when the clots were treated with 100 microliters of sterile distilled water (a negative control). During an in-vitro thrombolytic activity test, the methanolic extracts of Combretum indicum exhibited a clot lysis rate of 6.57%. Using a statistical model that comprised a negative control (sterile distilled water), a positive control (Streptokinase), and Combretum, a calculation was made to determine the effective clot lysis percentage.

Key words: Streptokinase, Thrombolytic, Saponins, Flavonoids.

Chapter One: Introduction

Chapter Two: Met

ıodology

Chapter Three: Result and Discussion

Chapter Four: Conclusion

Chapter Five: Reference

List Of Figure-

List Of Table-

Introduction

1.1. Plant

Photosynthetic eukaryotes, which are also eukaryotes, make up the kingdom of eukaryotic organisms. Plantae are plants. At one point in time, fungi and some algae were thought to be members of the plant kingdom; however, these organisms are now generally excluded, along with prokaryotes, from all contemporary definitions of the word "Plantae." (the archaea and bacteria). The plant group known as the Viridiplantae, which literally translates to "green plants," is the sister group to the Glaucophyta. The green algae and the clade Embryophyta make up the Viridiplantae (land plants). The second group of plants includes things like flowers, trees, ferns, hornworts, and liverworts, as well as mosses. This category also contains organisms known as mosses. The majority of plant species have bodies that are made up of an extremely high number of individual cells. The fundamental method by which green plants get their supply of usable energy is known as photosynthesis. This process is carried out by primary chloroplasts, which are created via the process of endosymbiosis with cyanobacteria. The chlorophylls a and b that are found in the chloroplasts of their cells are responsible for giving these organisms their characteristic green color. Even though they lack the photosynthesis and chlorophyll necessary for normal growth, parasitic or mycotrophic plants may still be able to produce flowers, fruit, and seeds despite the fact that they are unable to feed themselves. This is because they feed off of other plants rather than on themselves. In addition to these characteristics, it is generally known that plants are capable of sexual reproduction, generational alternation, and extensive asexual reproduction. There are around 320,000 unique species of plants, and between 260 and 290 thousand of those species are able to reproduce in ways outside the production of seeds. [1] The production of the vast majority of the molecular oxygen that is needed by the ecosystems of Earth is mostly the responsibility of green plants. In addition to this, green plants are accountable for the production of a sizeable portion of the food supply around the planet. In order for humans to fulfill their nutritional needs, they have been cultivating plants for a very long time that produce grain, fruit, and vegetables. This has been done in order to satisfy their dietary requirements. Plants have provided people with a broad array of valuable items for millennia, including food, fiber, paper, medicine, and a variety of hallucinogenic substances. These products include food, paper, and medicine. [2] Botany is a branch of biology that focuses specifically on the investigation and study of plant life. Plants and animals were the only groups that were able to be distinguished from one another based on their

ability to digest food and breathe oxygen. It is believed that Aristotle, who lived from 384 to 322 BC, was the first person to draw the analogy between plants and animals, which do not generally move (which are often mobile to capture their food). When Linnaeus (1707-1778) supplied the foundation for the present system of scientific taxonomy, these two divisions expanded into the kingdoms Vegetabilia (later Metaphyta or Plantae), and Animalia. Linnaeus is credited with being the father of modern scientific taxonomy (also called Metazoa). After it was recognized that the basic concept of the plant kingdom had a vast number of species that were not closely related to one another, the fungi and a few other kinds of algae were divided into their own kingdoms. This was done in order to make room for these new kingdoms. In spite of this, there are still many who, when speaking informally about these sorts of creatures, refer to them as plants. Under these specific conditions, a reference is expected to be provided. It is common practice to refer to as a "plant" any multicellular creature that has cell walls that are rich in cellulose and primary chloroplasts that are capable of conducting photosynthesis. This definition encompasses the vast majority of multicellular organisms. [3-4]

1.2. Medicinal plants

The use of therapeutic plants, sometimes referred to as medicinal herbs, has been an integral part of the traditional medical practices of a diverse range of nations throughout history. Plants produce a huge array of chemicals for a number of purposes, including the purpose of providing a defensive mechanism against herbivorous animals, insects, fungi, and illnesses. There have been a large number of phytochemicals discovered, and the biological activities that they engage in have either been confirmed or highly predicted. However, due to the fact that a single plant might contain a large number of different phytochemicals, the benefits of ingesting a complete plant in the form of medicine are still debatable. There are a great many plants that are thought to have therapeutic value; however, very few of these plants have been subjected to the kind of in-depth scientific study that would prove their usefulness and safety, let alone describe their phytochemical composition and the effects that it has on the body. [5] On clay tablets constructed by the Sumerian civilisation between around 3000 BC and 3000 AD, hundreds of different medicinal herbs, including opium, are listed. These tablets date back to roughly 3000 BC. One example of a document that was created in ancient Egypt is known as the Ebers Papyrus. [6] Not only are medicinal plants important to the economy, but they also have a diverse variety of applications in

everyday life. They include therapeutically beneficial compounds, such as Stary, which may be used to treat a broad variety of human health issues. It has been investigated, developed, and presented as a possibility that some plant extracts may be used as medicinal agents. Due to the one-of-a-kind qualities of medicinal plants, such as their potential to serve as a source of phytochemicals with therapeutic advantages that might lead to the creation of new medications, in-depth study into these plants is now under way. A number of studies have shown that phytochemicals, which are molecules that may be found in plants, have positive effects on human health and may play a part in the prevention of cancer. [7]. It has been shown that a plant-based diet is beneficial to Okinawans, who have the highest percentage of centenarians. As a result, the current Mediterranean diet and the DASH (Dietary Approaches to Stop Hypertension) diet both contain a meal that is rich in phytochemicals. [8-9]. These chemicals may be found in their natural form in a wide variety of fruits and vegetables. Because of the ongoing effort to discover and produce skin products derived from natural sources as alternatives to synthetic and traditional pharmaceuticals, there has been a recent uptick in interest in the research of medical plants as well as the commercial usage of these plants. Natural sources such as plant extracts, essential oils, and essential oils all fall under the category of being intriguing. Antioxidant properties, such as those revealed by high amounts of phenolic and flavonoid compounds in medicinal plants, are believed to have a role in the prevention of age-related illnesses, especially those diseases that are induced by oxidative stress. Research on medicinal plants is of utmost significance and ought to be approached in the same manner as research on conventional medicines. This is because medicinal plants naturally contain a wealth of phytochemicals, and the pharmaceutical and cosmetics industries are increasingly gravitating toward the use of natural ingredients. The techniques of preextraction and extraction come first in the investigation of medicinal plants since they are necessary in order to get access to the bioactive substances that may be discovered in plants. Maceration and Soxhlet extraction are two methods that are often used in labs and production facilities of a size that is considered to be moderate (SMEs). Extraction methods that have been developed recently and are already in use, such as supercritical fluid extraction, microwaveassisted extraction, and ultrasound-assisted extraction, are just a few instances of the improvements that have been made to the processing of medicinal plants (SFE). These advancements are intended to increase productivity while also lowering costs. In addition to that, the methods are continuously updated and improved. It is essential to choose the alternative that will result in the most desirable

outcomes given the variety of choices available. This review provides a discussion of the guiding principles, virtues, and drawbacks of the commonly employed techniques, as well as instances from recent years, in order to assist in picking tactics that are appropriate. [10]

1.3. Plants That Are Employed Within the Medical Field

Herbalists employ a wide variety of plants, some of which have medical capabilities; these plants, together, are referred to as medicinal plants. It is common known that the plants used in traditional medicine have a wealth of information that may be used to the creation of new medications. In addition, the expansion of human civilization throughout the world would not have been conceivable if it weren't for the use of these plants. Because of the broad acknowledgment of the role that various plants play as major contributors to nutrition, a number of plants are currently being promoted for their therapeutic capabilities. There are a few plants, like ginger, green tea, walnuts, and a few others, that could contain them. It is generally believed that the active components of both aspirin and toothpaste come from distinct types of plants.

The concept of treating medical ailments by use of plants is at the heart of the "Alternative Medicine" buzzword that has been popular in recent years. On the other hand, the conventional wisdom holds that one should only use drugs in pill form. The majority of the time, pills and capsules are also produced from plants, despite how often people use them. A variety of pharmaceuticals are made using plant extracts as an active ingredient. Several different drugs can't be made without the help of the chemicals that may be found in plants. Yew, foxglove, periwinkle, and morphine were all used in the extraction process to get taxol, vincristine, and morphine. [11]

1.4. Ethnobotany

The scientific study of plants and their uses as found via traditions and folklore related with a particular people's natural flora and fauna is known as ethnobotany. In the latter half of the 19th century, this academic subfield was first established. An ethnobotanist will use this method while documenting the myriad of ways in which natural flora is exploited in day-to-day life, such as in the manufacture of food, medicine, wine, and even garments. This technique will also be taken when doing research. The "scientific study of plants from different civilizations," as defined by Richard Evans Schultes, who is frequently referred to as the "creator of ethnobotany," is what

ethnobotany entails, according to Richard Evans Schultes. Ever since the time of Schultes, the purpose of ethnobotany has evolved from just investigating plants to applying the information obtained to current culture, most notably in the realm of medicines. Two of the most significant issues that are faced in the area of ethnobotany are the protection of intellectual property and the distribution of financial gains in an equitable manner. [12-13]

1.5. The Study of Ethnopharmacology

Ethnopharmacology is a branch of pharmacology that explores the link between various cultures and the therapeutic qualities of plants. This has connections to the discipline of ethnobotany as well as the use of plants for therapeutic reasons due to the fact that it is a source of lead compounds that are used in the creation of pharmaceuticals. [14] However, this technique has also been used to study new cures with a great lot of success. Historically, the major focus has been centered on traditional medicine. It is necessary to conduct research on the following topics: identification, ethnotaxonomy (cognitive categorization), preparation of traditional pharmaceutical forms, bioevaluation of the potential pharmacological action of such preparations (ethnopharmacology), the potential for clinical effectiveness, and the potential for social and medical implications implied in the uses of these compounds (medical anthropology). [15-16]

1.6. The importance of ethnobotany in the search for new pharmaceuticals

The choice of pharmaceutical companies to concentrate on leads gleaned from traditional remedies was not motivated by any sense of altruism, despite the fact that ethnobotany is a method for unearthing innovative combinations. The reason for this is that pharmaceutical corporations are not nice. This is because pharmaceutical companies are more interested with increasing their profits than with providing patients with safe and effective medicines. Ibuprofen, reserpine for hypertension, and D-tubocurarine (which is often used as a muscle relaxant in medical procedures) are just some of the modern drugs that have been developed from plants that were previously used in traditional medicine. On the other hand, the plants that were previously used in traditional medicine continue to provide for our needs today. On the other hand, the plants that have traditionally been used in various medicinal practices continue to be a source of information today. It is estimated that one quarter of all medications that are now for sale on the market in North America have been approved by certified medical professionals and include active components

acquired from plants (Farnsworth,1988). Approximately three quarters of them were brought to the notice of the business sector for the very first time as a direct result of their extensive usage in complementary and alternative medical practices (Farnsworth, 1988). (Farnsworth,1990). If ethnobotanical documentation and local knowledge are combined, an analyst will be able to identify more potential leads in a sample of plant testing than they would if the samples were picked at random. This is due to the fact that both ethnobotanical documentation and indigenous knowledge are founded on actual experience. This is because ethnobotanical documentation and indigenous knowledge are characterized by a greater degree of precision than scientific documentation. This is owing to the reason because (Balick,1990). Current researchers want to demonstrate significant pharmacological movement speeds using conventional pharmacopoeia, and in vitro and in vivo studies that make use of ordinary pharmacopoeia might assist them reach this aim. These researchers have the ability to achieve this objective. [17]

1.7. The Importance of Phytomedicine in the Context of the Worldwide Healthcare System

Phytomedicine is the discipline of using plants, plant parts, and phytochemicals in the diagnosis, treatment, and prevention of a wide range of illnesses and maladies. Phytomedicine is also known as herbal medicine. This subspecialty of medicine is often referred to as "homegrown medicine," another common term for it. Herbs and plants may be used in a broad variety of different methods in therapeutic settings, with the end goal being to accomplish a number of distinct objectives. Some of the procedures that fall under this category include the preparation of teas from plants that are cultivated at home, the application of the plants to the back in the form of packs or wraps, and the inhalation of steam or other components of the plant (such as a leaf or a stem or a radix). Numerous traditional therapies, such as Traditional Chinese Medicine (TCM) or Kampo Medication, include a negligible amount of phytomedicine into their respective formulations. Phytomedicine is also used in numerous other conventional treatments. Ever since the beginning of time, practitioners of traditional medicine have included the use of natural medicines as an essential component of the approach that they take to the treatment of a wide variety of conditions. Despite the fact that these subjects were largely ignored in the majority of the twentieth century due to the introduction of sophisticated produced medicines, the study of restorative plants has recently made a resurgence as a focus of exploration in today's semi-engineered society. This is despite the fact that the study

of restorative plants has recently made a resurgence as a focus of exploration. Patients in Western nations have a number of choices accessible to them in terms of the kind of medical treatment that they get, and phytomedicine is one of those possible courses of action. Patients in Eastern countries do not have as many alternatives available to them. [18] In contrast, persons who reside in western countries are more likely to use therapies that are readily accessible on the market, while those who live in eastern nations are more likely to use drugs that they themselves have created at home. In the Western world, there has been some discussion on whether or not medicines that are grown at home are both effective and safe. These discussions revolve on the question of whether or not medicines that are made at home are effective. Some of the labels that are used to refer to the constant use of medicinal plants and "phytomedicine" include "natural medicine," "homegrown medicine," "phytomedicine," "normal outcome," "regular medication," and "conventional medication." Alternatively, one may refer to it as "normal medicine" or "standard medication." In comparison to other therapies, they result in a lower total number of outcomes but have a greater positive impact, which demonstrates that they are, on the whole, more successful. For example, symptoms of hypersensitive rhinitis include nasal discharge, wheezing, and obstruction in the nasal passages. Therapies that are often used for rhinitis that is extremely sensitive include antihistamines and skin steroids. Despite this, there is reason for concern over the effects that these medications have over the long term, and they should be avoided wherever it is possible to do so. In addition to decreasing the number of eosinophils and eosinophil cationic proteins, naringenin chalcone, a component that can be found in tomato extract, prevents histamine from entering pole cells at any point during the underlying period of irritation. This action can take place at any time during the underlying period of irritation. During the underlying period of irritation, this activity may take place at any moment. Naringenin. Researchers in Japan conducted a randomized twofold visually impaired study to investigate the effects of tomato extract (Lycopersicon esculentum Mill operator) eliminates (TE, 360 mg each day for approximately two months) on people who suffered from mild to direct-lasting adversely sensitive rhinitis. The study lasted approximately two months. Participants in the research were required to have some degree of vision impairment. After taking part in the treatment, patients who had been diagnosed with TE and who had reported suffering from TE symptoms such as nasal blockage, nosebleeds, and sniffling reported substantial improvements in their condition. (p 0.05) (0,05) [19] Trials of a randomized double-blind and controlled trials of a combination natural therapy (ARND) against chronic hyper-sensitive rhinitis

were carried out as part of a hybrid plan for the organization of an unpleasant sensitive rhinitis nasal drop or fake treatment. The purpose of these trials was to determine whether or not ARND is more effective than traditional treatments for chronic hyper-sensitive rhinitis. It was shown that the ARND was effective in the treatment of persistent hypersensitive rhinitis. It was discovered that ARND contained a total of 23 percent Herba centipede, in addition to 16 percent Herba methane, 16 percent Radix Paeonia alba, 10 percent Radix Scutellaria, 6 percent Radix glycyrrhiza, 6 percent Radix placed, 5 percent Flo's Lonicera, 5 percent Fructus zizyphi, 5 percent Radix ledebouriella, and 4 percent each of the following: Herb Herb All of these indigenous treatments consist of different components, but they all have the common trait of moderating the immunological response of the body and preventing the spread of disease-causing organisms. Patients who used ARND for their chronic hypersensitive rhinitis and noted a decrease in the severity of their symptoms as well as an improvement in their general level of satisfaction in their lives. The preliminary findings of a research study that was hybrid in nature, randomized in its subject assignment, and double-blinded in its analysis revealed that an additional natural treatment for rhinitis was successful. Cinnamomum zeylanicum, Malphighia glabra, and Bidens pilosa were all components of the homemade pill that was analyzed alongside loratadine, which served as a positive control throughout the whole procedure. Loratadine was also present. After taking a natural supplement, the patients' nasal lavage samples showed considerably lower levels of the hormone prostaglandin D2. [6] The findings of the study were published in the journal PLOS One. For the purpose of this investigation, we used Xanthium sibiricum Patrin ex Widder (Fructus Asteraceae), Angelica dahurica (Fisch. ex Hoffm.) Benth. (Radix apiaceae), Saposhnikovia divaricata (Turcz.) Schischk. (Radix), and 7.5 grams of Saposhnikovia divaricata (Turcz. Patients who were diagnosed with long-lasting hypersensitive rhinitis and treated with SBL did not show a significant improvement in their symptoms when compared to patients who received fake therapy for approximately one month in a study that was controlled by a fake treatment and randomized with two blinds. The study was carried out with the assistance of two blinds and was randomized. In a research that examined the two groups after roughly one month of therapy, this was the situation that was found to exist. [20]

1.8. The investigation of natural substances and the creation of new medicines

The continual revolution in the area of research has directly resulted in the establishment of remarkable new freedoms with respect to the disclosure of freshly manufactured medications. It has been said that treatment targets are the "sub-atomic constituents of disease measurements." In recent years, there has been a rise in the amount of pressure placed on pharmaceutical corporations to improve the level of expertise with which innovative leads are produced and evaluated for the organic movement. This push comes after a number of years in which there was a decrease in the amount of pressure placed on pharmaceutical corporations. For a lengthy period of time, everyday objects have served as the primary ingredient in many medicinal preparations. In addition, a substantial portion of the medications that are in common use today were derived from natural occurring substances, which is another reason why they are so effective. Combinatorial science has been carried out by mother nature ever since the beginning of time through the production of natural particles that have an unrivaled degree of basic complexity and biological power. In the current era of medical research, there is a growing focus being placed on the analysis of common things, which may be linked to a number of different factors in a variety of various combinations. Among the issues posed by this category is the creation of innovative and sensitive approaches to the identification of naturally dynamic and unique entities. Unrecognized therapeutic demands, the variety of both substance structures and organic activities, and the applicability of bioactive natural objects as assays for biochemical and atomic processes are some examples of these issues. One such obstacle is the wide variation of both the structural makeup of substances and the organic processes they engage in. Techniques that are more efficient in separating, cleaning, and displaying the dynamic components of the data A period of unfathomable promise has just begun with the dawn of the modern age of drug disclosure and development. for research projects that combine the skills of common item science with those of other disciplines of study. Science at the subatomic and cellular level, both with and without the participation of scientific study. Research in organic chemistry and pharmacology has come a long way in recent years, and this has allowed scientists to make substantial headway in their efforts to capitalize on the wide variety of compound structures and inherent properties possessed by everyday items. [21]

1.9. Possibilities for the production of pharmaceuticals using medicinal plants

It is impossible for any library of microscopic particles to pave the way for massive fundamental and material variety, which has been and will continue to be a driving force behind groundbreaking discoveries in research, science, and medicine. There is a great deal of variety in both the items' essential components and their components. They are then formatively smoothed in order for them to assume the shape of medicine-like particles, and they continue to be the most trustworthy sources of prescriptions and pharmaceutical cravings. The annals of medical history are replete with examples of how the public revelation of a branded item altered both the advancement of scientific knowledge and the manner in which patients were exposed to and treated with drugs. One such example is the discovery of penicillin, which led to significant changes in the way that patients were exposed to and treated with drugs. There is an increasing amount of research that points to the significance that traditional practices may have in the treatment of human illness. Logical experts have been given an edge as a result of the creation of semisynthetic analogs, which sometimes take precedence over the basic fundamental in terms of the therapeutic relevance they possess. This is in spite of the fact that the things that occur in daily life continue to have a substantial influence on the health and happiness of people. When working on projects that are prone to regular things, designers have largely concentrated on the usefulness of their creations in analogous common spaces while simultaneously working to improve their properties that are similar to prescriptions. This has allowed them to make progress on both fronts. It is a miracle that is sometimes overlooked that mundane items may be utilized as astonishing beginning materials in the development of new classes of pharmaceuticals and uses for those treatments. The idea that this is even possible is awe-inspiring. This Perspective offers an illustration of a clinical reality as well as an unexplored opportunity, demonstrating that this is not only a theoretical concept but rather a clinical reality as well as an unexplored potential in the clinical situation as well. This shows that this is not only a theoretical concept. Plants have been employed in a variety of traditional medical procedures for many hundreds of years all around the world, and this practice is being carried out today. Patients were first given crude medicines such as tonic teas, poultices, powders, and other traditional categories when they were provided plant-based medications to treat their conditions. The public populace now has access to unique treatments that were not previously available to them as a result of these plant-based prescriptions. The dissemination of information

on medicinal products uses a multidisciplinary strategy. Ethnobotany, logical order, and ethnobotany have all developed into major components of drug disclosure from plants, in addition to the fundamental regimen disciplines. In the current day, there is a continuous process that involves the isolation and presentation of pharmacologically distinct combinations that are obtained from beneficial plants. In more recent times, medication disclosure tactics have been used in an effort to synchronize regional pharmaceutical practices and to discover correct marker molecules. According to the findings of many pieces of study, it is believed that there are around 250,000 distinct species of flowering plants that are regularly found. It has been estimated that there are around 125,000 of these animals living in tropical forests. They continue to provide researchers with enormous combinations of confidential substances so that the researchers might contribute to the development of new treatments. Only a very small percentage of the intriguing species that may be discovered all over the world have been researched to see whether or not they have the potential to be utilised in the medical field. The progress made by medical professionals who specialize in the treatment of cancer and other infectious diseases has paved the path for the eventual discovery of medicines that are derived from plants. The National Center for Illness Control and Prevention (NCI) in the United States has offered evidence that threatening to put the development of medications at risk has been beneficial in the past. Field research often makes use of the self-assertive combination technique as the motor behind its forward motion. This is the case in spite of the fact that data on ethnobotany and ethnopharmacology are often considered to be of secondary relevance in this endeavor. Between the years 1960 and 1982, the National Cancer Institute collected more than 114,000 samples from a typical 35,000 plant tests (with a trend toward 12,000–13,000 species) and tested them against various tumor systems. The results of these tests were published. These tests were carried out on tumors that were diagnosed as malignant (NCI). [22]

1.10. Bangladeshi medicinal plants

Natural remedies have been used for the treatment of a broad variety of disorders ever since ancient times by people from all over the world. The elimination of microbes required the use of several parts of plants, including their roots, barks, stems, flowers, and seeds, amongst other plant parts. The initial discoveries in medicinal chemistry were made at the same time, and both chance and the unquenchable curiosity of humans were responsible for their discovery. The use of medicines

derived from plants in daily life and the alleviation of human suffering can be traced nearly all the way back to the beginning of human civilisation. This is due to the fact that plants have been there virtually since the beginning of human history. In a number of countries, traditional medicines, herbal remedies, and spices, in addition to spices, are acknowledged as key components of treatment alternatives. A document known as the Apparatus Veda was compiled by the IndoAryans to record their history of the usage of medicinal plants between the years 4500 and 1600 BC. A further benefit that results from Bangladesh's location on the Indian subcontinent is the country's exceptionally diverse range of flora and vegetation. One of the countries that may be found in the subcontinent is called Bangladesh. There are around 2,000 distinct kinds of medicinal plants and 449 distinct species of restorative plants that may be found in Bangladesh. In the course of his work, which is known as "customary prescriptions" in today's parlance, Kaviraj has historically relied on a significant variety of unique herbs. Because of the creation of medicines like Chakma Marma Rakhshin Rakhshin Tipura Garo and Khashia, the use of plants and herbs with therapeutic properties has become much less complicated. The use of various types of plants with therapeutic properties is governed by a variety of rules and guidelines that have been established. Traditional societies, such as the rustic, ancestral, and mythical roral cultures, placed a great emphasis on the usage of plant extracts or basic preparations like implantation, decoction, and powder. These are examples of traditional arrangements. This is because plants can be obtained with very little effort. Both the treatment of major diseases and the reduction of the symptoms of such illnesses may be accomplished with the help of the medicinal plant. Microorganisms that may cause disease, often known as pathogens, can be found in things like infections and growths, as well as smaller organisms like bacteria and viruses. Several conditions, such as pneumonia, meningitis, ear infections, urinary tract illness, food-conceived sickness, and sexually transmitted diseases like as gonorrhea and syphilis, may be healed by the use of certain herbs. This medicine is used most often in the treatment of common colds, sinus infections, as well as a variety of other non-life-threatening skin conditions. Researchers have also discovered that the plants that are indigenous to our area contain bioactive substances such as alkaloids, glycosides, flavonoids, tannins, terpenoids, gum, and pitch. Both the ability to operate as cell glue and the capacity to strengthen cells were once thought to be unnatural in nature; yet, these chemicals have shown both of these capabilities. In spite of the fact that the vast majority of people do not consider the illnesses in question to be life-threatening, a significant number of people nonetheless rely on the treatments

that are now accessible. To provide you with an example, the plant family Malvaceae includes the species Abutilon Indicum L., which is more often known as monkey shrubbery, country mallow, or media. Other common names for this plant include: In Bengali, the name for the area is Polari, and while it's being discussed in English, the phrase "the region" is used. Leaf glue, leaf imbuement, roasted leaves, roots, and seeds are the primary components utilized in a variety of diagnostic techniques to test for the sickness. Other processes include roasting the leaves, roots, and seeds. Other diagnostic methods include the following: When compared to that of albendazole, the ethanolic content of Abutilon Indicum L. displays a degree of sufficiency that is more prominent. It was discovered via in vitro tests that it had antihelmintic effects. This plant belongs to the family Rubiaceae, which also includes Adina sessilifolia L. and other species that are closely related to one another. The locals in Bangladesh use the Bangla term kom to refer to this concept. Members of the Chakma clan almost always refer to it as "Kam grass" whenever they are talking about it. The use of leaf glue as a therapy for a broad variety of illnesses is common, especially for conditions that are difficult to alleviate.[2223]

1.11. Introduction of Combretum indicum

Combretum indicum, sometimes referred to as the Rangoon creeper is a climbing plant that is endemic to the tropical regions of Asia and has clusters of crimson flowers. The shape of the leaves is elliptical, and they have a pointed apex and a rounded base. They vary in length from seven to fifteen centimeters and are arranged in an opposing fashion. The blooms have a pleasant aroma, are tubular in shape, and range in color from white to pink to red. The fruit is ellipsoidal in shape and ranges in length from 30 to 35 mm. It has five noticeable wings.[24]

Combretum indicum

Fig 01: Combretum indicum

1.11.1. Description

The Rangoon creeper is a kind of ligneous vine that may grow to a height of up to 8 meters, reaching a maximum height of 2.5 meters. The shape of the leaves is elliptical, and they have a pointed apex and a rounded base. They vary in length from seven to fifteen centimeters and are arranged in an opposing fashion. The blooms have a pleasant aroma, are tubular in shape, and range in color from white to pink to red. The fruit is ellipsoidal in shape and ranges in length from 30 to 35 mm. It has five noticeable wings. When the fruit is fully grown, it has the flavor of almonds.In Malaysia, India, Pakistan, and the Philippines, you may find rangoon creeper growing in dense undergrowth or secondary woods. Now that time, people in tropical places like Bangladesh, Burma, Vietnam, and Thailand have been cultivating it, and it has since become naturalized there.It is believed that the blooms change color as they mature as a technique to attract more pollinators to the plant. The bloom opens up during the evening hours and is white when it first appears. Because of this, pollinating hawkmoths with lengthy tongues are drawn to the flower. After two days, it will become pink, and then on the third day, it will turn red, which will attract day flying bees and birds. Additionally, the bloom shifts from a horizontal position to an arching posture as it opens. [25]

1.11.2. Possibility of harmful effects

The chemical quisqualic acid, which is an agonist for the AMPA receptor, a sort of glutamate receptor in the brain, may be found in the seeds of this species and related species, Quisqualis fructus and Q. chinensis. Excitotoxicity has been associated with the chemical (cell death). Roundworm and pinworm infections have been successfully treated using the seeds. This causes the parasite to die in the digestive system since it is poisonous to the parasite.[26-27]

1.11.3. History

Dr. John Ivor Murray submitted a sample of the "nuts" to the Museum of Economic Botany in Edinburgh in the year 1861. Along with the sample, he sent a statement stating that the "nuts" were "used by the Chinese for worms," as well as a description of the manufacturing method and dose.[28]

1.11.4. Scientific classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Myrtales

Family: Combretaceae

Genus: Combretum

Species: C. indicum

Fig 02: Combretaceae

Binomial name

Combretum indicum

(L.) DeFilipps

1.11.5. Classifications and characteristics

- **Plant Division**- Angiosperms (Flowering Seed Plants) (Dicotyledon)
- **Plant Growth Form**-Climber, Vine & Liana
- **Lifespan (in Singapore)**-Perennial **Mode of Nutrition**-Autotrophic[29]

1.11.6. Biogeography

- **Native Distribution**-Philippines, India and Malaysia
- **Native Habitat**-Terrestrial **Preferred Climate Zone**-Tropical
- **Local Conservation Status**-Non-native (Spontaneous (Casual))[30]

1.11.7. Combretum indicum phytochemical screening

Antibacterial efficacy

In the case of ethanolic extracts, the following sequence was shown to provide the greatest amount of inhibition: Bacillus licheniformis (MTCC 530) was more effective against Pseudomonas aeruginosa (MTCC 2453) than Bacillus subtilis (MTCC 441), and Pseudomonas fluorescens (MTCC 103) was more effective against Bacillus mycoides (MTCC 7343) than Escherichia coli (MTCC 739), with a zone of inhibition measuring 27.67 0. (MTCC 530). In terms of its chemical composition, the extract obtained from ethanol has flavonoids, tannins, and alkaloids. Based on the findings of the FTIR study, the existence of R-CH2-OH groups, aromatics, C-N stretching amine, and NH stretching secondary amine was verified. The findings are statistically supported by the one-way ANOVA and the Tukey tests (p less than 0.05).[31]

Antioxidant and antimicrobial properties of methanolic extract of Combretum indicum leaf

The methanolic leaf extract of C. indicum exhibited significant antioxidant activity that was dosedependent, in addition to having some antibacterial activity. There aren't many studies from the past on the antioxidant activity of C. indicum that you can find in the literature. As a consequence, it is difficult to compare the findings of the most recent study with those of earlier investigations. As a result, it is possible that this is an undiscovered pharmacological activity of this plant that has not been investigated before. In addition to that, it demonstrated a mild

antibacterial activity. However, further research is required to investigate the overall pharmacological efficacy of this plant using a variety of in vitro and in vivo methods. In the course of this investigation, the standard tests have been carried out so that we can determine whether or not this plant may be used as a source of complementary or alternative medicine. Because of its high antioxidant and antibacterial content, the leaf of the C. indicum plant has the potential to be an effective alternative medicine that can treat a variety of conditions.[32-33]

1.11.8. Combretum indicum Benefits

The Rangoon creeper is a kind of plant that, in addition to bearing flowers, is used in traditional herbal medicine. Roots, seeds, or fruits of the plant may be used in the treatment of nephritis and diarrhea, respectively. You may ease the pain associated with a fever by gargling with fruit portions, and the leaves can be utilized to treat the discomfort.

Methodology

2.1 Plant Materials

Combretum indicum (Leaves) that were completely matured were taken from the Tetultola, Rajshahi, Bangladesh the plant leaves were divided, properly cleansed with tap water, dried in the shade, homogenized to a fine powder, and then kept in an airtight container.

2.2 Extraction of Plant Material

The powder was steeped in 500 ml of methanol to make extracts in accordance with Table 1. The jars were maintained sealed with foil sheets for fourteen days, occasionally being stirred. After that, the soaked leaves were filtered using Whatman No. 1 filter paper, cotton, and cloth. The filtrate was dried in a rotary evaporator at 50 °C for 40 minutes. It was then placed in a beaker and kept in the fume hood for the solvent to continue to evaporate. After a week, a sticky extract was created and kept dry and at room temperature. The crude extract was used for pharmacological and phytochemical study.

Table 1: Amount of plant material soaked in selected solvents

No. of Container	**Amount of plant materials**	**Amount of Solvent**
Container 1	250gm	Total 500 ml of methanol

2.3 Collection of human blood

Healthy volunteers (n = 5) without a history of smoking, taking oral contraceptives, lipid-lowering drugs, or anticoagulant therapy had 7 cc of venous blood collected from them. Aseptic procedures were followed as the blood was then transferred to several pre-weighed(W1) sterile microcentrifuge tubes (1 ml/tube).

2.4. Phytochemical screening

Standard phytochemical tests were performed on the newly produced extracts to identify several compounds, including alkaloids, glycosides, saponins, resins, tannins, flavonoids, and reducing sugar.

2.4.1. Detection of Alkaloids

Mayer's test:

2ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent (Potassium Mercuric Iodide) was added. Formation of yellow color precipitate indicates the presence of alkaloids.

Dragendroff's Test:

2ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendroff's reagent (Solution of Potassium Bismuth Iodide) was added. Formation of orange brown precipitate indicates the presence of alkaloids.

Wagner's Test:

2 ml solution of extract and 1 ml of Wagner's reagent (Iodine potassium Iodide). Formation of brown or reddish precipitation indicates the presence of alkaloids.

2.4.2. Detection of Flavonoids

Few drops of concentrated hydrochloric acid were added into 1ml of crude extract. Immediate formation of red color has shown the presence of flavonoids.

2.4.3. Detection of Saponins

1ml of extract solution was diluted with 20 ml distilled water. Then shaken vigorously for 15 minutes which develops clear foam and it indicates the presence of saponin.

2.4.4. Detection of Phenols

2 ml solution of extract and added 3 or 4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

2.4.5. Detection of Reducing Sugar

Benedict's test

0.5 ml of aqueous extract of the plant material was taken in a test tube. 5 ml of benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. A red color precipitate of cuprous oxide was formed in the presence of a reducing sugar.

Fehling's test (Standard test)

2 ml of an aqueous extract of the plant material was added 1 ml of a mixture of equal volumes of Fehling's solutions A and B. Boiled for few minutes. A red or brick red color precipitate was formed in the presence of a reducing sugar.

2.4.6. Detection of Glycosides

I. A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1ml of water. Then, a few drops of aqueous sodium hydroxide were added. A yellow color was considered as an indication for the presence of glycosides.
II. A small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution. Brick-red precipitate was considered as an indication for the presence of glycosides.
III. Another portion of the extract was dissolved in water and alcoholic and boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution as an indication for the presence of glycosides.

2.5. Thrombolytic assay

The leaves in vitro clot lysis activity was tested using the Prasad et al. methods with a few minor adjustments. The micro-centrifuged tubes underwent a 45-minute incubation period at 37°C. The serum was entirely withdrawn from the tubes after the clot had formed (without disturbing the clot), and each tube that had a clot was weighed again to calculate its weight (clot weight = weight of the tube containing the clot minus the weight of the tube alone).

A 100 ml solution of methanolic extract, with a concentration of 1 mg/mL, was applied in accordance with each micro-centrifuge tube containing a pre-weighed clot. The numbered control tubes received 100 ml of Streptokinase as a positive control and 100 ml of sterilized distilled water as a negative non-thrombolytic control, respectively. All of the tubes were then re-incubated for

90 minutes at 37°C, and clot lysis was checked. The collected fluid was taken from the tubes after incubation, and they were weighed once again to determine the weight difference after clot breakup. Finally, the weight difference was computed, and the result was reported as a percentage of clot lysis using the equation below.

% clot lysis= (Weight of the lysis clot, W5/ Weight of clot before lysis, W3)*100

Result & Discussion

3.1. Result of Phytochemical evaluation

Phytochemical screening of the *Combretum indicum* extract showed the presence of Reducing Sugar, Glycosides and Phenols. Results of the preliminary phytochemical screening of *Combretum indicum* are presented in Table 2.

Table 02. Phytochemical analysis of *Abroma augusta* in Methanol.

	Extracts
Phytochemical	**Methanol**
Alkaloids	-
Glycosides	+
Saponins	-
Resins	-
Tannins	-
Flavonoids	-
Reducing Sugar	+
Phenols	+

Key: (+) Present, (-) Absent

3.2. Result of thrombolytic Evaluation

In the event of in-vitro thrombolytic activity, adding 100 l Streptokinase (30,000 I.U.) to the clots and incubating for 90 minutes at 37° C resulted in 68.81% clot lysis. When clots were treated with 100 µl sterile distilled water (negative control), clot lysis was minimal (3.44%). The Methanolic extracts of *Combretum indicum* showed 6.57% clot lysis in an in-vitro thrombolytic activity investigation. The effective clot lysis % was calculated using a statistical model that included a negative control (sterile distilled water), a positive control (Streptokinase), and *Combretum indicum* herbal medicines, which are shown in Table 3:

Table 3: Effect of *Combretum indicum* extract on in-vitro clot lysis.

Fraction	Weight of empty vial, W1	Weight of clotcontaining vial before blood clot disruption, W2	Weight of clotcontaining vial after clot disruption, W3	Weight of clot before clot disruption, W4	Weight of clot after clot disruption, W5	% of clot lysis
Blank	0.775	1.528	0.753	0.575	0.178	23.64%
Streptokinase	0.83	1.75	0.93	1.15	0.64	68.81%
Manilkara zapota **extract in Methanol**	0.771	1.455	0.684	1.401	0.771	6.57%

Conclusion

The findings of the current research suggest that the leaves of C. indicum could be useful in the treatment of diabetes and the complications that are associated with the disease. These benefits could include the protection of the pancreas through the restoration of damaged beta islets and an improvement in the lipid profile. It doesn't have much of an impact on the thrombolytic actions. However, in order to understand the process and to separate the bioactive molecules, more research is necessary.

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