Phytochemical Screening and Scientific Exploration of Synergistic Antioxidant and Thrombolytic Potential of Methanol Extract *of Acacia concinna, Terminalia arjuna & Bombax ceiba.*

M. Pharm (Master's) Thesis Report

A dissertion submitted to the department of Pharmacy, Daffodil International University in the partial fulfillment of the requirements for the degree of Master of Pharmacy.

Submitted to

Department of Pharmacy

Daffodil International University

Submitted By

ID: 0242220011093009
Batch: 15th
Department of Pharmacy
Daffodil International University

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APPROVAL

This certifies that the work submitted for the partial fulfillment of the requirements for the Master's of Pharmacy degree from the Department of Pharmacy at Daffodil International University under the title **"Phytochemical Screening and Scientific Exploration of Synergistic Antioxidant and Thrombolytic Potential of Methanol Extract of Acacia concinna, Terminalia arjuna & Bombax ceiba**" is entirely original to me, and I have been given credit for any use of my words, ideas, or writings.

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Prof. Dr. Muniruddin Ahmed

Professor and Head

Department of Pharmacy

Daffodil International University

Project Supervisor

Md. A. K. Azad

Assistant Professor

Department of Pharmacy

Daffodil International University

_____ Internal Examiner-1

_____ Internal Examiner-2

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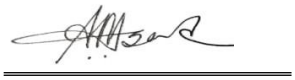
Declaration

I thus declare that the completion of the Master of Pharmacy (M. Pharm) program at Daffodil International University's Faculty of Allied Health Science, with the title **"Phytochemical Screening and Scientific Exploration of Synergistic Antioxidant and Thrombolytic Potential of Methanol Extract of Acacia concinna, Terminalia arjuna & Bombax ceiba"** is contingent upon completing the Thesis work.

Supervised By



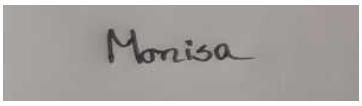
Md. A. K. Azad

Assistant Professor

Department of Pharmacy

Daffodil International University

Submitted By



Monisa Rani Poddar

ID: 0242220011093009

Department of Pharmacy

Daffodil International University

DEDICATED TO – My lovely parents (Mohendra Nath Poddar & Binita Rani Saha) My companion Spandan Saha and all of My respected teachers who have always supported and encouraged me

Abstract

85% of deaths globally are attributable to thrombosis, a serious consequence of cardiovascular illness that can result in myocardial infarction (or heart attack) and stroke. A major contributing factor to toxicity and illness may be oxidative stress.It can harm a lot of your tissues, which over time might result in a lot of illnesses. One crucial disclaimer is that antioxidant therapy should not be used after harm has started.

Objectives: To evaluate the impact on thrombolysis and antioxidants as well as the potential chemical components.

Materials and Methods: For the phytochemical testing, which took place in a lab, plant material from *Acacia concinna, Terminalia arjuna, and Bombax ceiba* was extracted using methanol. The thrombolytic activity was carried out using the clot lysis method, and the thrombolytic test was performed. The antioxidants were screened for using the DPPH technique.

Results: *Acacia concinna, Terminalia arjuna, Bombax ceiba*, Mixed extract-1(A+B)(*Acacia concinna & Bombax ceiba*),& Mixed extract-2(B+T) (*Bombax ceiba & Terminalia arjuna*) extract showed clot lysis percentagethe respectively 12.18%, 34.35%, 17.81%, 27.10% & 39.35%.And compeared with the control 7.33% where as standard (Streptokinase) showed a of clot lysis at 75.29%. Additionally *Acacia concinna, Terminalia arjuna, Bombax ceiba*, Mixed extract-1(*A+B*),& Mixed extract-2 (*B+T*) extract showed antioxidant activity respectively (IC50=16.20µg/ml, 28.85µg/ml, 22.27µg/ml, 16.20µg/ml & 14.75µg/ml),compeared with the standard ascorbic acid (IC50=9.072µg/ml). A phytochemical analysis revealed a wide range of chemical components in the extract of Acacia concinna, Terminalia arjuna, and Bombax ceiba, including alkaloids, carbohydrates, tannins, glycosides, and saponins.

Conclusion: The plant exhibits mild and moderate thrombolytic activity, antioxidant according to the data gathered.

Key words: *Acacia concinna, Terminalia arjuna and Bombax ceiba* Phytochemical screening, Thrombolytic, Antioxidant.

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CHAPTER ONE

INTRODUCTION

1.1. Introduction:

Phytochemicals are naturally occurring chemical substances found in plants that can have either beneficial or negative impacts on health.[1] The richest bioreservoirs of diverse phytochemicals are medicinal plants, which are used to treat a variety of illnesses and maladies. Are among some of the prominent phytochemicals that are found throughout the plants.[3] Nature is a singular source of structures with a high diversity of phytochemicals, with the main classes of phytochemicals being phenolics (45%), terpenoids and steroids (27%) and alkaloids (18%).[4] Despite appearing to be non-essential to the plant that produces them, these compounds are crucial to the plant's survival because they mediate ecological interactions with competitors, shield it from diseases, pollution, stress, and UV radiation, and give it its characteristic color, scent, and flavor. Plants produce metabolites to defend themselves against biotic and abiotic stressors, and these compounds have been developed into medications that can be used to treat a variety of illnesses.[5,6] Different extraction processes can be used to separate the phytochemicals from the plant material. In recent times.The most widely used conventional methods are still maceration, percolation, infusion, digestion, decoction, hot continuous extraction (Soxhlet extraction), etc.[7,8] Numerous solvents, such as water, ethanol, methanol, acetone, ether, benzene, and chloroform, are used in the extraction process.[9] The pre-extraction parameters (plant part employed, its origin and particle size, moisture content, drying method, degree of processing, etc.) have an impact on the phytochemical extraction from the plant materials. and variables pertaining to extraction (the method used, the solvent selected, the ratio of solvent to sample, the solvent's pH and temperature, and the extraction's duration).[7,9]

While plant components were once employed directly for medicinal purposes, modern technology have allowed for the identification, isolation, and synthetic production of the active ingredients in pure form.[6] The characterization and assessment of plants and their phytoconstituents can investigate the data pertaining to the medicinal claims made for those plants against a range of illnesses.[9] Highly beneficial for both qualitative and quantitative phytoconstituent detection.[1] The standard phytochemical assays, which are inexpensive, simple, and need fewer resources, continue to be a useful option for basic phytochemical screening in cases where these techniques are not available or cannot be afforded.[2]

1.2 Phytochemistry

The word "plant"[5]in Greek is where the name originates. Plants make stuff that helps them fight bad stuff like viruses, bugs, and the sun. People who study this are called phytochemists. They check out what these plant substances are made of, how they're made, what they do in bodies, and how they can be used in stuff like medicine and industry. Understanding these plant chemicals is vital for finding new treatments for bad diseases.[6]

1.3 Medicinal plant

Plants that have special chemicals in them or could become cancer-fighting drugs are called medicinal. Throughout history, people have used medicinal plants to get better.[7] The belief is that these plants help with health because they can treat many different illnesses and issues In places where traditional medicine is used, medicinal plants are more common than in areas that only use scientific medicine.[8] In those places, using medicinal plants is not common for treatment and has disappeared from official therapy; but in other places, it's still a big part of all therapy methods. Some believe that 90% of the plants used for medicine in developing nations have healing properties.[9] Cypress, licorice, myrrh, and cedar are some of the plants used in medicine long ago. The oldest known medical writings were done around 2600 BC in Mesopotamia on clay tablets. People still use those ancient medicines today to help with various issues like coughs, colds, infections, and inflammation. Bishop's weeds are used to treat vitiligo, a skin problem with pigment loss. This information comes from Egyptian medications. A special medication made from this plant is now used to help with T-cell lymphoma, psoriasis, and other skin problems.The interest in using plants for cancer treatment continues today More than half of the drugs tested in trials worldwide come from natural sources like plants Blooming plants like Dioscorea spp. have helped create a lot of strong medications in the past forty years.[10]

Significant of Medicinal plant:

Each type of plant serves a certain function. Plant is considered medicinal if it satisfies these three requirements.

Preventive: Studies show that these drugs can prevent a number of illnesses. It decreases the negative effects of artificial medications.

Synergistic: Certain medicinal herbs have the capacity to exacerbate or decrease negative physiological effects in humans.

Official: Certain plants are used as "formula medicines," often used to treat incurable diseases. 11]

Herbalism

It, also known as herbalism is the study of botany and the use of medicinal herbs. [12] Lots of folks are getting interested in and alternative remedies these days. Indigenous medical practitioners have passed down their therapeutic knowledge for years, resulting in effective herbal treatments. These natural remedies are not just popular developing countries for basic health needs—they're also gentle on our bodies, widely accepted, and usually free from bad side effects. However, recent studies show that not all herbal remedies are safe, as some can cause unwanted effects. Most herbal products on the market today haven't gone through the rigorous safety checks required for medications. According to WHO traditional medicine refers to treatments used for centuries before modern medicine came about—this includes herbal remedies.[13]

New product Discovery

Textbooks on herbal medicinal list a number of medicinal plants that have not received much research. Pharmacologic screening in line with these plants' traditional applications can be used to assess their utility. Spectrophotometric and chromatographic methods might be employed to identify the offender if a noteworthy result is obtained. Three separate phases of study are included in a bioactivity-guided approach. The raw material first demonstrates biological activity. The active fractions are separated from the inactive fractions using a bioassay technique. Fractionation of the raw material is the second step. [14]

1.7 Basis for Phytochemistry

All trousers produce fortifiers to help with development, like defense against herbivores or photoprotective chemicals, when salicylic acid corrosion occurs. These phytochemicals are perfectly suited for use as medications, and their current use in medications makes sense.example, glutamine, one of the nine alkaloid components found in daffodils, has been approved for use as a treatment for Alzheimer's disease. Herbivores can readily ingest volatile plant stems, or alkaloids. They are poisonous and smelly as well. Furthermore, they can provide defense against parasites. [15][16]

1.8 Alkaloids

Any of the nitrogen-containing bases, or alkaloids, found in a variety of species. The physiological effects of alkaloids on humans and other animals are diverse.The majority of it found in nature, and these trees are especially rich in them. To be honest, alkaloids have been detected in several thousand different types, and it is believed that up to 25% of higher plants contain them. An animal species typically only has a few distinct forms of alkaloids; however, the ergot parasite (Claviceps) and the opium poppy (Papaver somniferous) each have over thirty different form. Alkaloids are found in many plant groups; it is believed that the poppy family has alkaloids in all of its members. Three other well-known families with alkaloids are the Amaryllidaceaae (amaryllis), Solanacese (nightshades), and Ranunculaceae (buttercups). Numerous animal species, including poison-dart frogs (Phyllo bates) and New World beavers (Castor canadensis), have been reported to have two alkaloids. Furthermore, they are produced by ergot and a few other parasites. [17][18]

1.9 Glycosides

Some plants that contain anthraquinone glycosides are rhubarb, cascara, and alexandrin. Plant-based purgatives are made from plants including aloe, rhubarb, and senna. These wonderful medications are called cardiac glycosides, and they are derived from medicinal plant like foxglove and lily of the vally. Certain irregular pulses and cardiovascular collapse are treated with drugs called cardiovascular glycosides. They are among the select few classes of medications used to treat cardiac conditions and related illnesses. [19]

1.9.1 Tannins

The complex chemical molecules known as tannins are composed of phenolic acids, also known as tannic acid. These materials become insoluble and difficult to breakdown as a result of the process. Tannins are found in several groups of blooming plants and coniferous tree species. Tannins are present in plants and can leak out.

1.9.2 Saponins

Plant flavonoids called saponins are bound to sugars that dissolve in water and include a triterpenoid or lipophilic drug. These additives can lower surface tension because of their asymmetric information and hydrophobicity, which is similar to soap.

1.9.3 Steroids

Brassino steroids, another name for plant steroids, are significant hormones. Mutants lacking in steroids are sterile, as these hormones regulate many aspects of growth and development.

1.9.4 Phenol

To protect themselves from stress, plants primarily produce phenolic compounds. Plant growth depends on phenolic chemicals, particularly for the synthesis of lignin and pigments. Plants benefit from their structural stability and scaffolding support.[20]

1.9.5 Carbohydrates

Plants use complex carbs to grow strong. They can also stash them away or turn them into energy. To do this, plants employ respiration. It's like a magical process that combines glucose from photosynthesis with oxygen to unleash stored energy. Glucose is the main deal from photosynthesis and a go-to carb in the plant world. You can take two monosacchar and make disaccharides out of them. Think of sucrose and lactose – they're cousins of glucose in a way. Lactose is one special carb found in milk and gives it that milky goodness.[20]

1.9.6 Diterpenes

Diterpenes are compounds made up of four isoprene units. They can be found in fungi and plants. Most diterpenes are not smelly, but small amounts might be in essential oils sometimes.[20] Some important diterpenoid groups are: retinol (helps with vitamin A), Gibberellin (a plant hormone for growth), Trisporic acid (hormone for fungi reproduction change), and phytoalexins (disease-fighting substances). Diterpenes and their versions can do helpful stuff like reduce inflammation and help the immune system.[21]

1.9.7 Flavonoids

In expansion to utilizing complex carbs to construct their auxiliary system, plants can store them or utilize them as vitality to create. Plants require a instrument called breath to combine the glucose made amid photosynthesis with oxygen in arrange to discharge vitality from the put away carbohydrates. [21]

1.10 Activity of Antioxidants

Since it diminishes the hazard of heart infection, ensures against degenerative clutters, and amplifies item life by stopping or deferring oxidation occasions, antioxidant movement is noteworthy in the bundled nourishment segment. When your body digests nourishment, when it is uncovered to radiation. It is often known how important oxidation is to both the body and food. Oxidative metabolism is necessary for the survival of cells. Reactive oxygen species, which are free radicals that induce oxidative alterations, are one unexpected consequence of this dependency. Further information points to these species' involvement in a number of similar in vivo regulatory mechanisms. Cellular respiration can be stopped by an excess of free radicals because they can damage DNA, cellular proteins, membrane lipids, and enzymes. They can also overcome protective enzymes like as well as cause and induce deadly cellular consequences like apoptosis. Moreover, it appears that the impact of reactive oxygen species on cell signaling pathways remains a mystery. [23, 24] Techniques for evaluating antioxidant behavior can be divided into two fundamental classes based on whether they emphasize food activity or human bioactivity. [25]

Thrombolytic

It attaches itself to the place of creation and blocks blood flow. Thrombosis is the medical term for the onset of a thrombus. For people who are genetically predisposed to blood clotting and are sedentary, their risk of developing a thrombus is higher. An injury to an artery, vein, or other tissue can potentially result in a thrombus. [26] Venous thromboembolism (VTE) has a major detrimental influence on community economy as well as health[27].The cause of approximately in Australia, which results in 5,000 patient deaths annually. [28] After myocardial infarction and stroke, this ailment is ranked third among vascular illnesses in Caucasian populations. [29] According to estimates, two to three out of every 1000 hospital admissions involve [26].

Thrombolytic treatment increments blood stream, breaks up hurtful blood clots, and shields imperative organs and tissues from hurt. Thrombolysis is the prepare of infusing drugs that break up clots into the circulatory system through an IV line or a long catheter that gets the pharmaceutical to the blockage area. Another elective is to physically break up or evacuate the fabric clot utilizing a long catheter with a piece of hardware at the tip.[22] Intense pneumonic embolism and ischemic strokes are for the most part caused by blood clots in the supply routes providing the heart, which are broken up by thrombolysis treatment. In addition, it ousts blood clots in the heart's growing paths. veins in the upper appendages, legs, and pelvis that can cause clots or profound breaks separated and voyages to a lung course, it may cause an unexpected pneumonic embolism. As before long as signs of a heart assault, stroke, or aspiratory embolism

show up, thrombolysis may be considered if a blood clot is considered life-threatening; in a perfect world, this will happen inside one to two hours of the determination. [29]

1.11.1 Thrombosis formation Mechanism

Thrombolytic medications break up blood clots by causing plasminogen to cleave and produce plasmin. Blood clots maintain their structural integrity due to fibrin crosslinks, which are broken down. Thrombolytic medications are sometimes referred to as "fibrinolytic drugs" and "plasminogen activators" based on how they work. The three primary classes of fibrinolytic medications are SK (UK), tPA. Nonetheless, the effects of the three medication classes are identical. Each of them has a unique technique for dissolving blood clots. strategies to modify their level of fibrin clot selectivity. The graphic on the right shows the fibrinolytic processes of SK and tPA. Particularly for cerebral and coronary vascular clots. Tissue plasminogen activator causes clot lysis in the following order:

- The clot breaks down when tPA binds to the fibrin that covers its surface.
- Activates the fibrin-attached plasminogen.
- Disassembles fibrin molecules and separates plasminogen from fibrin-associated plasminogen.
- The clot dissolves when fibrin molecules are broken down by a protease known as plasmin. But it's crucial to keep in mind that plasmin also degrades other proteins.

Proteins in the bloodstream However, because of the relative, includes less breakdown of circulating fibrinogen in addition to the United kingdom (UK) and the Korea. Although tPA primarily targets clot-bound plasminogen, additionally, it increases the amount of plasminogen in the blood, which releases plasmin and may eventually synthesis it. The disintegration of circulating fibrinogen results in an undesired systemic fibrinolytic condition. Therapeutic antiplasmin also inactivates plasmin, even though antiplasmin that is in circulation does so naturally. Thus, SK not only causes significant clot fibrinolysis but also significant fibrinogen lysis. Because of this, tPA is frequently used as a thrombolytic medication rather than SK, especially for the disintegration of thrombi in the coronary and cerebral arteries. Patients who have recently become infected with streptococci may need much higher dosages of SK in order to achieve thrombolysis because SK is derived from streptococci.It is crucial to keep in mind that the age of the clot affects how well thrombolytic medications work. Dissolving as a result of improved fibrin cross-linking and older clots' increased compactness. Giving thrombolytic drugs is recommended within the initial. [31] [32]

Streptokinase

Streptokinase is used as an efficient and fairly price thrombosis analgesic in some ischemic stroke scenarios. And a pulmonary embolism is a type of blood clot that occurs in the lungs. Streptokinase

is a member of the class of drugs known as streptokinase inhibitors. Hydrolyzing fibrinolytic is possible when human mast cells form complexes with streptokinase. When bond cleavage activates additional unbound plasminogen, fibrin is generated. The make up the three domains that make up streptokinase. residues 288–414, 288–287, and 288–414, in that order. Although plasminogen is bound by each domain, plasminogen cannot be activated by a single domain alone. [33][34]

Streptokinase Mechanism

When streptokinase breaks down the Arg/Val bond in plasminogen, an active complex is formed, resulting in the production of the proteolytic enzyme plasmin. Plasmin then acts as a thrombolytic agent by dissolving the fibrin matrix of the thrombus. Blood proteins called plasmin, which are the main component of blood clots, help remove and disintegrate them until the wounds have healed properly. Streptokinase promotes the production of plasmin, which, for example.Usually, plasminogen is activated by proteolysis of the Arg561—Val562 connection. The target enzyme (Pm) creates a covalent bond between the amino groups of Val562 and Asp740 to catalyze the paracrine synthesis of an energetic protease. Following its discovery, (SK) joins forces with Plasminogen to form a complex called Even though the Arg561–Val562 connection is still present, this strong dynamic then rearranges to form another intense dynamic.In light of this potent dynamic, a different residue is required to take the place of Val562's free amino group provide a counterion for Asp740. It has been proposed that this counterion may be Ile1 of streptokinase and Lys698 of plasminogen. A salt bridge needs to form between SK's Ile1 and plasminogen's Asp740 in order for SK to use a nonproteolytic mechanism to generate an active site in plasminogen. [34]

CHAPTER TWO

PURPOSE OF THE STUDY

The objective

The investigation's primary focus was on the phytochemical composition plants, with focused pharmacological analysis being included. Analyzing possible undertakings The aim of this study was *Acacia concinna, Terminalia arjuna, and Bombax ceiba*. Herbal remedies frequently include the plant. These plants are also rich in alkaloids, glycosides, tannins, and other compounds that might be worth further research. The whole plant's methanol extract is used in the analysis. There are multiple justifications for researching these plants. As an illustration:

Chemical constituent analysis: Numerous phytochemical activities, such as the nearness or nonappearance of alkaloids, lessening will test in light of the study's discoveries.

Investigation of pharmacological activity: Examining the scientific underpinnings of the antioxidant and thrombolytic properties of the whole plants as potential therapeutic uses is another goal of this thesis.

CHAPTER THREE

PLANT PROFILE

3.1.1. Acacia concinna:

Soap pods, or Acacia concinna (Wild.) D.C., are naturally occurring and widely grown in South Asian nations. Because of its exceptional cleansing properties, ground legume fruit powder helps to give hair luster, encourage hair development, and lessen dandruff. In addition, this pod fruit has other medical uses such as a laxative, phlegm expellant, cough syrup, appetizer, treatment for fever, and skin conditions. The traditional uses of A. concinna include treating fever and intestinal disorders with the root, laxative effects with the stem and leaves, phlegm-expelling effects with the stem bark, and ligamentous dysfunction with the spoiled blooms.Because of its many uses, this plant is currently being grown commercially in great quantities in India and the Far East.[12,13,14] Native to Asia, Acacia concinna, also known as Shikakai in Hindi, is a climbing shrub that grows wild in the warm plains of central and southern India. The plants, which are bushy cum creeper, grow fairly quickly. Once these plants reach maturity, even elephants cannot pass through due to their curved thorns.

Figure-3.1.a: Acacia concinna

3.1.2. Scientific classification of Acacia concinna:

Kingdom: Plantae;

Order: Fabales;

Family: Fabaceae;

Genus: Senegalia;

Species: S. rugata.

Common name

Botanical Name: Acacia concinna

Bangladesh: Shikakai

India: Shikakai

3.2.1. Bombax ceiba:

Like other trees of the genus Bombax, Bombax ceiba is also referred to as cotton tree. To be more precise, it is sometimes referred to as the red silk-cotton tree, the Malabar silk-cotton tree, or the ambiguously named silk-cotton or kapok, which can also be used to refer to Ceiba pentandra.[15]This Asian tropical tree has a tall, straight trunk, and in the winter, its leaves turn brown. Before the new foliage emerges in the spring, red blooms with five petals bloom. When the capsule is fully ripe, it releases cotton-like white fibers. Its trunk is covered in spikes to prevent animal assaults. Its sturdy trunk suggests that it can be used for lumber, but the wood is too soft for much use.[16]

Figure-3.2.a: Bombax ceiba

3.2.2. Scientific classification of Bombax ceiba:

Kingdom: Plantae;

Order: Malvales;

Family: Malvaceae;

Genus: *Bombax*;

Species: *B. ceiba*.

Common name

Botanical Name: *Ceiba pentandra (L.) Gaertn*

Bangladesh: Shimul

India: Semal

3.3.1. Terminalia arjuna:

T. arjuna reaches a maximum height of 20 to 25 meters; it typically has a trunk with buttresses and a broad canopy at the crown from which branches droop. It features smooth, grey bark, oblong, conical leaves that are green above flowers that bloom in March and June, and glabrous, 2.5–5 cm fibrous, woody fruit that is divided into 5 wings that ripens from September to November.[17,18]

Figure-3.3.a: Terminalia arjuna

3.3.2. Scientific classification of Terminalia arjuna:

Kingdom: Plantae;

Order: Myrtales;

Family: Combretaceae;

Genus: Terminalia;

Species: T. arjuna.

Common Name

Botanical Name: Terminalia arjuna (COMBRETACEAE)

Bangladesh: Arjun

English Name: Arjun tree

India: Arjun

3.4.1. Acacia concinna:

Shikakai has vitamins A, C, D, E, K, among others. These are nutrient-dense and serve as antioxidants to protect and nourish hair follicles, which encourages the growth of new hair.

Antioxidants found in shikakai are crucial for protecting hair follicles from oxidative damage. Furthermore, they support and stimulate the production of collagen in the follicles' connective tissues, which promotes the growth of new hair.

3.4.1.1. Antimicrobial activity: Allicin activity, which has been shown to combat a range of infections, including Shigella and Escherichia coli as well as other that resistant drugs, is responsible for Acacia concinna's antibacterial properties.For example, the ethanolic Acacia concinna extract demonstrated a greater inhibitory effect against Saliva typhi and Escherichia coli than the aqueous extract, which demonstrated little to no inhibition effects.While methanolic Acacia concinna extract demonstrated antimicrobial activity against all tested strains with the exception of S. aureus, aqueous Acacia concinna extract demonstrated antibacterial activity against both Gram-positive [10]

3.4.1.2. Anti-Protozoal Activity: Phytochemicals and extracts from Acacia concinna have been shown in numerous studies to be effective against a variety parasites. For example, one in study found that extract of Z. officinale was the most effective anthelmintic agent against Haemonchus contortus, while the aqueous extract of Acacia concinna shown a noteworthy effect against Angiostrongylus cantonensis and Trichuris muris.[59]

3.4.1.3. Antioxidants Activity: Numerous chronic illnesses have been linked to the overproduction of reactive oxygen species (ROS).Numerous natural products, including medicinal herbs, and herbal infusions, have been shown to possess. Acacia concinna has a strong antioxidant activity that has been shown in several research. Using the ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH). [37]

3.4.1.4. Anticancer activity: Many studies have shown natural goods with anticancer properties include fruits and medicinal herbs. Recent studies have examined the anticancer effects of Acacia concinna against a range of cancer. Two possible modes of action for cancer are growth suppression and apoptosis induction.[59]

3.4.1.5. Antioxidant: The molecule known as allicin, which is created enzymatically when the bulb is crushed or minced, is responsible for the antioxidant power of Acacia concinna. The sulfur-containing chemical compound allicin has been the subject of much research because of its capacity to fight. They may set off a series of events that damage cells by oxidative stress. Extracts of Acacia concinna have an antioxidant defense system that is at least somewhat effective since allicin may scavenge these free radicals.

3.4.1.6. Anti-platelet aggregation: Recent research indicates that the extract from Acacia concinna may be able to prevent atherosclerotic disease. Research has shown that adenosine, a substance found in high concentrations (0.056%) in Acacia concinna, prevents platelet aggregation and enhances coronary artery blood flow. Studies have suggested that acacia concinna oil may prevent platelet aggregation. [33]

3.4.2. Terminalia arjun:

It has moreover been utilized to protect ideal wellbeing and treat a extend of human sicknesses, such as blood issues, frailty, and viral and venereal illnesses. Its hypocholesterolemic, antioxidant, antitumoral, qualities have all been illustrated. It is utilized to treat liver issues, ulcers, and breaks. [60, 61, 62] T. arjuna is said to process strong hydrolipidemic properties. The inotropic effects of T. arjuna are thought to be attributed to its saponin glycosides, while its flavonoids and phenolics may have both antioxidant and vascular amplification properties. This validates the plant's several functions in cardio-defense.[63, 64, 65]

3.4.2.1. Antimicrobial activity: T. arjuna has better antixident effect in methanol extract.[65]Different T. arjuna extracts have been demonstrated to inhibit harmful bacterial growth, with variable degrees of susceptibility. In contrast to the aqueous extract, which showed little to no inhibition impact against Saliva typhi and Escherichia coli, the ethanolic T. arjuna extract shown a stronger inhibitory effect. Aqueous T. arjuna extract showed antibacterial activity against both Gram-negative & Gram-positive strains, while methanolic T. arjuna extract showed antimicrobial. There was no antibacterial activity shown by hexane, ethyl acetate, or chloroform extracts. [66]

3.4.2.2. Cardioprotective Agent: One plant in the Combretaceae family, Arjuna, may have cardioprotective properties. During the vedic era, references to this ayurvedic treatment have been found in Astang Hridayam, Sushruta Samhita, Charaka Samhita, and other ancient Indian medical literature. Vagabhatta was the first to propose stem bark powder for cardiac problems.[67]By strengthening and toning the heart's muscles, it contributes to the heart's normal functioning. Arjuna tree has strong anti-hypertensive qualities and lowers excessive blood pressure. For heart health benefits, it is advisable to take Arjuna chaal boiled in milk one or two times a day. Arujunarishta strengthens and nourishes the heart's muscles. It accomplishes this via regulating cholesterol and blood pressure. [68]

3.4.2.3. Antioxidants Activity: The plant has a lot of phenolic compounds and flavonoids. Plants have powerful anti-oxidant qualities.[69] Numerous studies have demonstrated the potent antioxidant effect of ginger. The antioxidant activity of ginger has been test in vitro using the ferric-reducing antioxidant (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH).[69]

3.4.2.4. Hepatoprotective Activity: According to research, arjuna bark's hepatoprotective properties may help to protect and enhance liver function. [70]

3.4.2.5. Anti-platelet aggregation: Arjuna bark and its extract may offer protection against atherosclerotic disease, according to more recent research. It has been shown that adenosine, a substance found in arjuna bark at high concentrations (0.056%), prevents platelet aggregation and enhances coronary artery blood flow. Arjuna bark has been shown in trials to prevent platelets from aggregating.[69]

3.4.3. Bombax ceiba:

Traditional medicine use of the well-known plant bombax ceiba to treat a different of ailments. Cholera, tubercular fistulas, coughs, urogenital symptoms, nighttime pollution, abdominal pain associated with dysentery, and impotence. Gum is astringent, demulcent, and tonic.[70,71] The value of tree is associated with the existence active phytochemicals in those plants that have a particular effect on humans and can be used of diseases. Previous studies on a range of therapeutic plants have demonstrated a relationship between the existence of secondary metabolites and advantageous properties such as antioxidant, antidiabetic, antibacterial, and anti-inflammatory.[72]

3.4.3.1. Antimicrobial activity: In contrast to the water extract, that showed little to no inhibition impact against Saliva typhi and Escherichia coli, the methanol extract shown a stronger inhibitory effect.[73]

3.4.3.2. Antioxidants Activity: Antioxidant founds in different types of food. Numerous research have demonstrated the significant antioxidant activity of Bombax ceiba.Activity of antioxident of Bombax ceiba has been test using the ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH).

3.4.3.3. Hepatoprotective Activity: According to research, arjuna bark's hepatoprotective properties may help to protect and enhance liver function. [75]

3.4.3.4. Anticancer Activity: Fruits and medicinal plants are among the natural products with anticancer qualities, according to numerous research. Recent research has focused a great deal of attention on the anticancer properties of ginger against a variety of cancer types, including colorectal, breast, cervical cancer. [74]

CHAPTER FOUR

MATERIALS & METHODS

5.1. Plant collection

The Acacia concinna fruits, Terminalia arjuna burk , Bombax ceiba root was collected from Dhaka.

5.2. Preparation of plant materials & Extraction

To allow for ground and sun drying, materials are broken up into little bits. The plant components were eventually dried for a final 30 minutes at 40 to 45 °C in an oven to get rid of any remaining moisture after around a week of drying. The ingredients are pounded into a powder with a grinder, packed in an airtight receptacle, and stored somewhere dry and dark until needed again. After giving a plastic-capped jar a thorough cleaning, methanol was used to rinse it.2000 ml of methanol was added to the jar until it was sufficiently covered, up to two inches above the surface of the sample, following the addition of 675 g of dried plants to the pot. Aluminum foil was placed over the plastic cover of the jar to make sure it was sealed tightly and prevented air from entering. It seemed like a fifteen-day surgery. The pot was shaken roughly two or three times a day for maximum extraction.

5.3 Filtration

After collecting the filtrate into a glass holder, the refiltration handle was carried out once more.

5.4. Evaporation

The fluid concentrate was allowed to evaporate using a revolving evaporator set to run at 50 revolutions per minute and 65°C. The dense example was contained in a 100 ml container. After measuring glasses were filled with pure concentrate, they were put just beneath the fan and covered with aluminum paper. To get rid of any last traces of dissolveable, use a hair drier to remove any last bits of water from the concentrate.

5.5. Phytochemical screening

5.5.1. Test for glycoside

A brown ring that is brown in shade appears.

- Mixed extrac-1(A+B) (Acacia concinna & Bombax ceiba)
- Mixed extract-2(T+B) (Terminalia arjuna & Bombax ceiba)

Sample	Reagent	Result	Confirmation

Extract	Acetic Acid+ $FeCl_3$ + H_2SO_4	Brown color	Acacia concinna	Terminalia arjuna	Bombax ceiba	Mixed extrac-1(A+B)	Mixed extract-2(T+B)
			+	+	–	–	+

5.5.2. Test for tannins

A test tube was filled with 0.1% ferric chloride first, and then 10ml of plant extract.

Sample	Reagent	Result	Confirmation				
Extract	0.1% Ferric Chloride	Brownish Green	Acacia concinna	Terminalia arjuna	Bombax ceiba	Mixed extrac-1(A+B)	Mixed extract-2(T+B)
			+	+	+	–	+

5.5.3. Test for Flavonoids

Sample	Reagent	Result	Confirmation				
Extract	Ammonia solution + H_2SO_4	Yellow color	Acacia concinna	Terminalia arjuna	Bombax ceiba	Mixed extrac-1(A+B)	Mixed extract-2(T+B)
			+	+	–	–	+

6.5.4. Test for Alkaloid

6.5.4. a. Mayer's reagent

Sample	Reagent	Result	Confirmation				
Extract	Mayer's reagent	Cream or pale Yellow	Acacia concinna	Terminalia arjuna	Bombax ceiba	Mixed extrac-1(A+B)	Mixed extract-2(T+B)
			–	+	+	+	+

5.5.4. b. Dragendorff Reagent

Sample	Reagent	Result	Confirmation				
Extract	Dragendorff reagent	Cream or pale Yellow	Acacia concinna	Terminalia arjuna	Bombax ceiba	Mixed extrac-1(A+B)	Mixed extract-2(T+B)
			+	+	+	+	+

5.5.5. Steroids Test

Sample	Reagent	Result	Confirmation				
Extract	H_2SO_4	Red color	Acacia concinna	Terminalia arjuna	Bombax ceiba	Mixed extrac-1(A+B)	Mixed extract-2(T+B)
			+	+	+	+	+

5.5.6. Test for Gums

I filled a test tube with the 5ml plant extract. Sulfuric acid and Molish reagent were then added to that test tube.

Sample	Reagent	Result	Confirmation				
Extract	Molish reagent+ H_2SO_4	Red violet rin g	Acacia concinna	Terminalia arjuna	Bombax ceiba	Mixed extrac-1(A+B)	Mixed extract-2(T+B)
			+	+	+	+	+

6.5.7. Test for Tannins

First 0.1% Ferric Chloride and 10ml plant extract that test tube.

Sample	Reagent	Result	Confirmation

Extract	0.1% Ferric Chloride	Brownish Green	Acacia concinna	Terminalia arjuna	Bombax ceiba	Mixed extrac-1(A+B)	Mixed extract-2(T+B)
			+	+	+	–	+

5.6. Thrombolytic test of plant Extract [24]

Test materials (herbs extract)

Instrument

- Weighing Machine
- Ephendorph Tube
- Beaker
- Test Tube
- Tissue Paper
- Incubator
- Glass rod
- Syringe
- Syringe filter

Reagents

- Streptokinase
- Blood
- Distilled water
- Water

Procedure

- In the beginning, Eppendorf tubes were weighed individually.
- Each pre-weighted Eppendorf tube was then filled with 1 ml of blood and 7 ml of blood from a healthy volunteer who has never taken oral contraceptives or anticoagulants.
- Divide the serum portion again using tissue paper and weigh each tube individually once the incubator has been off for ninety minutes.
- **% of clot lysis= (wt of clot after release serum portion/clot wt) * 100**

5.7. Antioxidant test of plant Extract [31]

Instruments

- Test tube
- Test tube holder

- Weighing machine
- Beaker
- Incubator
- Uv spectrophotometry
- Spatula

Reagent

- DPPH
- Distilled water
- Methanol

Methods

Preparation of Stock Solution: Carefully prepared, a stock solution of freshly obtained extract (10 mg/ml) in methanol was created.

Experimental Setup: For the purpose of serial dilution, six volumetric flasks with distinct concentration markings were utilized. Test tubes and volumetric flasks were carefully covered with foil paper to shield the compounds from reactions brought on by light.

Sample and DPPH Solution Preparation: Two milliliters of extract from each concentration were cautiously mixed with two milliliters of 0.004% DPPH solution in different test tubes. These tubes were wrapped properly in foil and then allowed to sit in the dark for thirty minutes. Like the test arrangements, the clear arrangement was additionally coated in thwart paper to avoid light obstructions.

Measurement of Absorbance: After the foreordained brooding period, the absorbance of each solution—both test and blank—was fastidiously measured utilizing UV Spectroscopy at a wavelength of 517 nm.

Procedure

- Weigh out either 0.30 mg or 0.30 g of extract and mix it with 30 ml of distilled water.This solution is known as Stock Solution.
- Next, fill six test tubes with ten milliliters of distilled water.
- To the first test tube, added 10 milliliters of stock solution. Give a gentle shake.
- Next, transfer 10 milliliters of the first test tube's solution to the second test tube.
- Proceed with the serial dilution process.
- Each test tube is placed in an incubator or other dark area for 45 minutes following serial dilution.
- After 45 minutes, obtain a UV reading between 517 and 517 nm.

Measurement of % Inhibition:

$$\%\text{Inhibition} = \frac{\text{Blank solution absorbance} - \text{Extract solution absorbance}}{\text{Blank solution absorbance}} \times 100$$

Data Analysis: The collected data was methodically entered into Microsoft Excel in order to conduct a thorough statistical analysis. By using a regression equation ($y = mlnx + c$), it was possible to identify the x value that corresponds to 50% inhibition.

CHAPTER FIVE

RESULTS & DISCUSSION

6.1. Phytochemical screening test

Test Name	Result				
	Acacia concinna	Terminalia arjuna	Bombax ceiba	Mixed extrac-1(A+B)	Mixed extract-2(T+B)
Tannin	+	+	+	_	+
Glycoside	+	+	_	_	+
Flavonoid	+	+	_	_	+
Alkaloid	_	+	+	+	+
Steroid	+	+	+	+	+
Gums	+	+	+	+	+

Discussion: Here we can see Acacia concinna does not contain Alkaloid and it cannot impact on mixed extract.

Bombax ceiba does not contain Glycoside & Flavonoid and it impact on Mixed extrac-1(Acacia concinna+ Bombax ceiba).

All plants and both mixture contain Steroid & Gums.

6.2. Thrombolytic activity test result

Parameter	Wt. of empty tube (W_1)gm	Wt. of the first clot after 45min incubate (W_2)	$W_3 = W_2 - W_1$	Wt. of the second clot after 90min incubate (W_4)	$W_5 = (W_4-W_1)$	% of lysis
Positive control SK	0.86	1.01	0.24	0.77	0.09	75.29%

Control(DW)	0.78	1.48	0.70	1.33	0.740	7.33%
Acacia concinna	0.79	0.59	0.51	0.07	0.72	12.18%
Terminalia arjuna	0.79	0.78	0.51	0.27	0.52	34.35%
Bombax ceiba	0.77	1.34	0.57	1.24	0.67	17.81%
Mixed extrac-1(A+B)	0.78	1.386	0.606	1.22	0.614	27.4%
Mixed extrac-1(A+B)	0.80	0.66	0.26	0.27	0.53	39.35%

Discussion: The Standard (Streptokinase) showed a notably greater percentage of lysis at 75.29% in this investigation, demonstrating its strong thrombolytic action.

In contrast, the control group showed a lysis percentage of 7.33%, which represents the clot's spontaneous disintegration over time in the absence of thrombolytic intervention.

With regard to the Acacia concinna extract, the sample group showed 12.18% lysis percentage. Even though this is below the norm, it still shows that the Acacia concinna extract has a mild thrombolytic potential. Acacia concinna may have clot-dissolving properties.

Terminalia arjuna, the sample group showed 34.35% lysis percentage. This is still shows that the Terminalia arjuna extract has moderate thrombolytic potential even though this is nearest value of streptokinase. Terminalia arjuna may have clot-dissolving properties, which could explain its observed activity and its potential cardiovascular benefits.

Bombax ceiba extract, the sample group showed 17.81% lysis percentage. This still shows that the Bombax ceiba extract has thrombolytic potential. Bombax ceiba have clot-dissolving properties.

Mixed extract-1(Acacia concinna & Bombax ceiba), the sample group showed 27.10% lysis percentage. Mixed extract show Greater value than its individual value. Mixed extract-1 may have better clot-dissolving properties than its individual extract.

Mixed extract-2 (Bombax ceiba & Terminalia arjuna), showed 39.35% lysis percentage. Terminalia arjuna show moderate value of SK and Bombax ceiba sow below the normal, Mixed extract-2 show slidely low value than Terminalia arjuna extract.Mixed extract may impact. But we can say it might have better clot-dissolving properties than mixture-1.

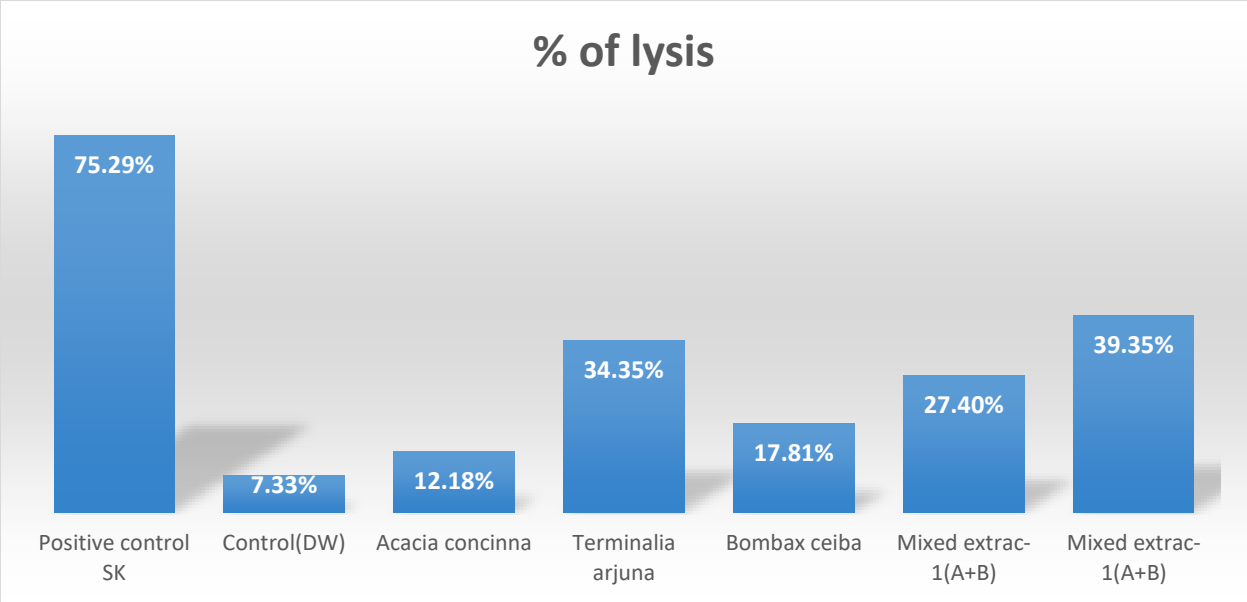


Figure-6.2.a. : Graphically presentation of Thrombolytic activity test

6.3. Antioxidant activity test result

Concentration (µg/ml)	% of inhibition *Acacia concinna*	IC_{50} (µg/ml)	% of inhibition *Bombax ceiba*	IC_{50} (µg/ml)	% of inhibition *Terminalia arjuna*	IC_{50} (µg/ml)	% of inhibition Mixed extract-1(A+B)	IC_{50} (µg/ml)	% of inhibition Mixed extract-2(T+B)	IC_{50} (µg/ml)
31.25	69.00 %	16.20	50.42 %	28.85	60.1 %	22.27	79.83 %	16.20	94.92 %	14.75
62.5	81.90 %		58.78 %		64.8 %		70.39 %		87.44 %	
125	82.60 %		67.82 %		67.5 %		62.80 %		76.89 %	
250	83.76 %		73.34 %		76.8 %		57.68 %		68.88 %	
500	88.68 %		76.9 %		80.5 %		52.62 %		62.66 %	

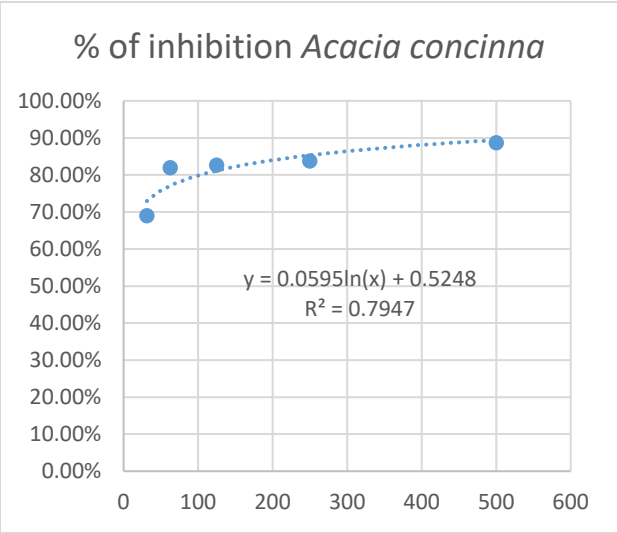


Figure-6.3.a.: Graphical presentation of Acacia concinna

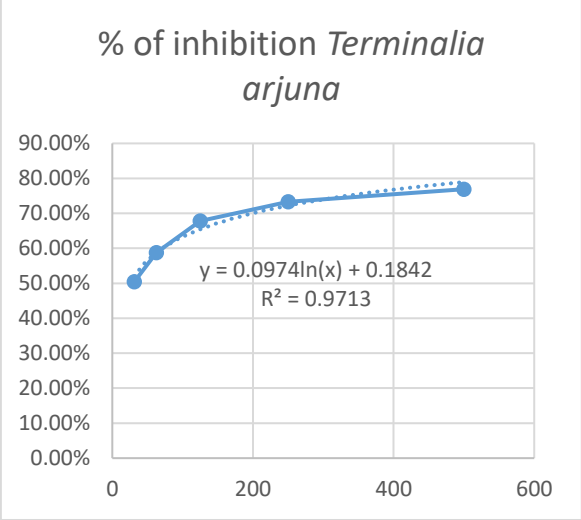


Figure 6.3.b.: Graphical presentation of Terminalia arjuna

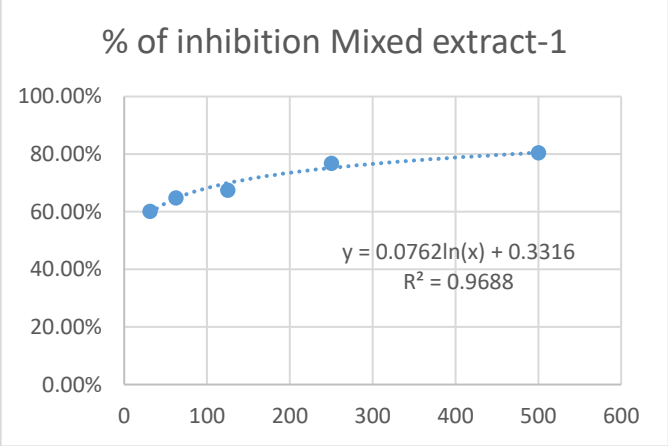


Figure 6.3.c: Graphical presentation of Mixed Mixed-2

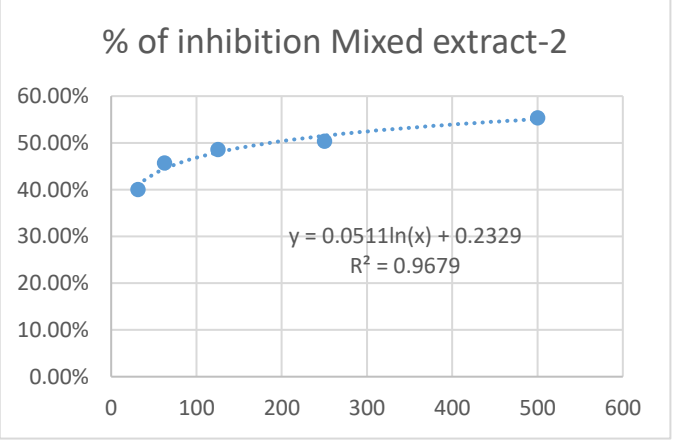
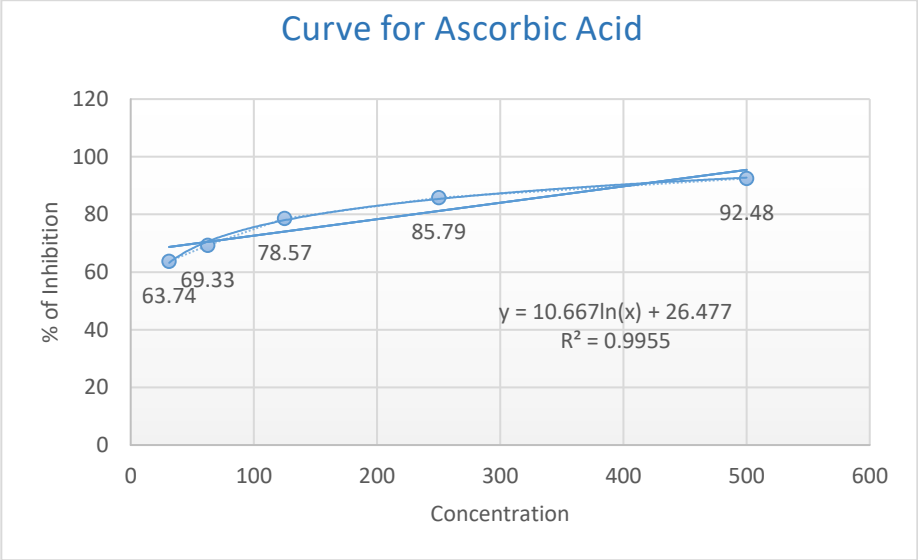


Figure 6.3.d.: Graphical presentation of

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



CHAPTE SIX

CONCLUSION

7. Conclusion

Acacia concinna extract showed 12.18% lysis percentage. Terminalia arjuna, the sample group showed 34.35% lysis percentage. Bombax ceiba extract, the sample group showed 17.81% lysis percentage. Mixed extract-1(Acacia concinna & Bombax ceiba), the sample group showed 27.10% lysis percentage. Mixed extract-2 (Bombax ceiba & Terminalia arjuna), showed 39.35% lysis percentage.And The Standard (Streptokinase) showed a notably greater percentage of lysis at 75.29%. Additionally Acacia concinna, Terminalia arjuna, Bombax ceiba, Mixed extract-1(Acacia concinna & Bombax ceiba),& Mixed extract-2 (Bombax ceiba & Terminalia arjuna) extract showed antioxidant activity respectively (IC50=16.20µg/ml,28.85µg/ml, 22.27µg/ml, 16.20µg/ml & 14.75µg/ml).

Here we can see Acacia concinna does not contain Alkaloid and it cannot impact on mixed extract. Bombax ceiba does not contain Glycoside & Flavonoid and it impact on Mixed extrac-1(Acacia concinna+ Bombax ceiba).All plants and both mixture contain Steroid & Gums.

Assist inquire about is prescribed to way better get it the component basic the watched exercises and to recognize and characterize the dynamic phytochemical components capable.

CHAPTER SEVEN

REFERENCE