Scientific exploration of Cytotoxic, Anti-Oxidant and Thrombolytic Activity of methanolic extract of *Cassia Fistula* (Leaves) and *Cicer arietinum* (Fruits) Remedies

M. Pharm (Masters) Thesis Report

A dissertation submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University to fulfill of the prerequisites for the degree of Master of Pharmacy

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DEDICATION

First and foremost, I dedicate this work to the Almighty Allah, followed by my family members, in especially my parents, teachers, and friends.

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Declaration

I thus certify that the thesis work title is **" Scientific exploration of Cytotoxic, Anti-Oxidant and Thrombolytic Activity of Methanolic extract of *Cassia Fistula* (Leaves) and *Cicer arietinum* (Fruits) Remedies"** is necessary to fulfil the requirements of the Daffodil International University Faculty of Allied Health Science's Master of Pharmacy (M. Pharm) program.

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Abstract

The investigation looked into the scientific underpinnings of the plants' potential medical uses, including their cytotoxic, thrombolytic, and antioxidant properties. Both the chickpea remedy and the Cassia fistula remedy showed moderate cytotoxic and notable thrombolytic and antioxidant activities, according to the study, indicating that they might be utilized as possible natural medication sources for the treatment of a range of illnesses.

Objectives: To assess the possible chemical components and assess the effects on thrombolysis, antioxidants, and cytotoxicity.

Materials & Methods: Methanol was used to extract the plant material from Cassia fistula and Cicer arietinum for the phytochemical testing, which were conducted in a lab. The thrombolytic test was conducted, and the coat lysis method was used to carry out the thrombolytic activity. The Brine Shrimp method was used to measure cytotoxic activity, while the free radical scavenging method was used to screen for antioxidants.

Results: Numerous chemical components, including alkaloids, carbohydrates, tannins, glycosides, and saponins, were found in the extract of Cassia fistula and Cicer arietinum, according to phytochemical study. When the output of thrombolytic activity from these plant remedies is compared to the usual value of streptokinase (75.29%, Previous report), it is 39.35%. Additionally displayed is the antioxidant activity (IC_{50}=5.56), which is contrasted with the standard ascorbic acid (IC_{50}=9.072). The methanolic extract of Cassia fistula exhibits cytotoxic effect because its LC_{50} value is almost the same as that of vincristine sulfate.

Conclusion: The plant exhibits phytochemical and pharmacological activities (thrombolytic, antioxidant, and cytotoxic) according to the data gathered.

Key words: *Cicer arietinum and Cassia fistula* Phytochemical screening, Thrombolytic, Antioxidant, Cytotoxic

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CHAPTER ONE

INTRODUCTION

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1.1 General Introduction

Every sickness has a plant remedy. [1] Since the dawn of time, people have used herbs and plant-based products as medicine to boost immunity or resistance against a range of illnesses, such as fever, joint discomfort, coryza, and colds. The traditional medical systems of Siddha, Unani, and Ayurveda rely on plant-based medicines as their foundation to treat common ailments. Herbal treatments are typically used by the vast majority of people in our country, especially those who live in rural areas. [2]

Approximately 80% of the 5.2 billion people on the earth reside in less developed nations, and practically all of them rely almost solely on herbal medicine to cover their basic medical needs, according to the World Health Organization. Medicinal herbs are the "foundation" of traditional medicine, used by around 3.3 billion people in underdeveloped nations on a daily basis. [3]

When medicinal plants are produced, hundreds of chemical compounds are discovered. These substances are antagonistic to fungus, insects, illnesses, and herbivorous mammals. [4]

Many contemporary pharmaceuticals have been developed as a result of indigenous tribes' historic usage of plants as medicine. You can carry out ethnobotany-related activities outdoors or at botanical gardens. This field can be useful for research on pharmacognosy, drug discovery, and plant physiology. [5].

1.2 Phytochemistry

The name comes from the Greek word for "plant"[5]. Phytochemistry is the study of compounds generated by plants, particularly their secondary metabolites, which are created as a defensive mechanism against infections, insects, pests, herbivores, UV radiation, and environmental threats.

Phytochemistry considers the structural composition of these metabolites, their biosynthetic processes, their roles and methods of action within living systems, as well as their economic, industrial, and medical uses. A good understanding of phytochemicals is essential to the search for novel therapeutic agents to cure critical disorders. [6].

1.3 Medicinal Plant

Any plant that contains compounds with therapeutic efficacy or semi-synthetic precursors to chemotherapy medicines in one or more of its organs is regarded as medicinal. [7] Medicinal plants have been used for medical purposes for the entirety of recorded history. [8] Because medicinal plants are widely utilized to treat and prevent a wide range of ailments and disorders, it is believed that they have a good effect on health care. [9]

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The use of medicinal plants is more common in countries where traditional medicine is practiced than it is in countries where exclusively scientific medicine is used. In the former, using medicinal plants directly has almost completely disappeared from official therapy and is mostly restricted to self-treatment; in the latter, on the other hand, it continues to be the mainstay of all forms of therapy used, to the point where, in several developing countries, 90% of the population's stock of medicinal plants is made up of plants that are considered to be medicinal. [7]

The materials included oils from the following plants: Commiphora species (myrrh), Glycyrrhiza glabra (licorice), Cupressus sempervirens (cypress), and Cedrus species (cedar). The earliest known writings were composed in cuneiform about 2600 BC on clay tablets in Mesopotamia. These drugs are still used today to treat a variety of illnesses, including inflammation, parasite infections, and colds and coughs. Ammi majus, sometimes known as bishop's weeds, is used to treat vitiligo, a skin disorder characterized by a loss of pigment. Egyptian medications are the source of this information. Recently, this plant has produced β-methoxy psoralen, a drug that treats psoriasis, T-cell lymphoma, and other skin diseases the interest in using plants as a potential supply of chemotherapeutic medications. Natural products or their derivatives account for more than half of all medications utilized in clinical trials across the globe. Higher plants make up at least 25% of the total. In the last 40 years, blooming plants like Dioscorea spp. have been used to make at least a dozen potent drugs. [10]

1.4 Significant of Medicinal plant:

Every kind of medicinal plant has a unique purpose. If a plant meets these three criteria, it is deemed medicinal.

Preventive: Research indicates that these medications can avert several ailments. It lessens the adverse effects of synthetic medicines.

Synergistic: Some therapeutic herbs have the potential to either worsen or lessen adverse physiological consequences in people.

Official: Often referred to as formula medicines, some plants are utilized to cure difficult illnesses like cancer. [11]

1.5 Herbalism

Herbal medicine, or herbalism, is the study of botany and the uses of medical herbs. [12] The number of patients pursuing herbal and alternative medicines is rising. Herbal remedies are the result of hundreds of years of therapeutic experience accumulated by successive generations of indigenous medicinal practitioners. Herbal medicines are not only becoming more and more common in underdeveloped countries for basic medical requirements, but they are also more adapted to human bodies, more culturally acceptable, and less likely to cause side effects. However, recent studies indicate that not all herbal medicines are safe, with some having been connected to harmful side

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effects. The majority of herbal products that are currently on the market have not had the drug approval process used to confirm their efficacy and safety. The use of herbal medications is included in the definition of traditional medicine, which was recently provided by the World Health Organization (WHO) as any therapeutic strategy that has been in use for hundreds of years and predates the creation and dissemination of modern medicine. [13]

1.6 Approaches to new product discovery

Herbal medicine texts list several plants that are medicinal but have not been well studied. Pharmacologic screening in accordance with the customary uses of these plants can be used to determine their usefulness. If a notable result is produced, spectroscopic and chromatographic techniques can be used to identify the perpetrator. A bioactivity-guided strategy involves three distinct stages of research. First, the crude material exhibits biological activity. The inactive fractions are discarded, and a bioassay approach is used to identify the active fractions. The second stage is the fractionation of the raw material. [14]

1.7 Basis for Phytochemistry

When salicylic acid corrosion occurs, all pants manufacture fortifiers that aid in development, such as defense against herbivores or photoprotective compounds. These phytochemicals are ideally adapted for use as pharmaceuticals, and if their pharmacological effects and compounds in restorative plants are established, then the current usage of these phytochemicals in pharmaceuticals makes sense. Daffodils, for instance, contain nine alkaloid components, including glutamine, which has been given the all-clear to treat Alzheimer's disease. Alkaloids are volatile plant stems that are easily consumed by herbivores. They are also odorous and toxic. Additionally, they can offer parasite defense. [15][16].

1.8 Alkaloids

Any of the alkaloids, or bases containing nitrogen, that are present in a range of organisms. Alkaloids have a variety of physiological impacts on people and other animals. Things like triclosan, ephedrine, morphine, and vape are examples of alkaloids. Alkaloids are mostly found in nature, and these flowering trees are particularly abundant in them. In all honesty, alkaloids are thought to be present in up to 25% of higher plants, with several thousand different varieties having been identified. Usually, an animal species contains only a few different kinds of alkaloids; however, the opium poppy (Papaver somniferous) and the ergot parasite (Claviceps) each have over thirty different forms. Alkaloids are prevalent in several plant families; it is thought that all members of the papaveraceous (poppy) family contain alkaloids. The Amaryllidaceae (amaryllis), Solanaceae (nightshades), and Ranunculaceae (buttercups) are three more prominent families that include alkaloids. Two alkaloids have been found in a number of animal species, such as New World beavers (Castor

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canadensis) and poison-dart frogs (Phyllo bates). In addition, ergot and a few other parasites create them. [17][18].

1.9 Glycosides

Alexandrian senna, cascara, and rhubarb are among the plants that contain anthraquinone glycosides. Plants including senna, rhubarb, and aloe are used to make plant-based purgatives. The cardiac glycosides, which come from medicinal plants like lily of the valley and foxglove, are amazing drugs. They contain digoxin and digitoxin, which function as diuretics and promote heart palpitations. Cardiovascular glycosides are medications used to treat specific irregular pulses and cardiovascular collapse. They belong to one of the few groups of drugs used to treat ailments of the heart and associated disorders. [19]

1.9.1 Tannins

Phenolic acids, sometimes referred to as tannic acid, make up the complex chemical compounds known as tannins. The method renders these materials insoluble and resistant to breakdown. Numerous coniferous tree species and families of flowering plants contain tannins. Plants contain tannins that can seep out.

1.9.2 Saponins

Saponins are plant flavonoids that are bonded to water-soluble sugars and contain a lipophilic medication or triterpenoid. These additives have a soap-like hydrophobicity and asymmetric information that can reduce surface tension.

1.9.3 Steroids

Plant steroids are important hormones that are also referred to as brassino steroids. Due to the fact that these hormones control many facets of growth and development, mutants lacking in brassino steroids are frequently stunted and sterile.

1.9.4 Phenol

Plants mostly manufacture phenolic compounds—which are compounds with one or more oxygen atoms on the aromatic benzene ring—to ward against stress. Phenolic compounds are crucial to the growth of plants, especially for the formation of pigments and lignin. Their structural integrity and scaffolding support are beneficial to plants.

1.9.5 Carbohydrates

Plants can store complex carbohydrates or use them as energy to grow in addition to using them to create their structural foundation. In order to extract energy from stored carbohydrates, plants take the glucose produced during photosynthesis and mix it with oxygen in a process known as respiration. The main result of photosynthesis and a frequent carbohydrate found in plants is glucose. Disaccharides can be created by

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combining two monosaccharides. They go by several names, including lactose and sucrose. Lactose is one type of carbohydrate found in milk.

1.9.6 Diterpenes

Composed of four isoprene units, dieterpenes are secondary metabolites that are present in fungus and plants. The bulk of diterpenes are non-volatile, however occasionally extremely minute amounts of them can be identified in essential oils. [20] Vitamin A activity (retinol), phytohormones that control plant growth and germination (Gibberellin), fungal hormones that promote the transition from asexual to sexual reproduction (Trisporic acid), and disease-resistant substances (phytoalexins) are examples of physiologically active diterpenoid groups. [21] Diterpenes and their derivatives have biological actions that include anti-inflammatory and immunomodulatory qualities.

1.9.7 Flavonoids

Plants can store complex carbohydrates or use them as energy to grow in addition to using them to create their structural foundation. In order to extract energy from stored carbohydrates, plants take the glucose produced during photosynthesis and mix it with oxygen in a process known as respiration. Glucose is the primary byproduct of photosynthesis and is a common carbohydrate found in plants. Two monosaccharides can be combined to make disaccharides. These are known by many names such as lactose and sucrose. One kind of carbohydrate that is present in milk is lactose.

1.10 Activity of Antioxidants

Antioxidant activity is important in the packaged food industry because it lowers the risk of heart disease, guards against degenerative illnesses, and prolongs product life by stopping or postponing oxidation events. Compounds known as antioxidants have the ability to protect your cells from free radicals, which have been connected to heart disease, cancer, and other diseases. Your body creates free radicals when it breaks down food, when it is exposed to radiation, or when it smokes cigarettes. The importance of oxidation to the organism and to food is well understood. Cells require oxidative metabolism in order to survive. One unintended consequence of this dependence is the production of free radicals, including reactive oxygen species, which cause oxidative changes. Additional data suggests that these species participate in a variety of common in vivo regulatory systems.[22] Overabundance of free radicals can oxidize enzymes, cellular proteins, membrane lipids, and DNA, which ends cellular respiration. They also have the ability to destroy and produce lethal cellular consequences like apoptosis, as well as override defensive enzymes like catalase, peroxidase, and superoxide dismutase. Furthermore, it seems that there are still unanswered questions regarding how reactive oxygen species impact cell signaling pathways. [23, 24] The two basic

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groups that techniques for assessing antioxidant behavior fall into are indicative of the emphasis on either human bioactivity or dietary activity. [25]

1.11 Thrombolytic Activity

A thrombus is a blood clot that occurs in the circulatory system. It adheres to the formation site, obstructing blood flow in the process. The medical name for the beginning of a thrombus is thrombosis. The risk of getting a thrombus is greater in those who are sedentary and have a genetic susceptibility to blood clotting. A thrombus may also occur as a result of injury to an artery, vein, or adjacent tissue. [26] Both community economics and health are significantly impacted negatively by venous thromboembolism (VTE)[27]. In Australia, VTE may be the reason for over 30,000 hospital admissions, leading to 5,000 patient fatalities yearly. [28] This condition ranks third among vascular disorders in Caucasian populations, after myocardial infarction and stroke. [29] Estimates state that VTE, an acute incident that is followed by a main diagnosis, occurs in two to three out of every 1000 hospital admissions [26]. Pulmonary embolism (PE) and deep vein thrombosis (DVT) are two clinical signs of VTE that can be lethal for people of either gender. [27]

Thrombolytic therapy protects critical organs and tissues from damage, improves blood flow, and dissolves dangerous blood clots. The technique of administering drugs that disintegrate clots into the bloodstream via an IV line or a lengthy catheter that delivers the drug to the blockage site is known as thrombolysis. Using a lengthy catheter with a piece of equipment at the tip to physically break up or remove the material clot is an additional option.[22] Treatment for thrombolysis breaks up blood clots in the arteries supplying the heart, which is the primary cause of acute pulmonary embolism and ischemic strokes. It also breaks up blood clots in the airways that expand the heart. veins in the legs, pelvis, and upper extremities that can induce deep vein thrombosis (DVT) or clots; if the clot fragments and goes to a lung artery, it can result in an abrupt pulmonary embolism. If a blood clot is determined to be life-threatening, thrombolysis may be a possibility as soon as symptoms of a heart attack, stroke, or pulmonary embolism occur, preferably within one to two hours (once a diagnosis has been made)[29].

1.11.1 Mechanisms of Thrombolysis

Thrombolytic drugs disintegrate blood clots by activating plasminogen, which leads to the cleaved product plasmin. Fibrin crosslinks preserve the structural integrity of blood clots and are destroyed by the proteolytic enzyme plasmin. Thrombolytic drugs are also known as "plasminogen activators" and "fibrinolytic drugs" according to the nature of their action. The three main kinds of fibrinolytic drugs are urokinase, tissue plasminogen activator (tPA), and SK (UK). The three drug classes, however, have the same effects. They all have different specialized ways for breaking up blood clots. methods to change how selective they are for fibrin clots. The fibrinolytic mechanisms of tPA and SK are depicted in the diagram on the right. Because of their relative

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selectivity in activating fibrin-bound plasminogen, derivatives of tPA are the most commonly utilized thrombolytic medicines, especially for cerebral and coronary vascular clots. The following order is used by tissue plasminogen activator to cause clot lysis:

- The clot dissolves when tPA attaches to fibrin on its surface
- Activates plasminogen that is attached to fibrin
- Cleaves plasminogen from the fibrin-associated plasminogen, and breaks apart fibrin molecules.
- A protease called plasmin has the ability to disintegrate fibrin molecules, causing the clot to disappear. However, it's important to remember that plasmin also breaks down other proteins.

proteins in circulation, such as fibrinogen Alongside the United Kingdom and the Republic of Korea, clot dissolution with tPA, however, involves less breakdown of circulating fibrinogen due to the relative specificity of fibrin. Despite the fact that tPA is mostly selective for plasminogen that is clot-bound, it also activates circulating plasminogen, which releases plasmin and may eventually produce plasmin. An undesirable systemic fibrinolytic state is caused by the breakdown of circulating fibrinogen. Although antiplasmin that is in circulation naturally inactivates plasmin, therapeutic antiplasmin also does so.

Even though SK lacks any enzymatic activity and is not a protease, it attaches to plasminogen and releases plasmin. It binds both circulation and non-circulating plasminogen equally, unlike tPA, which shows a predilection for binding to clot-associated fibrin. As a result, SK induces substantial fibrinogen lysis in addition to clot fibrinolysis. For this reason, tPA is often selected as a thrombolytic drug instead of SK, particularly for the breakdown of thrombi in the cerebral and coronary arteries. Since SK is generated from streptococci, patients who have recently contracted streptococci may require considerably greater dosages of SK to achieve thrombolysis. It is important to remember that the effectiveness of thrombolytic drugs is dependent on the age of the clot. Dissolving because of greater compactness of older clots and enhanced fibrin cross-linking. It is advised to give thrombolytic medications in the first two hours following an acute myocardial infarction. Beyond that point, the drug begins to lose its potency and higher dosages are usually required to achieve the desired lysis. [31] [32]

1.11.2 Streptokinase

Several species of streptococci release the enzyme streptokinase (SK), which can bind to and activate human plasminogen. In certain situations of ischemic stroke, SK is utilized as an effective and reasonably priced thrombolysis analgesic (chest discomfort). and a kind of blood clot in the lungs is called a pulmonary embolism. Streptokinase belongs to a group of medications called streptokinase inhibitors. Streptokinase complexes with human mast cells have the ability to hydrolyze fibrinolytic. Fibrin is produced when other unbound plasminogen is activated by bond

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cleavage. Three domains comprise streptokinase: residues 1–150, residues 1–150, and residues 1–150. Residues 151–287, 288–414, and 288–414, respectively. Each domain binds plasminogen, but no single domain is able to activate plasminogen on its own. [33][34]

1.11.3 Mechanism of action of Streptokinase

The proteolytic enzyme plasmin is produced when the Arg/Val bond in plasminogen is broken down by streptokinase, which then forms an active complex. Plasmin then breaks down the thrombus' fibrin matrix to function as a thrombolytic agent. The primary constituent of blood clots, plasmin, is a blood protein that aids in their removal and dissolves them until they have healed appropriately. Plasmin, which breaks down blood clots in the lungs, for example, is produced more when streptokinase is present. (pulmonary embolism). Proteolysis of the Arg561—Val562 link ordinarily activates plasminogen. Target enzyme (Pm) catalyzes the paracrine formation of an energetic protease by forming a covalent link between the amino group of Val562 and Asp740. After (SK) is discovered, it combines with Plasminogen to produce a complex known as (SK. Plasminogen), which transforms Plasminogen into Pm. Residues 1–59 control SK's nonproteolytic capacity to create an active site in bound Pg and activate substrate Pg without the need for fibrin. This intense dynamic then reorganizes to produce an intense dynamic, even if the Arg561–Val562 connection is still intact. Therefore, given this strong dynamic, another residue needs to replace the free amino group of Val562 and supply a counterion for Asp740. It has been suggested that Lys698 of Plasminogen and Ile1 of Streptokinase could be this counterion. In order for SK to induce an active site in plasminogen through a nonproteolytic mechanism, a salt bridge must be established between Ile1 of SK and Asp740 of plasminogen. This finding lends credence to the hypothesis that SK must first establish a salt bridge between Ile1 of SK and Asp740 of plasminogen in order to induce an active site in plasminogen through a nonproteolytic mechanism. The SK facility's dissociation constant was 15–50 times lower due to the Lys698 anomalies than the Ile1 genetic alterations. These results imply that Lys698 is involved in the original creation of the SK Plasminogen facility. [34]

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CHAPTER TWO
PLANT PROFILE

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2.1 Introduction of plant

Belonging to the family *Cassia fistula Linn*, commonly called the golden rain tree (Bengali: Sonali, Bador lathi).

The primary therapeutic benefit of Caesalpiniaceae is that it is a mild laxative that is safe for use by both pregnant women and children. This family member is used extensively in medicine. Because of the wax aloin, it also functions as a purgative and a tonic [35]. It has also been used to treat a variety of other intestinal conditions, including the healing of ulcers [36].

According to WHO estimates, traditional medicine serves primary medical needs for over 70% of the world's population. For primary medical concerns, 80% of individuals in developing countries turn to traditional medicine [37, 38]. The general consensus is that synthetic drugs have more adverse effects and are more hazardous than herbal and plant-based ones [39].

Figure-1: Image of *Cassia fistula* Plant

2.1.1 Plant name

Scientific Name: *Cassia fistula*

Common Name: Golden Rain Tree

Bangla Name: Sonali, Bandor lathi

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2.2 Taxonomical classification

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Genus: Cassia

Subject: Cassia fistula

2.3 Research on Phytochemistry [40]:

- 2% anthraquinones
- 24% crude protein
- 4.5% crude fat
- 6.5% crude fiber
- 50% carbohydrates are present in the seeds.

In addition, the plant contains phlobaphenes, oxyanthraquinone compounds, rhein, rheinglucoside, galactomannan, sennosides A and B, tannin, emodin, chrysophanic acid, fistulacacidin, barbaloin, lupeol, beta-sitosterol, and hexacosanol [41].

2.3 Traditional use

One of the most often utilized plants in Unani and Ayurvedic medicine is Cassia fistula, which has been described in several traditional medical texts.

It has been proposed that it is beneficial for treating skin conditions, liver issues, tuberculous glands, and hematemesis, pruritus, leucoderm, and diabetes [42]. The herb is also traditionally used as a powder, decoction, or infusion, either by itself or in conjunction with other healing plants. Currently available commercial preparations have typically been standardized extracts of the entire plant, both in controlled clinical trials and in everyday life [43].

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2.4 Introduction of plant

One of the seven Neolithic founder crops in the Fertile Crescent of the Near East, the chickpea (*Cicer arietinum L.*), often known as the Bengal gram or garbanzo bean, is an Old-World pulse [44]. Over fifty countries in the Indian subcontinent, North Africa, the Middle East, southern Europe, the Americas, and Australia currently grow chickpeas. After field peas and dry beans, chickpeas are the third most important pulse crop produced worldwide. Around 11·3 million hectares were used for chickpea cultivation worldwide in 2006–09, yielding an average yield of 849 kg/ha and a production of 9·6 million metric tons [45]. With an average production of 6·38 million metric tonnes between 2006 and 2009, India is the greatest producer of chickpeas, making up 66% of the world's production [46]. Other significant nations that produce chickpeas are Iran, Mexico, Canada, Ethiopia, Ethiopia, Turkey, Australia, and the United States.

Figure-2: Image of *Cicer arietinum*

The nutritious benefits of chickpeas is driving up demand for them. Chickpeas play a significant role in the diets of persons who cannot afford animal proteins or who choose to follow a vegetarian diet in the semi-arid tropics. Compared to other pulses, chickpeas are a good source of protein and carbs, making up about 80% of the dry seed mass [47]. In addition to being low in cholesterol, chickpeas are an excellent source of vitamins, minerals, and dietary fiber (DF)[48].

2.4.1 Plant name

Scientific Name: *Cicer arietinum L*

Common Name: Chickpeas

Bangla Name: Chola

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2.5 Taxonomical classification [49]

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Faboideae

Genus: Cicer

Species: C. arietinum

2.6 Traditional use

Chickpea seeds have been used in traditional medicine as a tonic, stimulant, aphrodisiac, anthelminthic, appetizer, and to relieve stomach burning. In herbal medicine from China Chickpea seeds have reportedly been used for over 2500 years in Chinese herbal medicine to treat diabetes mellitus and hypertension.

Additionally, chickpeas are commonly used to treat and prevent osteoporosis, hypertension, hyperlipidemia, diabetes, itchiness on the skin, flatulence, poor libido, and tumor formation [50].

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CHAPTER THREE

OBJECTIVE OF THE STUDY

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3. The objective of the study

The phytochemical makeup of these plants and a few specific pharmacological analyses were the main focus of this investigation. Examining potential activities of *Cassia fistula* and *Cicer arietinum* was the goal of this investigation. The herb is far less commonly used in traditional medicine. These plants also contain a lot of tannins, glycosides, alkaloids, and other substances that would make it a good subject for additional study. The analysis makes use of the methanol extract from the entire plant. There are several reasons to investigate these plants. For example:

Chemical constituent analysis: Based on the findings of this study, a number of phytochemical activities, including the presence or absence of alkaloids, glycosides, flavonoids, saponin, reducing sugar, etc. in those plants, will be evaluated.

Investigation of pharmacological activity: An additional objective of this thesis is to examine the scientific basis for the cytotoxic, thrombolytic, and antioxidant actions of the entire plants as possible medical applications.

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CHAPTER FOUR
LITERATURE REVIEW

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Khan, B. A., Akhtar, N., Rasul, A., Mahmood, T., Khan, H. S., Iqbal, M., & Murtaza, G. (2012). Investigation of the effects of extraction solvent/technique on the antioxidant activity of Cassia fistula L. Journal of Medicinal Plants Research, 6(3), 500-503.

Title: Investigation of the effects of extraction solvent/technique on the antioxidant activity of Cassia fistula L.

Recent worries about synthetic antioxidant safety have shifted the focus of research toward less expensive and safer antioxidants derived from nature. The antioxidant activity of Cassia fistula pods, leaves, barks, and flowers was examined in relation to the effects of two extraction techniques—hot percolation (Soxhlet apparatus) and simple maceration—as well as four extracting solvents: ethanol, methanol, n-Hexane, pet ether, and methanol. Standard free radicals were 1,1-diphenyl-2-picrylhydrazyl (DPPH), while standard antioxidants were ascorbic acid (Vitamin C) and quercetin. Experiments showed that extracts have antioxidant properties that are technique- and solvent-dependent.

With ascorbic acid as the standard and quercetin as the standard, 70% methanolic v/v leaf extract exhibited 89% DPPH scavenging activity using the straightforward maceration process. Still,Using simple maceration and ascorbic acid as a reference, the percentage inhibition of a same concentration of pods, barks, and floral extract was 66, 81, and 83.4%, respectively. However, there were fewer free radical scavenging activities in the extraction performed using the Soxhlets device.

Duraipandiyan, V., Baskar, A. A., Ignacimuthu, S., Muthukumar, C., & Al-Harbi, N. A. (2012). Anticancer activity of Rhein isolated from Cassia fistula L. flower. *Asian Pacific journal of tropical disease*, *2*, S517-S523.

Title: Anticancer activity of Rhein isolated from Cassia fistula L. flower

To assess Rhein's anticancer properties against colon cancer celllines using an ethyl acetate extract of Cassia fistula (C. fistula) flowers. Rhein was evaluated against the normal cell line VERO and the human colon cancer cell line COLO 320 DM. Rhein showed only a slight cytotoxic effect on VERO cells. A spectroscopic approach was used to identify the chemical rhein.Rhein was discovered to be cytotoxic in a time- and concentration-dependent manner to COLO320 DM cells. At 200 g/mL concentration, Rhein showed 40.59%, 58.26%, 65.40%, 77.92%, and 80.25% cytotoxicity during 6,

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12, 24, 48, and 72 hours of incubation. Rhein's IC50 values were 100, 25, 15, and 12.5 g/mL for incubation times of 12, 24, 48, and 72 hours, respectively.

The COLO 320DM cells treated with Rhein exhibited apoptotic characteristics at 6.25 and 12.5 g/mL after a 24-hour treatment period. At 6.25 g/mL, apoptosis was 2.29% in the early stages and 1.94% in the late stages. Apoptosis was 5.61% in the late stages and 4.36% in the early stages when the concentration was raised to 12.5 g/mL. The findings suggested that Rhein may be applied to cancer therapy.

Fernandez-Orozco, R., Frias, J., Zielinski, H., Muñoz, R., Piskula, M. K., Kozlowska, H., & Vidal-Valverde, C. (2009). Evaluation of bioprocesses to improve the antioxidant properties of chickpeas. ***LWT-Food Science and Technology*****,** ***42*****(4), 885-892.**

Title: Evaluation of bioprocesses to improve the antioxidant properties of chickpeas

To increase their antioxidant qualities, chickpea seeds (Cicer arietinum cv. Blanco lechoso) were subjected to three different bioprocesses: i) two and three days of seed germination; ii) natural fermentation of flour or cracked seeds; and iii) inducing fermentation of flour or cracked seeds using Lactobacillus plantarum. Total phenolic compounds (TPC), reduced glutathione (GSH), and vitamins C and E were measured. Lipid peroxidation inhibition (LPI), trolox equivalent antioxidant capacity (TEAC), SOD-like activity, and peroxyl radical-trapping capacity (PRTC) were used to assess antioxidant capacity. Following germination, vitamin C and E levels rose, but vitamin E levels decreased as a result of fermentation.

TPC content increased as a result of both bioprocesses, however GSH content declined. SOD-like activity (47–41%), PRTC (16–55%), and TEAC (12–23%) all rose with germination, and there was also a little suppression of LPI. Following fermentation, there is a sharp decline in SOD-like activity. With the exception of flour natural fermented seeds, where PRTC declined, an increase in TEAC (29–57%) and PRTC (27–44%) was seen, whereas the LPI fell between 11.3 and 21.3%. The total antioxidant capacity (TEAC) was significantly enhanced by TPC. The findings showed that the investigated bioprocesses improved the antioxidant qualities of chickpea flours, making them desirable components for novel functional food formulations.

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Tarzi, B. G., Gharachorloo, M., Baharinia, M., & Mortazavi, S. A. (2012). The effect of germination on phenolic content and antioxidant activity of chickpea. *Iranian Journal of Pharmaceutical Research: IJPR*, *11*(4), 1137.

Title: The Effect of Germination on Phenolic Content and Antioxidant Activity of Chickpea

One of the best methods for raising the caliber of legumes is germination. During germination, vitamins and some other substances that may be advantageous as antioxidants frequently undergo significant changes. Antioxidants are substances that have the ability to shield the human body from oxidative damage or to prevent, delay, or retard the development of rancidity or other flavor deterioration in food. Three distinct solvents were utilized in this study in order to extract the phenolic chemicals found in chickpea sprouts and seeds. The Folin-Ciocalteau method was used to assess the total phenolic contents, and the oven test method and the hydroxyl radical scavenging activity assay were used to determine the antioxidant activity.

For the latter, tallow was mixed with various extract concentrations (0.02, 0.04, 0.06, 0.08, and 0.1% w/w), and the treatments' stabilities were assessed. Measurements of the induction period and peroxide value were utilized to assess the antioxidant activity. The outcomes showed that the antioxidant activity is altered by the germination process. When acetone solvent was used, there were more phenolic compounds present; nonetheless, methanolic extract showed superior hydroxyl radical scavenging action. By postponing the indicated oxidation, the antioxidant activity of the extracts was evaluated. This activity improved with larger quantities of the extracts applied. Thus, chickpea sprout extract or flour could be utilized in the preparation of oil-based supplements or as a natural antioxidant source in functional foods.

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CHAPTER FIVE

METHODS AND MATERIALS

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5.1 Preparation of extraction

5.1.1 Plant Collection

The one plant was collected from local market and another plant was collected from the cumilla, Bangladesh.

5.1.2 Preparation of plant material

A plastic-topped bottle jar was utilized, and it was thoroughly cleaned before being rinsed in methanol. Each jar was then filled with 500g of dried sample and 1000 ml of methanol up to one inch above the sample surface, covering the whole surface. The jar was correctly sealed with an aluminum foil-lined plastic cover to keep air out. It seems like the process took fourteen days. Every day, the jar was shaken two or three times, in order to improve extraction.

5.1.3 Filtration

Following the extraction process, the plant extract was filtered through sterilized cotton and filter paper, which were then both soaked in the proper solvent and put inside a funnel. The filtrate was collected and placed in a glass container. I then went through the refiltration process once more.

5.1.4 Evaporation

A rotating evaporator operating at 65–67 degrees Celsius was used to evaporate the fluid concentration. The thick sample was moved into a 100-milliliter container. The measurement glasses were coated with aluminum paper and put just under the fan once the pure concentrate was collected. Use a hair dryer to evaporate any remaining water in order to get rid of any dissolvable that is still present in the concentrate.

5.2 Methods of Phytochemical Screening

It describes the process of removing, sorting through, and identifying the compounds in plants that have healing properties. Plants include a variety of bioactive chemicals, including tannin, phenolic compounds, carotenoids, flavonoids, and alkaloids. Although they are present in smaller amounts, phytochemicals are typically optional, while cannabinoids are abundant. Plants contain a wide range of other compounds in addition to the active molecules. More than half of pharmaceuticals are derived from plants, but little is known about the restorative potential of certain plant species. Thus, phytochemical analyses of higher plants associated with ethnobotanical data receive a great deal of attention. Next, the phytochemicals are examined for a range of naturally occurring properties [51].

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5.2.1 Materials and Methods

5.2.2 Test Materials

- Extract of *Cassia fistula*
- Extract of *Cicer arietinum*

5.2.3 Chemical group test reagents

- Mayer’s Reagent
- Fehling’s Solution II
- Dragendroff’s Reagent
- Distilled water
- Fehling’s Solution I
- Molish Reagent
- Ethyl acetate
- Ferric chloride

5.2.4 Alkaloids Testing

(I) Mayer's Test

The raw material was put in a 2 mL aqueous solution, then 0.2 mL of strong hydrochloric acid was added. Mayer's reagent (1 mL) was then added. Alkaloids would be a major contributing component. An indication would be the formation of a yellow-colored precipitate.

II Dragendroff's Test

2 ml of the crude extract was added to an aqueous solution, and 0.2 ml of strong hydrochloric acid was added. Dragendroff's reagent (1 mL) was then added. Alkaloids

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would be a major contributing component. This is indicated by the formation of an orange-brown precipitate.

5.2.5 Glycosides Testing

In a test tube, there was a 2 mL extract solution. Subsequently, the test tube was filled with a 1mL sample. Combining Fehling solutions A and B. To place the tube, a water bath at 60° C was utilized. Glycosides are present, and a ppt form is available if you want your hue to be brick red.

5.2.6 Tannins Testing

5g of plant extract will be mixed with 10mL of distilled water for each component, filtered, and then reconstituted. Ferric chloride reagent was used to treat the filtrate. Tannins are present when a precipitate turns blue-black, green, or blue-green. The color is blue-black because to the presence of tannin.

5.2.7 Flavonoids Testing

The sample consisted of five to ten drops of strong hydrochloric acid in a test tube, 0.5 milliliters of alcoholic extract, and a little piece of zinc dust or ribbon. The solution was brought to a boil for a short period of time. If flavonoids were present, their existence would be implied.The colors shift from red to crimson.

5.2.8 Saponins Testing

The sample's alcoholic extract solution was diluted to 10ml from 0.5ml using distilled water. Shake in a graduated cylinder for three to five minutes. If frothing was present, it would be verified by allowing frothing to form over time.

5.2.9 Gum examinations

The extract was combined with sulfuric acid and molish reagent after being diluted to a 5 mL solution. A reddish-purple ring formed around the mouth signifies the existence of carbohydrates and gums.two liquids colliding.

5.2.10 Carbohydrates Tests

Molisch Experiment

It was turned into a 2 mL raw leaf extract. A test tube held two drops of a 10% solution. Buffer Naphthol Acetate The flowing side of the inclined tube was then filled with sulfuric acid. Putting a layer in the mixture beneath the aqueous solution. At the intersection of the two layers, a deep reddish-purple ring would form, indicating the current carbohydrate concentration. The solution would turn dark purple when standing or shaking. Once some time has elapsed, the testing tube Before being utilized, the test solution was mixed for two minutes. 5 milliliters of distilled water were added to the

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water combination. The presence of carbohydrates would be indicated by the precipitate producing a dull violet color.

5.2.11 Tests for Steroids

Test for sulfuric acid

Red color indicates 1 mL of chloroform extract solution and 1 mL of sulfuric acid.

A steroid is present.

5.2.12 Phenol Testing

Add three to four drops of ferric chloride solution to the extract. The presence of phenols was discovered, which was encouraging for the development of a blue-black tint.

5.2.13 Test Materials

- Extract of *Cassia fistula*
- Extract of *Cicer arietinum*

5.2.14 Apparatus

- Test tubes and stands
- Beaker
- Pipettes
- Volumetric flasks
- UV spectrophotometer
- Electric balance
- Spatula
- Foil paper
- Funnel
- Tissue paper
- Marker

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5.3 Thrombolytic activity test of the sample

5.3.1 Equipment of thrombolytic activity test

Serial No.	Content
1	Test Tube
2	Glass Rod
3	Vortex Mixture
4	Eppendorf Tube
5	Eppendorf Tube Rack
6	Beaker (50ml)
7	Incubator
8	Cotton
9	Syringe
10	Syringe Filter
11	Micropipette

Table 1: Equipment of thrombolytic activity test

5.3.2 Reagent of thrombolytic activity test

Serial No.	Content
1	Blood (Human)
2	Streptokinase
3	Distilled Water
4	Sample Extraction

Table 2: Reagent of thrombolytic activity test

5.3.3 Procedure

5.3.4 Extract solution preparation for thrombolytic activity

Initially, 25 mg of crude extract suspended in 2.5 ml of distilled water were stirred with a vortex mixer. The suspension was allowed to settle overnight before being filtered using syringe filter paper to collect the soluble supernatant. With this approach, ex-vivo cardioprotective action could now be accomplished.

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5.3.5 Solution preparation of Streptokinase (SK) as standard

The commercially available 15, 000,000 I.U. lyophilized ATPase (Streptokinase) alpine tube (Beacon Pharmaceutical Ltd.) served as the standard solution. After that, five water was added to the streptokinase vial and well stirred. This suspension was used in 100µl (30,000 I.U.) for in vitro thrombolysis [52].

5.3.6 Preparation of Eppendorf Tube

Three Eppendorf tubes were sanitized, weighed, and cleaned and disinfected with distilled water before being marked with a permanent marker to identify them as separate tubes.

5.3.7 Working procedure of Thrombolytic Test

A blank tube was first acquired and measured.

↓

Each tube held one milliliter of blood extracted from a healthy subject.

↓

To aid in the formation of clots, the blood tubes were incubated for 45 minutes at 37°C.

↓

The tube was used to weigh the initial clot after the serum was removed.

↓

First-clot weight is equal to first-clot tube weight minus blank tube weight.

↓

Use 100µl, or 30000 IU, as the standard dose, and extract 100µl, or 1000µg/100µl, while maintaining a 100µl concentration in distilled water.

↓

once more stands for 90 minutes at 37 °C in an incubator to lyse clots.

↓

extracted the blood fluid used for lysis from the tube.

↓

Using tube weight, measured the second clot.

↓

Weight of second clot = weight of second clot with tube - weight of blank tube

↓

Finally, weight of Lysis clot = first clot weight – second clot weight

Lastly, Clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100

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5.4 Detection of In Vitro Antioxidant Test

5.4.1 Equipment & Reagent of Antioxidant test

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

Table 3: Equipment & Reagent of antioxidant activity test

5.4.2 Preparation of In Vitro Antioxidant Test

The plant extract stock solution (10 mg/ml) was prepared in methanol and used in a serial dilution. The first six concentration kinds are prepared using six volumetric flasks: 1, 5, 10, 50.100, and 500 ug/ml. We encircle volumetric flasks and test tubes with foil paper. Six volumetric flasks are prepared with varying extract dilutions and labeled appropriately.

Two milliliters of the sample for each concentration and two milliliters of the 0.004% DPPH solution are pipetted into each of the six test tubes. Each test tube is wrapped in foil paper, and the solution is then left in a dark room for half an hour. In another test tube, combine two milliliters of 0.004% DPPH and two milliliters of methanol to create the blank solution. Next, UV Spectroscopy is used to measure absorption. The percentage of inhibition can be calculated using the formula below.

$$\%\ of\ Inhibition = \frac{Blank\ Absorbance - Solution\ Absorbance}{Blank\ Absorbance} \times 100$$

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DPPH solution

The DPPH stock solution has 0.004% DPPH in it. Thus, 0.004gm of DPPH are present in 100ml of solvent (methanol or ethanol).

Preparation of standard

2.5 cc of methanol with 25 mg of ascorbic acid dissolved in it. Thus, the solution has a concentration of 1000µg/ml. Subsequently, serial dilution was carried out in accordance with the protocol: 500µg, 250µg, 125µg, 62.5µg, 31.25µg.

5.4.3 Working Procedure

Make the extract stock solution first, at a concentration of 10 mg/ml.

Next, dilute this concentration using serial dilution at concentrations of 500µg, 250µg, 125µg, 62.5µg, and 31.25µg.

prepared a methanol-based 0.004% DPPH solution.

Subsequently, a 1:3 concentration of extract solution and DPPH solution—that is, 1 milliliter of extract solution and 3 milliliters of DPPH solution—are added.

Prepare the 1 ml methanol solvent blank solution and add 3 ml DPPH solution to it.

Lastly, the absorbance at 517 nm was determined for the blank solution and various extract solution concentrations.

The prepared solution is then left in a dark area for forty-five minutes.

Measurement of % of inhibition:

$$\% \; of \; Inhibition = \frac{Blank \; Absorbance - Extract \; Solution \; Absorbance}{Blank \; Absorbance} \times 100$$

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The percentage of inhibition of each diluted solution was then computed, and all of the results—along with the X-excess of concentration and the Y-excess of percent inhibition—were entered into Microsoft Excel. Additionally, gather the Y= mln(x)+c equation and find the X value of 50% inhibition. For instance, in the case of a 50% restriction, Y equals 50. The same procedures were used to get the standard (ascorbic acid) IC50. Next, contrast the IC50 values of the sample and the standard.

5.5 Brine Shrimp Lethality Bioassay

5.5.1 Materials and apparatus used in the study

- *Artemiasalina leach* (brine shrimp egg)
- Sea water with salinity 38 ppt. (=3.8% NaCl)
- Transparent glass or plastic hatching container
- Micropipettes
- Test tubes or measuring cylinders
- Magnifying glass
- Continuous air supply
- Test sample (plant extract)
- Distilled water
- Lamp to attract shrimps (Light: 2000 lux)
- Electronic balance

5.5.2 Seawater preparation:

One liter of distilled water was used to dissolve 38g of sea salt (pure NaCl), which was then filtered out to produce a clear solution.

5.5.3 Hatching of Brine Shrimp

38 grams of sodium chloride were weighed, diluted in one liter of distilled water, and then filtered to obtain a clear solution in order to prepare sea water. The purpose of this

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seawater simulation was to hatch brine shrimp. For two days, the shrimp were left to hatch and develop into nauplii, or larvae.

5.5.4 Preparation of test samples of the experimental plant:

First, 20 milligrams of plant extract and 20 milliliters of distilled water were combined in a beaker. Next, a number of solutions with varying concentrations were made from the stock solution using the serial dilution method. Ten milliliters of sample were placed into test tubes in each case, and ten milliliters of distilled water were placed into vials. This led to the discovery of different quantities in the different test tubes: 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, and 15.625 µg/ml. After that, 10 milliliters of the solution were added to the petri dish together with 20 shrimp nauplii and 5 milliliters of artificial seawater.

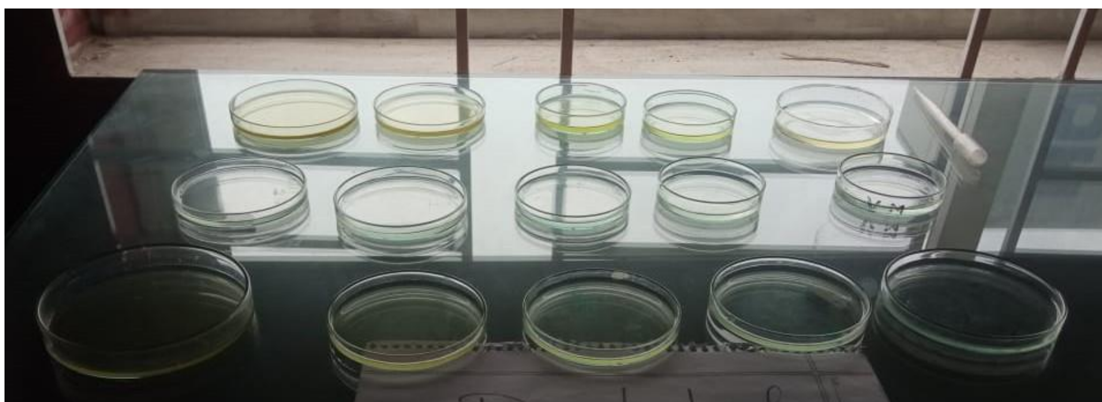

Figure-3: Image of preparation of test samples

5.5.5 Preparation of control group:

In cytotoxicity studies, control groups are employed to validate the test protocol. This guarantees that the effects of any other potential influences are negated and that the results obtained are solely attributable to the activity of the test agent. Two kinds of control groups are typically employed: i) Positive control ii) Negative supervision

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5.5.6 Preparation of the positive control group

In cytotoxicity research, a frequently used cytotoxic agent acts as the positive control, and the results of the test agent are compared with the positive controls. As of right now, Techno Drugs Ltd.'s vincristine sulfate was used as the study's positive control. Vincristine sulfate was weighed out and then dissolved in DMSO to produce a 20 µg/mL starting concentration. Following this, DMSO was serially diluted to yield solutions with concentrations of 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL, and 0.3125 µg/mL. To create the positive control groups, the answers for the positive control were then placed to the vials that had been previously tagged and contained ten live brine shrimp nauplii in five milliliters of simulated sea water.

5.5.7 Preparation of the negative control group:

Three pre-marked glass vials containing 5 mL of simulated sea water and 10 shrimp nauplii to use as control groups were filled with 100 µL of DMSO each. The test is deemed invalid if the brine shrimp in these vials exhibit a high incidence of mortality as the nauplii's cause of death was not the compounds' cytotoxicity.

5.5.8 Counting of Nauplii

The number of surviving was ascertained by looking through the vials under a magnifying glass after a 24-hour period. The mortality percentage (%) was calculated for each dilution. To analyze the concentration-mortality data, a statistical technique called linear regression was used with the Microsoft Office Excel PC application. A median lethal concentration (LC50) value is commonly used to indicate the effectiveness of a plant product or the relationship between concentration and death. This is the concentration of the chemical at a particular exposure duration that results in 50% of test subjects passing away.

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CHAPTER SIX
RESULT AND DISCUSSION

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6.1 Phytochemical Test

Serial No.	Test Name	Result *(Cicer arietinum)*	Result *(Cassia fistula)*
1	Alkaloid (Mayer's & Dandruff's test)	+	+
2	Glycoside	+	+
3	Tannin	+	+
4	Saponin	+	+
5	Steroid	+	+
6	Phenol	+	+
7	Carbohydrate	+	+
8	Diterpene	+	+
9	Gum	+	+
10	Phytosterol	+	+
11	Reducing Sugar (Benedict & Fehling Test)	+	+
12	Flavonoid	+	+

Table 4: Result of Phytochemical Test

Discussion

Every substance discovered in the plant being studied for these lab-tested plants. It should be the duty of steroids and glycosides to provide a thrombolytic action. The compounds that have hepatoprotective and antioxidant properties are phenol, steroid, and phytosterol. Additionally, gum is in charge of antioxidant action.

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6.2 Thrombolytic Activity Test

Solution	Blank Eppendorf Tube Weight	1st Clot Weight	2nd Clot Weight	Lysis Weight	% of Lysis
Standard	0.860gm	1.01gm	0.24gm	0.77gm	75.29%
Control	0.776gm	0.841gm	0.787gm	0.54gm	7.33%
Sample *Cassia fistula*	0.797gm	0.786gm	0.516gm	0.27gm	34.35%
Sample *Cicer arietinum*	0.799gm	0.591gm	0.519gm	0.072gm	12.18%
Combination	0.804gm	0.657gm	0.262gm	0.27gm	39.35%

Table 5: Result of Thrombolytic Activity Test

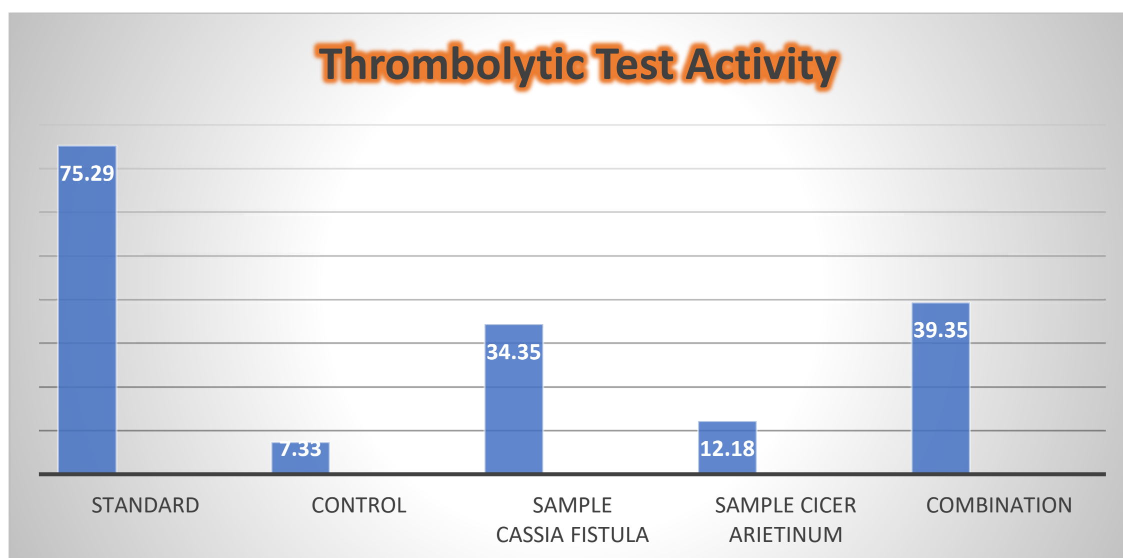

Figure-4: Graphical representation of Thrombolytic Activity Test

Discussion about Thrombolytic Activity Test

The benchmark employed was streptokinase, which showed 75.29% clot lysis in a previous study. In distilled water (Control), the clot lysis rate was 7.33%. In studies, methanol extracts from the plants Cassia fistula and Cicer arietinum demonstrated 34.35% and 12.18%, respectively, of clot lysis. When these plants were combined, the percentage was 39.35. When compared to the standard (Streptokinase), the thrombolytic activity of the methanol extract from these plants revealed considerable antioxidant activity.

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6.3 Antioxidant Test

IC_{50} for Methanol extract of *Cassia fistula and Cicer arietinum*:

Concentration (µg/ml)	% of Inhibition *Cassia fistula*	IC_{50} (µg/ml)	% of Inhibition *Cicer arietinum*	IC_{50} (µg/ml)	% of Inhibition Combination	IC_{50} (µg/ml)
500	79.83%		90.90%		94.92%	
250	70.39%		85.30%		87.44%	
125	62.80%	**2.27**	68.72%	**1.18**	76.89%	**5.56**
62.5	57.68%		60.72%		68.88%	
31.25	52.62%		58.47%		62.66%	

Table 6: IC_{50} value for Methanol extract of *Cassia fistula and Cicer arietinum*

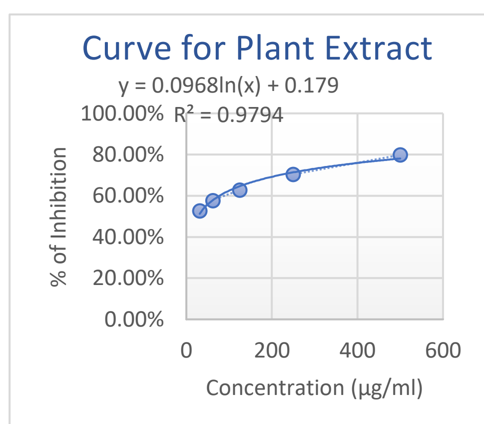

Figure 5: Graphical representation of ***Cassia fistula***

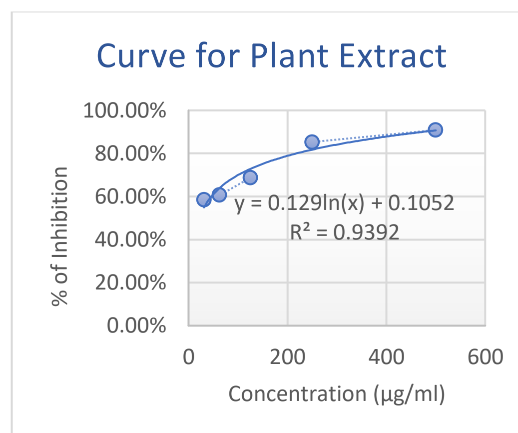

Figure 6: Graphical representation of *Cicer arietinum*

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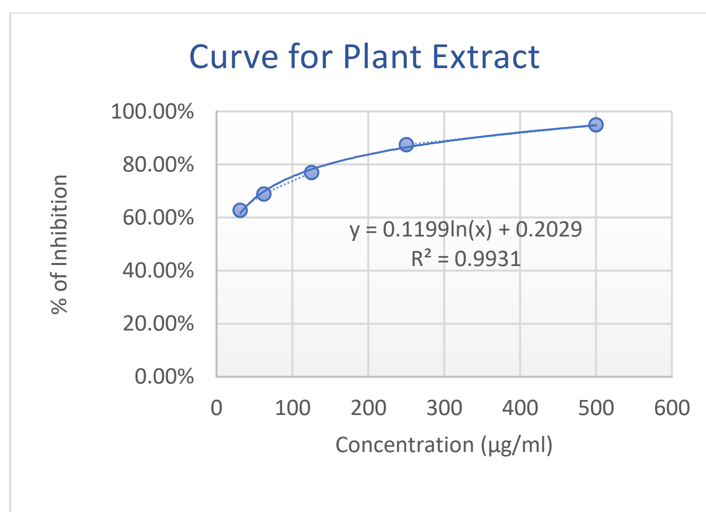

Figure 7: Graphical representation of Combination of extract

IC_{50} for ascorbic acid (standard):

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

Table 7: IC_{50} value for Ascorbic Acid

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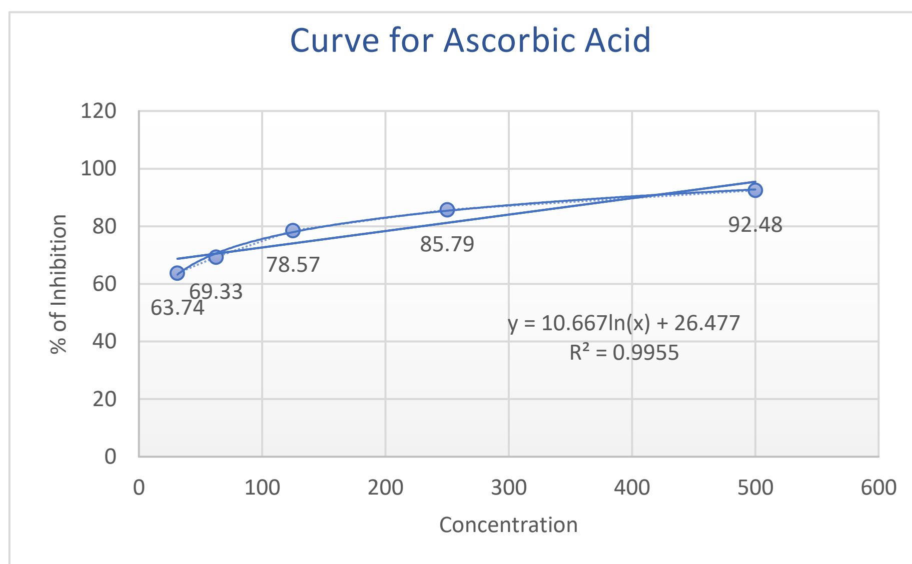

Figure 8: Graphical representation of inhibition and conc for ascorbic acid test

Discussion about Antioxidant Activity Test

The IC50 value of the antioxidant activity was determined using the DPPH method. The quantitative testing results for ascorbic acid's antioxidant activity and the methanol extracts of Cassia fistula and Cicer arietinum are shown in the above chart. In conclusion, we found that the solvent utilized during the extraction process may have an effect on these component contents in the extract. This study shows that, as opposed to solo activity, the combination of Cassia fistula and Cicer arietinum methanol extract has good antioxidant activity.

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No	Conc (µg/ ml)	Log C	% Mortality *Cassia fistula*	LC_{50} (µg/ml) *Cassia fistula*	% Mortality *Cicer arietinum*	LC_{50} (µg/ml) *Cicer arietinum*	% Mortality combination	LC_{50} (µg/ml)
1	**1000**	**3.00**	**100**	**0.986**	**45**	**3.16**	**70**	**2.23**
2	**500**	**2.70**	**90**		**30**		**50**	
3	**250**	**2.40**	**85**		**25**		**40**	
4	**125**	**2.10**	**77**		**10**		**40**	
5	**62.5**	**1.80**	**70**		**00**		**25**	

6.4 Brine Shrimp Test

Table 8: Effect of Methanolic Extract of *Cassia fistula* & *Cicer arietinum* on Shrimp Nauplii

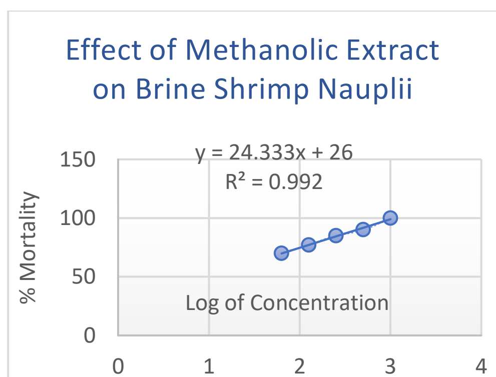

Figure 9: Graphical representation of *arietinum* on Shrimp Nauplii

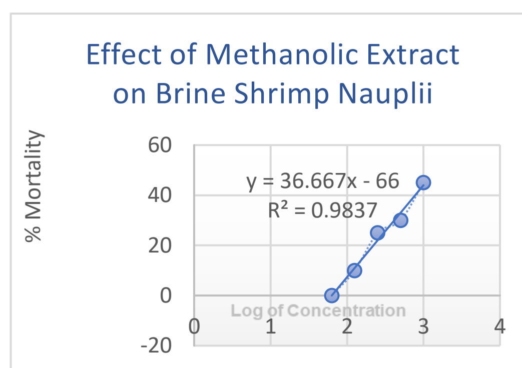

Figure 10: Graphical representation of ***Cicer*** *Cassia fistula* **on Shrimp Nauplii**

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No.	Conc. (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
1	20	1.3010	100	0.0464
2	10	1.00	100	
3	5	0.6989	90	
4	2.5	0.39794	80	
5	1.25	0.09691	60	
6	0.625	-0.2041	70	
7	0.3125	-0.5052	40	

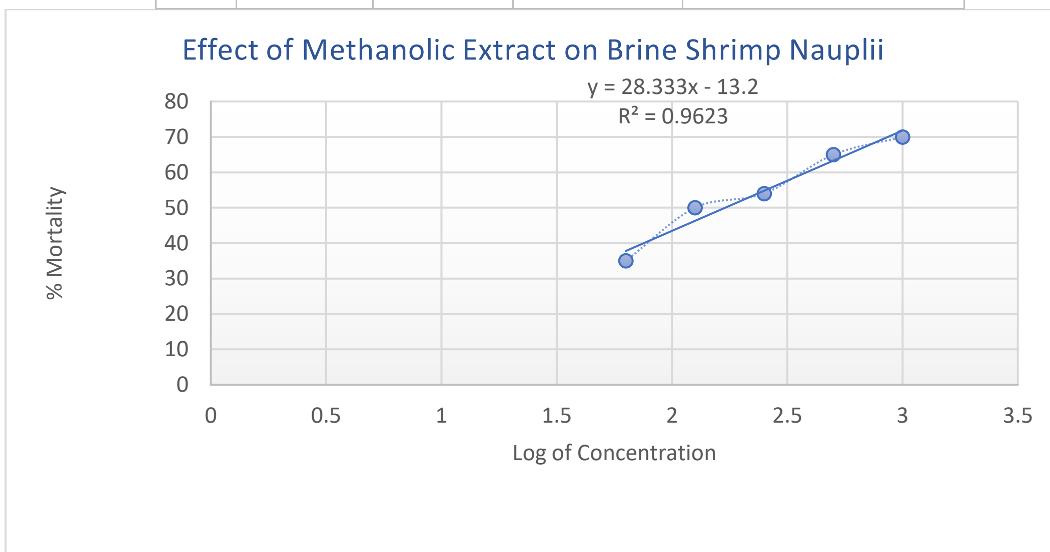

Figure 11: Graphical representation of combination **on Shrimp Nauplii**

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Table 9: Effect of Vincristine Sulfate on Brine Shrimp

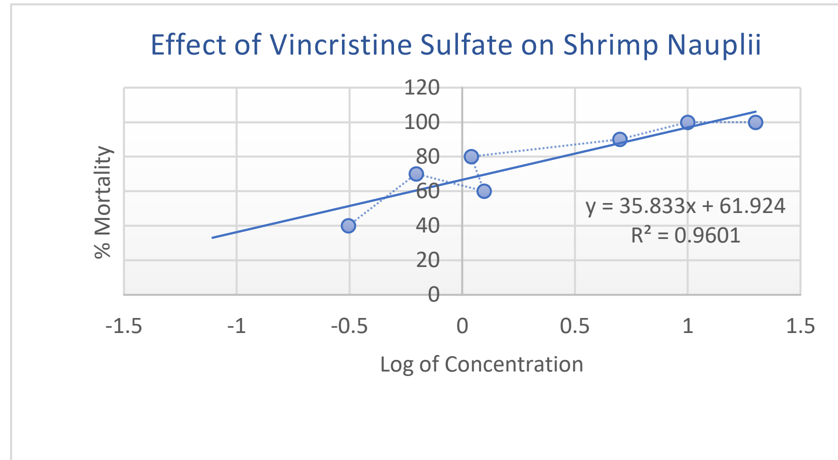

Figure 12: Graphical representation of Vincristine Sulfate on Brine Shrimp

Discussion: There is cytotoxic action in Cassia fistula methanolic extract. due to the fact that its LC50 value is nearly identical to that of vincristine sulfate. Yet, Cicer arietinum had no cytotoxic effects. The combined effect of these plants was diminished.

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CHAPTER SEVEN

CONCLUSION

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7.Conclusion

The investigation's findings demonstrated that the chickpea-based therapies had no discernible cytotoxic impact because of their extremely low efficacy (LC50, µg/ml) of 3.16 when compared to the conventional value. However, Cassia fistula has a strong cytotoxic and thrombolytic impact. The findings imply that these treatments may serve as prospective suppliers of natural medications for the management of a range of illnesses. To ascertain the precise mechanisms of action and possible adverse effects of these treatments, more research is necessary. All things considered, the study offers insightful information about the possible medical uses of chickpea treatments and Cassia fistula.

The phytochemical assessment showed the presence of alkaloids, carbohydrates, glycosides, steroids, phenol, tannin, diterpenes, gum, phytosterol, reducing sugar, and the absence of saponin and flavonoids, despite the fact that numerous research have sought to offer scientific proof for these medicinal plants.

Further research is recommended to better understand the mechanism underlying the observed activities and to identify and characterize the active phytochemical components responsible.

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CHAPTER EIGHT

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