# ‘‘Phytochemical Screening, Assessment of Thrombolytic and Antistress Activity of Methanol Extract of *Terminalia Arjuna* and *Oxalis Corniculata*”

A Dissertation Is Submitted to The Department of Pharmacy, Daffodil International University, as a Partial Fulfillment of the Requirements for the Degree of Bachelor of Pharmacy

PROJECT WORK


**Submitted by**

Khushruz Jahan Joli

Student ID: 203-29-1731

Batch: 24th

Department of pharmacy

Daffodil International University


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Submission Date

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# APPROVAL

‘‘Phytochemical Screening, Assessment of Thrombolytic and Antistress Activity of Methanol Extract of *Terminalia Arjuna* and *Oxalis Corniculata*” is the title of the project. This paper has been formally submitted to the Department of Pharmacy at Daffodil International University. This paper has been reviewed and affirmed as partial fulfillment of the bachelor of pharmacy degree requirements. The work depicted here is the outcome of my own investigation, and I have provided appropriate credit for the language, ideas, or writings that I have referenced.

## BOARD OF EXAMINERS

---

Project Supervisor

Md. A.K. Azad

Assistant Professor, Department of Pharmacy

Daffodil International University

**Board of Chairman**

Prof. Dr..Muniruddin Ahmed

Head, Department of Pharmacy

Daffodil International University.

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----------Internal Examiner -I

----------Internal Examiner - II

----------External Examiner

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# DECLARATION

‘‘Phytochemical Screening, Assessment of Thrombolytic and Antistress Activity of Methanol Extract of *Terminalia Arjuna* and *Oxalis Corniculata*” is the title of the project. This work has been submitted as halfway fulfillment of the requirements for the Bachelor of Pharmacy program at the Daffodil International University, Faculty of Health & life Sciences. It has been authorized in terms of its academic style and scientific content.

**Supervised By**

---

Md. A. K. Azad

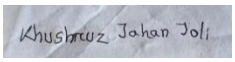
Assistant Professor

Faculty of Allied Health Science

Department of Pharmacy

Daffodil International University

**Submitted By**



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Khushruz Jahan Joli

Student ID: 203- 29- 1731

24th batch

Department of pharmacy

Daffodil International University

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# ACKNOWLEDGMENT

I am profoundly appreciative of Allah, the Almighty, for providing me with the fortitude, tolerance, and perseverance necessary to successfully complete this research. Throughout this voyage, my motivation has been derived from the boundless bounties of God.

I am deeply grateful to my parents, due to their unwavering moral support, boundless love, and the innumerable sacrifices they made in order to ensure my success. Their motivation has consistently served as an inspiration to me. May Allah abundantly recompense each of these individuals for their contributions.

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The Author,

Khushruz Jahan Joli

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***DEDICATION***

*I humbly dedicate this work, to my beloved family, whose boundless love have been my constant source of motivation and my all well-wishers, whose companionship have been invaluable along the way.*

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# ABSTRACT

The present study provides a comprehensive overview of the investigation into the potential pharmacological properties of *Terminalia Arjuna* Bark and *Oxalis Corniculata* and their combine extract. this research investigates the traditional applications and phytochemical constituents of the substance, with an emphasis on its potential for the treatment of stress-related disorders and thrombus disintegration. Through experimental methodologies including plant collection, extraction, and biochemical analysis, the study evaluates the thrombolytic activity and effects on cortisol levels, blood glucose, and C-reactive protein (CRP) levels in animal models. Results indicate that Arjun tree bark extract and combine extract both exhibits reducing serum cortisol, blood glucose and CRP levels, thereby indicating anti-stress properties and have mild thrombolytic activity, Amrul extract shows mild thrombolytic activity and have little bit effects on changing cortisol and CRP levels. The lysis results for the control and standard were 7.2% and 76%, respectively, as the investigation revealed. The three samples contained 29.1%, 27.5%, and 33.5%. Furthermore, the serum cortisol levels of the standard and herbal groups were 2.94 ± 0.4, 3.01 ± 0.25, 3.36 ± 0.68, and 2.14 ± 0.22 ug/dl, respectively, while the control group's level was 3.79 ± 0.56 ug/dl. The investigation demonstrated that the following values were obtained for the control and standard: glucose and CRP, respectively: 4.17 ± 0.71 and 6.39 ± 0.59, and 3.54 ± 0.31 and 3.85 ± 0.95. CRP 1.91 ± 0.59, 3.32 ± 0.36, 2.73 ± 0.95, and glucose 4.55 ± 0.88, 5.76 ± 1.69, and 2.11 ± 0.42 were detected in the sample. The results suggest that the combine sample have anti-stress properties, as they reduce serum cortisol and CRP levels. Furthermore, the arjun bark and amrul extract exhibits the potential to regulate Serum cortisol and CRP levels, which serves to emphasize its therapeutic advantages. The effects of anti-stress and its related activities were investigated through the examination of serum cortisol levels, cortisol-induced C-reactive protein (CRP), and random blood sugar (RBS) levels in a design analysis that was divided into a single section.

**Keywords:** Bark extract, Cortisol levels, Blood glucose, CRP, Cardiovascular disorders, Stress-related disorders

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# CHAPTER- 01

# INTRODUCTION



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## 1.1.1 General introduction

There has been an unbreakable bond between humans and vegetation. Medicinal plants have been instrumental in the welfare of humanity since the earliest phases of civilization, and their use to alleviate human suffering is as ancient as civilization itself. While healthcare has been revolutionized by advancements in science, technology, and medical practices. For their fundamental healthcare requirements, individuals worldwide continue to depend on herbal medicine. Incredibly, the majority of prescribed medications in developed nations are derived from plant sources, and the properties of the compounds are altered to ensure that they are culturally acceptable, compatible with the human body, and have minimal adverse effects. [1-9]

A vital source of medication for the treatment and prevention of a variety of diseases, plants are essential to global health. The World Health Organization predicts that botanical medications are taken by more than 75% of the global population as part of their routine primary healthcare. There has been a resurgence of interest in plant-derived compounds in recent years, as a result of their extensive applications, and the belief in the therapeutic properties of plants is immutable. [ 3-6]

For an extended period, medicinal plants have been a source of biologically active compounds that have helped alleviate human maladies, serving as intermediates for pharmaceuticals, nutraceuticals, and traditional medicine. Significant attention has been paid to phytochemicals due to their therapeutic and preventative capabilities. In ancient medical systems, medicinal plants were not only responsible for the vast array of colors, fragrances, and aromas in nature, but they also provided remedies. It is the bioactive compounds that are present in these substances that are responsible for their therapeutic potential, as they induce physiological responses in humans. Plants remain at the vanguard of drug discovery as the world continues to investigate nature in search of new therapeutics. [ 11-15]

## 1.1.2 Status of medicinal plants in Bangladesh

Bangladesh, being a sub-tropical country, boasts an abundant reserve of naturally occurring medicinal plants. The botanical medicine companies in Bangladesh obtained 80 percent of their plant requirements from the country's forests during the early 1980s, with the remaining 20 percent



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sourced from imports. However, the situation has since changed, with imports now satisfying 80% of the local demand, while only 20% is supplied by locally cultivated medicinal plants. The Bangladesh Agricultural Research Institute (BARI) reports that the country has 722 medicinal plant varieties, which is a substantially lower number than the 4,000 species found in India, its neighbor. Producers of Ayurvedic and Unani remedies employ 255 of the 700 plants that are utilized for medicinal purposes in Bangladesh. [7,14,16]

### 1.1.3 Importance of medicinal plants

Drugs are abundantly available from medicinal plants, which are widely acknowledged for their bioactive properties. Traditional medicine is a fundamental component of the healthcare systems of numerous developing countries. These plants are regarded as a valuable natural resource, as they are essential for the production of traditional and modern remedies and provide essential healthcare. [10-18]

Pharmaceuticals, cosmetics, agriculture, and food industries have all benefited from the use of medicinal plants. The curative properties of plants were discovered to be the result of the active compounds they contain as medical research progressed. Medicinal plants continue to be essential in the healthcare sector, providing natural and sustainable solutions for a diverse array of health issues. They provide affordable healthcare options, contribute to sustainable development, and conserve biodiversity, particularly in underserved regions. The significance of medicinal plants in addressing global health challenges is underscored by ongoing research into their potential in drug discovery and development. [10-18]

### 1.1.4 Phytochemicals

Plants contain naturally occurring substances known as phytochemicals, which are responsible for a variety of biological processes that bolster the plant's defensive measures. Although these substances—including flavonoids, alkaloids, terpenoids, saponins, tannins, and glycosides—are not considered essential for human nutrition, they have been demonstrated to provide some



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prospective health benefits. phytochemicals exhibit a variety of medicinal properties, including antimicrobial, anti-inflammatory, anticancer, and cardioprotective effects. Due to their bioactive properties, phytochemicals are indispensable to conventional medicine and constitute a significant area of investigation in modern pharmacology, facilitating the development of novel medications and treatments. Secondary metabolism in plants provides the production of phytochemicals. [3,10,11,18-23]

### 1.1.5 Thrombolytic activity

"Thrombolytic activity" refers to the ability of a substance to disintegrate or fragment thrombi, or blood clots, within the vascular system. The fibrinolytic system of the body is activated by thrombolytic medications, which convert plasminogen to plasmin, an enzyme, and subsequently degrade fibrin, the primary protein component of blood clotting. By assisting in the restoration of normal blood flow to arteries and vessels that have been obstructed by blockages, this procedure mitigates the risk of severe adverse effects, including myocardial infarction (heart attack), stroke, and pulmonary embolism. Diseases that involve the formation of emboli are frequently treated with thrombolytic agents, including streptokinase, alteplase, and urokinase. Recent attention has been focused on natural products, particularly plant extracts, due to their bioactive components, which have the potential to induce thrombolytic effects. The assessment of thrombolytic activity can be used to identify novel therapeutic possibilities with lower potential adverse effects for the prevention and treatment of clot-related maladies when comparing plant extracts to synthetic medicines. [51-56]. A substance's thrombolytic activity is frequently evaluated using in-vitro assays, such as the thrombus lysis assay. To achieve this, the degree of thrombus dissolution is compared to that of recognized thrombolytic medications. The assessment of thrombolytic activity may be used for identifying possibilities of novel therapeutics with lower potential adverse effects for the prevention and treatment of clot-related maladies when comparing plant extracts to synthetic medicines. A substance's thrombolytic activity is frequently evaluated using in-vitro assays, such as the thrombus lysis assay. To achieve this, the degree of thrombus dissolution is compared to that of recognized thrombolytic medications. [ 20-34]



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### 1.1.6 Coagulation and Thrombus Formation:

The coagulation system is necessary for the formation of a stable clot, despite the fact that platelet plugs are essential for initial hemostasis. A series of coagulation factors are involved in the complex cascade of enzymatic reactions that results in the conversion of soluble fibrinogen into insoluble fibrin. This process is known as coagulation. The platelet blockage is reinforced by fibrin filaments, which form a lattice that prevents additional blood loss by forming a stable thrombus. The coagulation cascade can be initiated through either intrinsic or extrinsic pathways, which ultimately converge to produce a shared pathway that initiates fibrin formation. Sixteen Nevertheless, the hemostatic system is essential for preventing excessive hemorrhage; however, dysregulation can result in substantial health complications. Bleeding disorders, including thrombocytopenia and von Willebrand disease, can be the consequence of an insufficient platelet count or impaired platelet function. In contrast, thrombotic events, such as arterial thrombosis and venous thromboembolism, can result from excessive platelet activation or coagulation. These conditions have the potential to result in life-threatening complications, including stroke, myocardial infarction, and pulmonary embolism. [31-,35,57- 60]

### 1.1.7 Antistress activity

Stress is an ever-present aspect of our daily lives; however, it is crucial to regulate it in order to preserve both our mental and physical health and relaxation. In order to alleviate tension, certain activities are beneficial, including: Deep breathing, physical exercise, spending time in nature, meditation and mindfulness, laughter, and humor are all beneficial. Deep breathing or box breathing is a technique that involves the utilization of controlled breathing patterns to alleviate tension and elevate pulse rate. Focusing on the respiration induces a state of relaxation in the nervous system. Specific neurotransmitters and receptors in the brain are targeted by anti-stress drugs, which are also referred to as anxiolytics or stress-relieving medications. This approach is intended to alleviate anxiety, tension, and the physiological responses that are associated with stress. [15,16,30-38]



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### 1.1.8 Stress in cardiovascular disease

The stress phenomena are illustrated and the effects of stress on the circulatory system are examined [13]. It is specifically addressed the pathophysiological function of stress in coronary artery disease, hypertension, atherosclerosis, and myocardial infarction. Stress has a substantial impact on a variety of physiological processes that are associated with the immune system [14]. It may have either beneficial or detrimental effects, as a result. Stress is frequently associated with adverse consequences in the context of cardiovascular disorders [15], however. There are numerous variables that contribute to the precise outcome, such as the duration of the stress, its severity, the patient's genetic composition and history, and its genetic components. All of these factors can influence the organ name hypothalamic pituitary adrenal axis and the system of sympathoadrenal medullary, which are the two primary components of the stress response [16]. Circulatory system is substantially affected by stress. The development, progression, and resolution of cardiovascular maladies are significantly influenced by it. Subjective or individual differences must also be taken into account [17]. In certain situations, stress, particularly "adequate" acute stress—that is, stress that is not "overwhelming—may improve performance and be advantageous [18]. It is possible that the intimate relationship between stress and cardiovascular ailments is a significant advancement in modern medicine. Implementing novel treatment strategies is essential [19,49,50].

### 1.1.9 Effect of Stress on Cortisol Level

The outer cortex of the suprarenal gland produces the steroid-shaped hormone cortisol, which influences glucocorticoids. It is predominantly passively permeated from the capillary arteries to the cells for its fat-soluble charecter and modest size [20]. It is present in body fluids, including cerebrospinal fluid, hair, saliva, urine, and perspiration. Cortisol undergoes a circadian oscillation cycle. The highest levels of blood cortisol are observed in the early morning, while the lowest levels are observed around midnight. In the blood, the maximum amount of cortisol, is firmly bound to a protein, name corticosteroid binding protein (CBG), which is frequently referred to as transcortin. Only a small quantity of cortisol is transmitted as a bound to albumin. Research has demonstrated that free cortisol constitutes only 3–10% of the total cortisol in the bloodstream



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[21,22,23]. The sublingual cortisol are correlated with the blood free cortisol. The cortisol of salivary is a critical criteria in psycho neuroendocrinological observing due to its noninvasive nature, lack of discomfort, and ease of self-administration. Cortisol levels were demonstrated to increase by nearly nine times in stressful situations compared to tranquil ones. Some physical responses to stress include paralysis in the fingertips and toes, muscular spasms, myotonia, cramping, and trembling. The HPA axis is essential for the coordinated physiologic response to stress, which includes the inflammatory and pain states of numerous diseases and "stress-related syndromes" such as chronic migraines, dysmenorrhea, temporomandibular disorder, and CFS [24].



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# CHAPTER-02

# PLANT PREVIEW



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## 2.1.1 Plant Introduction

*Terminalia Arjuna*, genus Terminalia, is a majestic deciduous tree native to the Indian subcontinent. It's renowned for its medicinal properties, particularly for heart health. Arjuna trees are large, often reaching heights of 20-25 meters. They have a wide, spreading crown and drooping branches. The bark is thick, smooth, and often grey or pinkish-green. The leaves are oblong or conical, green on the top and brown on the bottom. Arjuna trees produce small, pale yellow flowers in clusters, fruit is a fibrous, woody drupe with five wings. [4,9-17]

*Oxalis corniculata*, is a small, herbaceous perennial plant found in various parts of the world. It's a versatile plant that can be found in gardens, lawns, and even as a weed. Creeping Wood Sorrel is a low-growing plant with delicate, clover-like leaves that consist of three heart-shaped leaflets, have a sour taste due to the presence of oxalic acid, are typically green, but they can sometimes be reddish or purple. The plant produces small, yellow flowers that are often solitary but can sometimes appear in clusters. [5,14,18,19,20]

## 2.1.2 Scientific classification

### 2.1.2.1 Scientific classification of *Terminalia Arjuna*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Myrtales

Family: Combretaceae

Genus: Terminalia

Species: *T. arjuna*

Fig-01: Arjun tree



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### 2.1.2.2 Scientific classification of *Oxalis Corniculata*

Kingdom : Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order : Oxalidales

Family : Oxalidaceae

Genus : Oxalis

Species : *O.corniculata*

Fig-02: Amrul tree

## 2.1.3 Common vernacular Names

### 2.1.3.1 Common vernacular Names of *Terminalia Arjuna*

- ✓ English: Arjuna, Arjun Tree
- ✓ Hindi: Arjun, Arjuna
- ✓ Sanskrit: Arjuna, Dhanvi, Kakubha, Nadisarja
- ✓ Bengali: Arjun
- ✓ Tamil: Maruthu, Neer Maruthu
- ✓ Telugu: Thellamaddi
- ✓ Kannada: Holematti, Maddi
- ✓ Malayalam: Neermaruthu
- ✓ Marathi: Sadura, Arjuna
- ✓ Gujarati: Arjun Sadada
- ✓ Oriya: Arjuna

### 2.1.3.1 Common vernacular Names of *Oxalis Corniculata*

- ✓ English: Creeping Wood Sorrel, Indian Sorrel, Sleeping Beauty
- ✓ Hindi: Tinpatiya, Amrul
- ✓ Bengali: Amrul, Khatti Buti



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- ✓ Tamil: Puliyarai, Pulichai Keerai
- ✓ Telugu: Areli, Puli Koora
- ✓ Kannada: Hullikivige, Pucchappala
- ✓ Malayalam: Puliyarila
- ✓ Marathi: Ambat chuka
- ✓ Gujarati: Khatibutti
- ✓ Punjabi: Khatti Booti

### 2.1.4 Habitat and Distribution

The arjuna, a member of the Combretaceae family, is well-suited to humid environments and is widespread throughout the Indian Subcontinent. It has also been established in Malaysia, Indonesia, and Kenya. It is able to access a plethora of water resources and nutrient-rich soil in its natural habitat, which includes ravines, streams, a river banks, thoroughout the world. [7-9]

*Oxalis corniculata*, a vegetation species that is adaptable and can be found in a diversity in ecosystems, highly distributed in the tropical and the temperate regions worldwide. This herb is present in various habitats, including forests, meadows, farmland. Also, found in regions with moderate temperatures and ample precipitation, frequently forming dense colonies in disturbed or nutrient-rich environments. It is an herbaceous plant that is low-growing and somewhat delicate-appearing. It is widely distributed in moist, shaded areas, roadsides, plantations, and lawns, and is found in nearly all regions of the globe. [6-9]



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### 2.1.5 Phytochemistry and Pharmacological Potential of *Terminalia Arjuna* bark

Arjuna bark contains a variety of plant-based compounds, such as,

| ***Compounds Root*** | ***Stem or bark*** | ***Activity of compound*** |
|---|---|---|
| Triterpenoids | Arjunin, arjunolic acid, arjungenin | Antifungal, cardioprotective |
| Flavonoids | Arjunolone, Arjunone, Bicalein, Luteolin, Gallic acid, Ethyl gallate Kempferol, Proanthocyanidins, Quercetin, Pelorgonidin, | Antiallergic, antibacterial, cytotoxic, antiasthmatic, antifungal, antioxidant |
| Tannins | Pyrocatechols, Casuarinin, Casurin, Punicallin, Punicalagin, Castalagin, Terchebulin, Terflavin C | Astringent, wound healing and antimicrobial |
| Glycosides | Arjunetin, Arjunaphthanoloside, Arjunoside I, II and Terminoside-A | Cardioprotective |
| Sitosterol | Sitosterol | Antimutagenic, antiinflammatory, antitussive |
| Trace elements | Calcium, Aluminium, Magnesium, Silica, Zinc, Copper | To fill up ion requirement |

Table No-01: Phytochemistry and Pharmacological Potential of Arjun



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### 2.1.6 Phytochemistry and Pharmacological Potential of *Oxalis corniculata*

Tannins, palmitic acid, and a mixture of 8 oleic, linoleic, linolenic, and stearic acids have been uncovered through phytochemical investigations. The methanolic and ethanolic extricates of this plant exhibit the presence of carbohydrate, glycosides, phytosterols, phenolic compounds, flavonoids, proteins (12.5%), amino acids, and volatile oil. Additionally, the presence of calcium, fiber, and tannin was observed. Tartaric acid and citric acid, calcium oxalate, flavones, glycoflavones, flavonols, and phenolic acids, including phydroxybenzoic, vanillic, and syringic acids, are among the leaves' components. The leaves and stems of this herb are famous for their acidic flavor, which is a result of the high concentration of oxalate.
The plant's pharmacological activities include anthelmintic, anti-inflammatory (in-vitro anti-inflammatory activity was assessed using albumin denaturation), anti-diabetic, anti-cancer, analgesic, antioxidant, antifungal, antimicrobial, astringent, depurative, diuretic, emmenagogue, febrifuge, relaxant, lithontripic, stomachic, and styptic action. Various activities were investigated using varying dose levels, such as a 300-500 mg per kg p.o. dose that exhibited significant wound healing activity. Anxiolytic activity (ethanolic extract 100 and 300 mg/kg P.O.), Anti-epileptic activity (methanolic extract of leaves at doses of 200 and 400 mg/kg body weight), Anti-ulcer activity (ethanolic extract leaves at doses of 200 and 400 mg/kg body weight), and Anti-diarrhoeal activity (at doses of p.o. 160, 320, and 640 mg/kg body weight). [50- 60]

### 2.1.7 Uses

The traditional Ayurvedic herb Arjun is believed to have the potential to enhance skin conditions, manage diabetes, and promote cardiac health. Bark is recognized in Ayurveda for its cardio-protective properties, which are frequently incorporated into formulations to alleviate angina-related symptoms, strengthen the cardiac musculature, and alleviate chest pain. A medicinal decoction is produced by boiling the bark in water. Powdered bark is ingested with water or milk, or as a beverage, to promote cardiac health. Ayurvedic formulations, such as Arjunarishta, are fermented and utilized as a cardiac tonic. [3-16]

The traditional remedy Amrul Shak is renowned for its digestive benefits, as well as its capacity



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to alleviate constipation, diarrhea, and fever. This herb also possesses diuretic, anti-inflammatory, antibacterial properties. Amrul Shak is employed to treat influenza, urinary tract infections, enteritis, traumatic injuries, sprains, and poisonous snake bites, and as an antiscorbutic to treat scurvy. The leaves of Amrul are highly edible, possessing a zesty lemon flavor. They are employed as an antidote to toxicity induced by the seeds of Datura, arsenic, and mercury. The leaf juice is applied to insect bites, burns, and skin eruptions. It can be prepared by infusing the leaves in hot water for approximately 10 minutes, sweetening, and then chilling. The infusion can be used as a wash to cleanse children of hookworms, as a gargle to remove opacities of the cornea, and as a drop in the eyes to alleviate itching lids. In numerous locations, *Oxalis corniculata* is also cultivable as an ornamental plant in gardens. [11-20]



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# CHAPTER-03

# LITERATURE REVIEW



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### 3.1.1 To investigate the analgesic and antipyretic properties of an ethanol extract from *Oxalis corniculata.*

Analgesic activity was evaluated using the acetic acid-induced writhing test in Swiss albino mice (weighing 25-30g), while the antipyretic potential was assessed through Brewer's yeast-induced pyrexia in rats. The *Oxalis corniculata* extract was administered orally at two dose levels, 200 mg/kg and 400 mg/kg, respectively. For the antipyretic study, animals were divided into four groups after ensuring consistent baseline rectal temperatures for 7 consecutive days. Pyrexia was induced by a subcutaneous injection of 10 mL/kg of a 15% w/v Brewer's yeast suspension in 0.09% saline solution, administered beneath the nape of the neck. The initial rectal temperature of each rat was measured prior to yeast injection, followed by treatment with either the vehicle, *Oxalis corniculata* extract (at 200 and 400 mg/kg), or the standard drug paracetamol (150 mg/kg). Subsequent rectal temperatures were recorded at hourly intervals over a 4-hour period to assess the reduction in fever. To do analgesic study, all mice were distributed into five randomized group, including a control group that received 1% v/v CMC as the vehicle and a standard group treated with Aspirin (100 mg/kg). The acetic acid-induced writhing test was used to evaluate pain-relieving effects. Each treatment group was observed for the frequency of writhing movements after the administration of the test substance, which was then compared to the control and standard groups. [30-33]

### 3.1.2 Antimicrobial Activity investigate of *Oxalis corniculata*

The Agar Well Diffusion method was employed to evaluate the antimicrobial efficacy of a variety of plant extracts. Various test microorganisms were utilized for this antimicrobial screening, including common pathogenic Gram-positive bacteria like Staphylococcus aureus and Streptococcus species, as well as fungal isolates like Aspergillus niger and Aspergillus flavus. Bacterial cultures were propagated and maintained in Nutrient Broth medium at 37°C for a 24-hour period, while fungal cultures were grown on Potato Dextrose Agar slants and incubated at 27°C for 48 hours.The assay was performed by uniformly distributing fresh microbial cultures (0.1 mL) across nutrient agar plates using a sterile glass spreader. Subsequently, a sterile cork borer was employed to meticulously puncture wells with a diameter of 6 mm into the agar medium.



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These wells were filled with 50 µg of petroleum ether extracts at varying concentrations using a micropipette, all of which were performed under aseptic conditions. To facilitate the pre-diffusion of the plant extracts into the agar, the prepared dishes were subsequently refrigerated for 30 minutes. After this, the dishes were transferred to an incubator that was set at 37°C and allowed to incubate for 24 hours. The antimicrobial potency of the extracts was assessed by measuring the zone of inhibition that formed around each well, which indicates the extracts' efficacy against the test pathogens. The antimicrobial activity of the plant extracts was indicated by this zone of inhibition. [31,32]

### 3.1.3 Antidiabetic effect of *T. arjuna* bark extract by using diabetic rats

- **Hypoglycemic effects:** The study demonstrated that the bark extract of *Terminalia Arjuna* significantly reduced blood glucose levels in rats with alloxan-induced diabetes.
- **Pancreatic protection:** The extract protected pancreatic beta cells from destruction, which is crucial for insulin production.

The objective of the present investigation was to examine the impact of *Terminalia Arjuna* root extract on blood glucose levels in both normal and diabetic rodents. A thorough physicochemical analysis was performed on the acetone extract of Terminalia arjuna roots. Over a 15-day period, the extract was administered to diabetic rodents, resulting in substantial decreases in their elevated total cholesterol (TC) and triglyceride (TG) levels in comparison to the negative control group. The results indicated that the administration of the extract in conjunction with the standard antidiabetic drug glibenclamide reduced severe body weight loss in diabetic animals. This protective effect was observed when the treatment was administered orally on a daily basis for a period of 15 days. In alloxan-induced diabetic rodents, the evaluation demonstrated that its root extract exhibited potent glucose-lowering effects. The findings indicate that *Terminalia Arjuna* root extract has the potential to be an efficacious natural remedy for the management of diabetes, as it effectively reduces hyperglycemia and prevents excessive weight loss in diabetic subjects.[32]



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### 3.1.4 Evaluation of thrombolytic activity of *Terminalia Arjuna* bark extract in rats

- **Thrombolytic effects:** The study demonstrated that the bark extract of *Terminalia Arjuna* significantly reduced clot lysis time in rats, indicating a thrombolytic effect.
- **Mechanism of action:** The authors suggested that the thrombolytic activity might be due to the presence of fibrinolytic enzymes in the extract.
- **Comparison with standard:** The extract's thrombolytic activity was compared to a standard thrombolytic agent, streptokinase, and found to be comparable.

The research findings suggest that *Terminalia Arjuna* bark extract possesses thrombolytic properties. This could be beneficial for conditions associated with blood clot formation, such as stroke and heart attack. However, further research is needed to confirm these findings in humans and to elucidate the precise mechanisms of action. [33]



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# CHAPTER-04

# THE STUDY OBJECTIVE



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### 4.1.1 Purposes of the study

The objective of this study was to examine the potential actions of the plants *Terminalia Arjuna* bark and *Oxalis Corniculata* through the examination of their phytochemicals and specific pharmacological analyses. Use of this plant is justified by a variety of factors, such as:

- Chemical component profiling: This investigation will evaluate a diverse array of phytochemical activities, for ensuring the presence or absence of various Phytochemicals.
- The main objective of my current research was to examine the combined activity of the *Terminalia Arjuna* bark and *Oxalis Corniculata* plant extracts. The following Specific activities are-

1. Thrombolytic Activity
2. Antistress Activity



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# CHAPTER-05

# MATERIALS AND METHOD



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### 5.1.1 Plant Collection & Identification

The parts of experimental plants *Terminalia* (bark) *Arjuna* and *Oxalis Corniculata* (stems, roots, leaves) collected from the regional area of Chattogram. Those plants were collected in June 2024 at the day time. During plants part collection of the barks of Arjun and leaves, roots, stems, any chance of chemical degradation, hydrolysis, adulteration were avoided.

### 5.1.2 Preparation of plant for the test

- Collect plant and clean by fresh water
- Undesirable material from the plant parts were removed
- Then dried under sun two days and air-dried for 10-15 days by fan, were cutting into small pieces.
- After well dried than crushed the plant into fine powders by blender.
- 409g bark & 371g herbs powder were dissolved in 2.5L methanol in an amber glass bottle.
- kept for a period of 13 days accompanying occasional shaking and stirring.
- coarse parts of the fruits were separated from the mixture by cotton filtration method (7 times). Then the liquid portion was also filtered through whatman filter paper.
- the filtrates were kept in Rotary evaporator machine which separates solvent and desirable crude extracts was obtained, remaining small portion methanol dried by using hair dryer.



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### 5.1.3 Cotton Filtration, Rotary Evaporation, Drying

The plants extract, filtered by using sterilized cotton and filter paper. First of all powders were immersed in the appropriate solvent, then placed in a funnel, following the methanolic extraction procedure. The filtrate was collected during a glass instrumentality. The mélange was subsequently filtered through a clean, white cotton piece for primary filtration. It was once more filtered through Whatman filter paper. The filtrates were taken in a glass container. The liquid extracts were evaporated at a rotational speed of 30 rpm and temperatures ranging from 64 to 65 degrees Celsius using a rotary evaporator. The condensed sample was transferred to a 100 ml beaker. The beakers were covered in aluminum foil and the purified extract was collected and deposited immediately beneath the fan. The residual solvent in the extract was evaporated, and a hair dryer was employed for removing unnecessary water in the extract. The non-polar desiccated extract is stored in the refrigerator for future use.

### 5.1.4 Phytochemical Screening for identifying different chemical groups

Phytochemical screening of for both plants was carried out to identify the functional groups as described.

**1. Test for the Alkaloids**

- ✓ Mayer's test

A test tube, 2 ml extract solution and 0.2 ml of diluted hydrochloric acid were added. Mayer's reagent ( called Potassium Mercuric Iodide) 1 ml was added. Yellow color precipitate will show indicates presence of alkaloids.

Fig-03: Different Color in Phytochemical Screening



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- ✓ Dragendroff’s Test

In a test tube, 2 ml of the extract solution and 0.2 ml of diluted hydrochloric acid were added. Next, 1 milliliter of the Potassium Bismuth Iodide Solution, or

Dragendroff's reagent, was added. Alkaloids are present when an

orange-brown precipitate forms.

- ✓ Wagner’s Test

1 ml Wagner's reagent (KI) and 2 ml extract solution. The presence of alkaloids is indicated by the formation of brown or reddish precipitate.

**2. Test for Flavonoids**

1 ml extract was mixed with a few drops of strong HCl. Immediate production of red color indicates presence of flavonoids

**3. Test for tannins**

0.5ml extract and 5ml of distilled water were taken in a test tube , then 1% ferric chloride was added. Deep green indicates the presence of tannin.

**4. Test for Saponins**

20 ml distilled water solution was diluted with 1 ml of extract solution. After that, give it a good shaken 15 minutes, clear foam will form, signifying saponin is present.

**5. Test for Steroids**

1 ml aqueous solution was dissolved in 2 ml chloroform. Concentrated sulfuric acid was carefully added to the test tube to form a lower layer. A reddish brown color at the interface ensure the presence of a steroid ring.



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**6. Test for terpenoids**

The test tube was filled with 0.5ml of extract, which was then combined with 2ml of chloroform. 3 ml concentrated sulfuric acid was added. The reddish brown tint near the contact shows the presence of terpenoids.

**7. Test for Cardiac Glycosides**

0.5ml extract was treated with 0.2ml of glacial acetic acid before adding 3.5% ferric chloride dropwise to the solution, add 1mL of concentrated sulfuric acid. A reddish brown ring that has formed, shows the presence of cardiac glycosides.

**8. Test for Reducing Sugar**

a) Benedict's test

0.5 ml aqueous solution of the plant extract was taken in a test tube. 5ml Benedict's solution was added and boiled for 5 minutes. Then it was allowed for cooling.

b) Fehling's test

2ml of the aqueous extract of the plant material was taken in a test tube and 1ml of a mixture of equal volume of Fehling's solution A [Copper (II) sulfate solution (blue)] and B [Alkaline sodium potassium tartrate solution (clear)] was added.

Results Interpretation:

Blue: reducing sugar present not present (no color change).

Yellow: reducing sugars low amount

Brick-red precipitate: reducing sugars high amount.

**9. Test phenol**

2 ml extract and 3 or 4 drops of ferric chloride solution mixed. Bluish black color ensure the phenols are present.

**10. Test for Gums**



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5ml extract taken and molish reagent and sulphuric acid was added. Red violet ring produced at the junction of two liquid, indicates the presence of gum.

## 5.1.5 Thrombolytic Activity Test

### 5.1.5.1 Working Procedure

1. Dose preparation:

A 50 mg of each extract were dissolved individually in 5 ml of distilled water. For combine dose preparation 25mg taken from both extract and dissolved in 5ml. After that, all were placed in a vortex mixer and shaken for making better solution. After 24 hours, the soluble supernatant was decanted, it was filtered through a syringe filter paper. 100µl of this aqueous preparation was put into each micro centrifuge tube. The solution was ready for ex-vivo thrombolytic test.

2. Streptokinase Solution as Standard:

The standard solution was available commercially 1500000 I.U. lyophilized Streptokinase alpine tube Beacon Pharmaceutical Ltd. The streptokinase vial was then filled with 5ml water and thoroughly mixed. For in vitro thrombolysis, 100µl (30,000 I.U.) of this suspension was utilized.

3. Eppendorf Tube Preparation:

Five tubes were washed with distilled water, dried, and sterilized and weighed those tubes then labelled with permanent marker to distinguish the distinct tubes. Then 4 ml blood was withdrawn from healthy person. Then transferred 1ml of blood to each of the Eppendorf tube previously weighed sterile Eppendorf tubes to form clot.

4. The blood tubes, were incubated at 37 degrees Celsius for 45 minutes in order to facilitate the formation of clots.
5. The serum was taken out of the tube and weighed using the tube to take wight of the first clot. Weight ( first clot) = first clot weight (with tube) - blank tube weight
6. Apply the usual dose of 100µl (Streptokinase) and extract 100µl, while controlling with 100µl of distilled water and again done a 90-minute clot lysis incubation at 37°C.



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7. Separate the lysis blood fluid from each tube and measured the 2nd clot with tube weight.

   2nd clot weight = 2nd clot with tube weight – blank tube weight

8. At last, Lysis clot weight = weight of first clot – weight of second clot

9. Finally, clot of lysis = (lysis clot weight / clot weight before lysis) × 100

### 5.1.6 Experimental design for In-Vivo test

Swice albino mice (22-23g) were purchased from Jahangirnagar University, Dhaka, Bangladesh and their ages five to six weeks and were housed in animal's cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light: 12 hr dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University, Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

A temperature-controlled animal room was employed to house the colony cages of mice for four days before the experiment. The bedding was changed daily to maintain a clean environment.

The rats were split up into five groups, each with four rats, for the investigation. There were five groups:

- ✓ Control Group
- ✓ Standard Group
- ✓ Sample Group1 for Arjun extract
- ✓ Sample Group for Amrul extract
- ✓ Sample Group 3 for Combine extract

I took 0.108 mg/kg of Clonazepam (segment 1) Additionally, medication was obtained from Model Pharmacy with my supervisor's authorized signature.



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## 5.1.7 Experimental profile to access the In vivo effect of medicinal plant

**Segment 1: Anti-stress and related levels of CRP and RBS activity**

Heat Induce Method:

Fig-04: Styrofoam Box Design

i. A Styrofoam box with a hole at the top was made.

ii. A 100-watt incandescent lightbulb was removed from the pass-through hole in Blob.

iii. Await the temperature increase to 60°C.

iv. Place the mice inside the box and examine their movements for three minutes.

| Group | Sample of Medicine & Herbal | Duration |
|---|---|---|
| Control Group | (60 degree for 3 min) | |
| Standard Group | Heat + Clonazepam (0.108 mg/kg body weight) | |
| Sample Group 1 | Heat+ Arjun tree's bark extract (400mg/kg body weight) | 7 days |
| Sample Group 2 | Heat+ Amrul extract (400mg/kg body weight) | 7days |
| Sample Group 3 | Combine extract (200+200) mg/kg body weight) | 7days |

Table No-02: Experimental design for Anti stress Activity



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### 5.1.7.1 Blood Collection

After the experimental medications and group samples were effectively given for seven days, the blood was taken from mice. Heart punctures were employed for obtaining blood samples of 1-2 ml. As a result of the blood centrifugation, finally serum was extracted, preserved for research purposes.

### 5.1.7.2 Biochemical Test

There were three biochemical testing conducted as part of the investigation. blood glucose, cortisol, and CRP. Cortisol was quantified through the ELISA technique with PHOMO Autobio. Nepholometry technique is employed to measure CRP using the Getein 1100 machine. The Spectrometry method is employed to measure the glucose level using micro laboratory instruments.



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# CHAPTER-06

# RESULTS & DISCUSSION



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### 6.1.1 Result of phytochemical screening with discussion

| Phytochemical constituents | *Terminalia Arjuna* | *oxalis corniculata* |
|---|---|---|
| **Alkaloids** | + | + |
| **Flavonoids** | + | + |
| **Tannins** | + | + |
| **Saponin** | + | + |
| **Steroid** | + | + |
| **Terpenoids** | + | + |
| **Cardiac Glycosides** | + | + |
| **Reducing sugar** | + | + |
| **Phenol** | + | - |
| **Gum** | + | - |

Table-03: Results of phytochemical screening of methanolic extract

(+) indicate presence, (-) indicate absence



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## 6.1.2 Result of Thrombolytic activity with discussion

| **Solutions** | **Blank tube weight (gm)** | **1st clot + tube weight (gm)** | **1st clot weight (gm)** | **2nd clot + tube weight (gm)** | **2nd clot weight (gm)** | **Lysis weight (gm)** | **% Of lysis** |
|---|---|---|---|---|---|---|---|
| **Standard (Previously reported)** | 0.860 | 1.870 | 1.01 | 1.10 | 0.24 | 0.77 | 76% |
| **Control** | 0.750 | 1.937 | 1.187 | 1.63 | 1.0191 | 0.0851 | 7.2% |
| ***Terminalia Arjuna*** | 0.765 | 1.908 | 1.143 | 1.575 | 0.81 | 0.333 | 29.1% |
| ***Oxalis corniculata*** | 0.772 | 1.781 | 1.009 | 1.504 | 0.732 | 0.277 | 27.5% |
| **Combine extract** | 0.765 | 1.795 | 1.030 | 1.450 | 0.685 | 0.345 | 33.5% |

Table 04: Thrombolytic Activities on The Basis Of % Clot Lysis

Streptokinase used as the standard which was previously reported 76% clot lysis. The clot lysis rate in distilled water (Control) was 7.2%, methanolic bark extract of *Terminalia Arjuna* was 29.1%, methanolic extract of *Oxalis Corniculata* was 27.5% and Combined extract showed 33.5% clot lysis, in research.

The result of thrombolytic activity of methanolic extract of *Oxalis Corniculata* and methanolic bark extract of *Terminalia Arjuna* and combine extracts showed partially positive result when compared to standard (SK). According to my research, there all extract showed mild thrombolytic activity.



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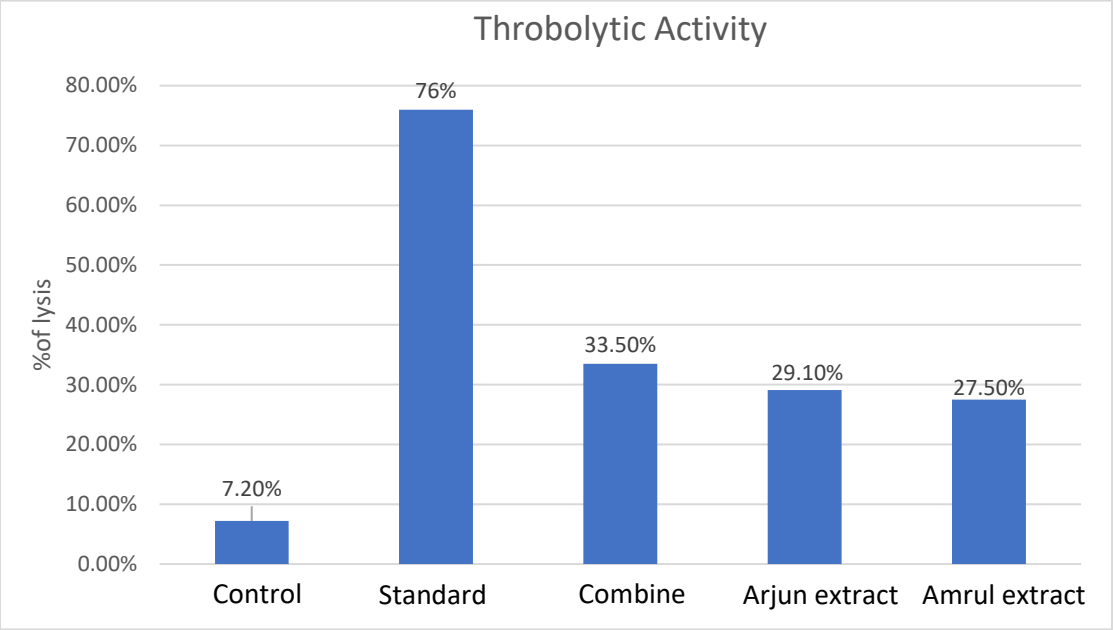


Figure 01: Graphical representation of Thrombolytic activity test.

### 6.1.3 Biochemical Test Result

#### 6.1.3.1 Control, Herbal sample, Standard drugs effects on serum cortisol

| Name of the group | S. Cortisol (µg/dl) |
|---|---|
| Control Group | 3.79 ± 0.56 |
| Standard Group | 2.94± 0.40 |
| Sample Group 1 | 3.01± 0.25 |
| Sample Group 2 | 3.36 ± 0.68 |
| Sample Group 3 | 2.143 ± 0.22 |

Table-05: Control, Standard drugs, plant sample effects on serum cortisol.

Data were expressed as mean Standard Error Mean (± SEM) {n= 3 for single group}



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## Discussion

Effects of various interventions on serum cortisol levels in three experimental groups—the Control Group, the Standard Group, and the Sample Group—are displayed in this table. Serum cortisol levels are quantified in micrograms per deciliter (µg/dl). The Control group is the baseline or control, representing the natural serum cortisol levels without any treatment. The control treatment entails the subjects being exposed to heat at 60 degrees Celsius for a duration of 3 minutes. The mean serum cortisol level in this group is 3.79 µg/dl, with a standard error of the mean (SEM) of 0.56. A standard treatment is administered to the Standard group for the purpose of comparison. In addition to the heat exposure experienced by the control group, the subjects in this group are administered Clonazepam at a dosage of 0.108 mg/kg body weight. Clonazepam is a conventional medication that is recognized for its ability to influence cortisol levels. The mean serum cortisol level in this group is 2.94 µg/dl, with a standard error of 0.40, which is lower than that of the control group. The experimental treatment is administered to the Sample group, which corresponds to the control group's exposure to heat. In addition, the subjects are administered extracts at a dosage of 400 mg/kg body weight. The mean serum cortisol level in sample group 1 is 3.01 µg/dl, with a standard error of 0.25, and is lower than that of the control group but higher than that of the standard groups. The cortisol level in Sample Group 2 is lower than that of the control group but higher than that of the standard groups, with a standard error of 0.68 and a value of 3.36 µg/dl. The mean serum cortisol level in sample group 3 is lower than that of all other samples. In conclusion, the findings suggest that there is a reduction in serum cortisol levels following the administration of Clonazepam in comparison to the control group. Nevertheless, the administration of *Terminalia Arjuna* & Combine extract leads to a decrease in serum cortisol levels in comparison to both the control and standard groups. A reduction in serum cortisol levels is observed in the Amrul extract administration group in comparison to the control group. According to these results, the Arjun tree bark extract, Amrul extract, and their combined extract may be beneficial in reducing stress and lowering cortisol levels, a hormone that is frequently linked to stress response.



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### 6.1.3.2 Control, Standard drugs, Plant sample effects on blood glucose

| Name of the group | Level of Blood glucose [mmol/L] |
|---|---|
| Control Group | 4.17 ± 0.71 |
| Standard Group | 6.39± 0.56 |
| Sample Group 1 | 4.55 ± 0.88 |
| Sample Group 2 | 5.76 ± 1.69 |
| Sample Group 3 | 2.11± 0.42 |

Table 06: Control, Standard drugs, plant sample effects on blood glucose
Data were expressed as mean Standard Error Mean (± SEM) {n= 3 for single group}

**Discussion**

The result is expressed as mean ± SEM (Standard Error Mean), indicating the average blood glucose level for each group along with the standard deviation. The number of samples (n) for each group is specified as 3, indicating that the experiment was conducted three times for each group to obtain consistent results.

These results suggest that three samples extract led to lower blood glucose levels comparing with the control and standard group.



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### 6.1.3.2 Control, Standard drugs and plant sample effects on CRP

| Name of the group | CRP (mg/l) |
|---|---|
| Control Group | 3.54± 0.31 |
| Standard Group | 3.85± 0.95 |
| Sample Group 1 | 1.91± 0.59 |
| Sample Group 2 | 3.32± 0.36 |
| Sample Group 3 | 2.73± 0.95 |

Table-04: Effects of Control, Herbal sample, Standard drugs on CRP. Data were expressed as mean Standard Error Mean (± SEM) {n= 3 for single group}

**Discussion**

From the table, it can be observed that the standard drug (Clonazepam) doesn't reduces CRP levels compared to the control group. These results suggest that sample Group 1, 2, 3 extracts led to lower blood glucose levels comparing with the control and standard group. There shows positive result in combine extract. This indicates that *Terminalia Arjuna*, *Oxalis Corniculata* & their combine Extract have the potential to lower inflammation, as reflected by the decreased CRP levels. However, the CRP levels in the sample groups treated with the herbal extract are slightly lower compared to the standard group, are still significantly lower than the control group. This suggests that the herbal extract may have a moderate anti-inflammatory effect.



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# CHAPTER-07

# CONCLUSION



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## Conclusion

In summary, the scientific investigation has demonsrated the potential of the methanol extract of *Terminalia Arjuna* (Bark) and *Oxalis Corniculata* to serve as a natural remedy for stress-related disorders and cardiovascular disease. It also demonstrates the thrombolytic and antistress properties of the extract. Additional research is necessary to determine the precise bioactive compounds that are responsible for the therapeutic effects and the potential for synergistic interactions. The investigation demonstrated that the lysis of blood was 76% (previously reported) and 7.2% for the standard and control. The three samples contained 29.1%, 27.5% and 33.5%. In addition, the serum cortisol levels of the standard and herbal groups were 2.94 ± 0.4, 3.01 ± 0.25, 3.36 ± 0.68, and 2.143 ± 0.22 ug/dl, respectively, while the control group's level was 3.79 ± 0.56 ug/dl. The investigation revealed that, for control and standard, the following values were obtained: glucose 4.17 ± 0.71 & 6.39± 0.59; CRP 3.54 ± 0.31 & 3.85 ± 0.95. Glucose 4 .55± 0.88, 5.76 ± 1.69, 2.11± 0.42 and CRP 1.91 ± 0.59, 3.32 ± 0.36, 2.73 ± 0.95 were found in the sample Group 1, 2, 3. These results indicate that plant extract has the potential to serve as a natural remedy for tension and heart issues, offering a comprehensive approach to mental and cardiac health. My research is necessary to identify the precise bioactive components that confer its thrombolytic activity and anti-stress effects. In the end, my work contributes to the overarching goal of improving global health outcomes for individuals and promoting evidence-based medicine.



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# CHAPTER-08

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