Phytochemical screening and neuro modulatory activity of methanol extract of *Terminalia catappa* in experimental wister albino mice.

PROJECT WORK

A PROJECT REPORT THAT PARTIALLY SATISFIES THE REQUIREMENTS FOR THE THE DEGREE OF BECHELOR OF PHARMACY, SUBMITTED TO THE DEPARTMENT OF PHARMACY, DAFFODIL INTERNATIONAL UNIVERSITY.

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Approval

A project paper **"Phytochemical screening and neuro modulatory activity of methanol extract of Terminalia catappa in experimental wister albino mice"** is submitted to the Department of Pharmacy, Faculty of Health and Life Science, Daffodil International University has been approved in terms of style and contents and accepted as meeting the requirements for a partial fulfillment of the Bachelor of Pharmacy degree.

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Krishno Banik
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Dedication

I DEDICATE MY WORK TO HARIPADA KAPALI, MY SELF-EDUCATED IDOL, WHO HAS DEVOTED HIS LIFE TO PRACTICING KNOWLEDGE, AND TO MY MOTHER, WITHOUT WHOM I WOULD NOT HAVE EMBARKED ON THIS JOURNEY THROUGH UNIVERSITY LIFE.

Declaration

I thus certify that the project work title is **"Phytochemical screening and neuro modulatory activity of Terminalia catappa in experimental wister albino mice "** is necessary to fulfil the requirements of the Daffodil International University Faculty of Allied Health Science's Bachelor of Pharmacy (B.Pharm) program.

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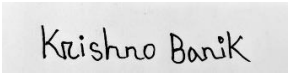

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Abstract

In our daily life everyone have to deal with depression, anxiety and stress these are very similar in that time. Neurological disorder interrupt somesones daily and personal life. Main purpose of this study is to investigate the phytochemical composition and neuro-modulatory potential of the methanol extract derived from *Terminalia catappa* leaves. CNS depressant activity were done by hole cross, hole board and open fieild tests at 50mg/kg & 125mg/kg in body weight. It also investigates how this plant may reduce stress level by measuring both blood serum cortisol level and glucose level respectively in experimental Wistar albino mice. For antistress activity,The blood glucose level for herbal sample were respectively 2.98± 0.13 & standard groups were 1.47± 0.3419 mmol/L respectively where control group was 4.83 ± 0.450325 mmol/L. Serum cortisol level 2.78± 0.102632 & 1.83± 0.0757 ug/dl for plant extract and standard group drugs where 2.30± 0.11ug/dl for control group. A herbal sample group exhibited a significant reduction in stress, as indicated by a serum cortisol level of 1.83±0.0757 ug/ml.On the other hand, CNS depressant activity significantly reduction of frequent locomotor and exploratory activity was found in hole cross ($P< 0.001$) and open field test $P < 0.05$, $P < 0.01$, $P < 0.001$. Furthermore, both extracts also decreased the significant numbers ($P < 0.001$) of head dips by mice in hole-board test. Terminalia catappa, commonly known as Indian almond, has a rich history of traditional medicinal use. Phytochemical screening was conducted to identify the constituents present in the methanol extract, revealing the presence of flavonoids, alkaloids, tannins, and phenolic compounds. Subsequently, neuro-modulatory assessments were carried out to explore the extract's antidepressant and anti-stress effects using behavioral paradigm

Key words: Stress, Serum Cortisol, Terminalia catappa , mice, Antidepressant

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1. Introduction

1.1 Introductory Overview:

The natural world has provided an incredible reservoir of remedies to cure every human illness. Almost all of the medicine used in the past came from natural sources, particularly plants, and plants continue to be a major source of novel drugs even now. Recently, there has been a growing recognition of the importance of plant, chemical, and pharmacological evaluation of plant-derived experts used in the treatment of human ailments. Plants are used for many different things. [1]

Nature has always supplied for humankind's needs, including places to get medications, covers, food, clothes, flavors, and composts. In agricultural nations where homegrown medication has a long history of usage, therapeutic plants continue to play a key role in the framework of medical care for big populations. The medicinal and financial benefits of these plants are becoming more widely recognized in both developed and developing nations. Plants were used to create facilities that have been employing traditional remedies for thousands of years. There are still plants that can be used to create new recipes for humans. Some of the beneficial properties of plants are considered incomplete, and the therapeutic treatment of plants depends on experimental discoveries made over hundreds or thousands of years. The oldest possible carvings on clay tablets are cuneiform, dating from around 2600 BC It originated in Mesopotamia and contains oils from Cupressus sempervirens (cypress), Glycyrrhiza glabra (licorice), Commiphora species (myrrh), and Papaver somniferous (poppy juice). These oils are still used to treat a variety of illnesses, from injuries and colds to exacerbations and parasite infections. [2]

Though their structure is the best hope for a hotspot for safe future pharmaceuticals, plants are nevertheless significant in the medical sector today. Let it go even if they are still vital in medicine today, plants' structures are better than any potential hotspot for secure pharmaceutical development in the future. Though we have access to a wide range of drugs these days, it is still essential to find and train new restorative experts. Only around 33 percent of all known human ailments have a therapy that is deemed OK. In light of this, it is imperative to battle illnesses vigorously. Long-established plant prescriptions are highly sought-after in the current crop. In most significant pharmaceuticals created in the last 50 years that have changed modern restorative therapy, plants have been disconnected or used as a derivative. These ingredient additions show how medicinal plants and animals can be. [3]

About 80% of the 5.2 billion people on the planet, according to the WHO, reside in less developed countries and almost exclusively rely on herbal medicine to meet their fundamental medical needs. The "foundation" of traditional medicine is comprised of medicinal plants, which are regularly utilized by nearly 3.3 billion people in underdeveloped countries. [4] Indigenous tribes' historic usage of plants for medicine has led to the development of several modern pharmaceuticals. Ethnobotany-related activities might take place in botanical gardens or outdoors. Investigations into plant physiology, drug development, and pharmacognosy can all benefit from this field [5]. Within its Public Health Program, WHO encourages and supports the growing use of complementary and alternative medicine.

This is due to the fact that natural remedies are truly more affordable than those used by the typical person and are regarded as considerably safer than recently manufactured, tested medications. Therefore, the location of several chemically significant medications with significant roles in the treatment of human disorders has been indicated by pharmacologically/organically dynamic expert studies acquired by screening normal sources, for example, by eliminating plants. Recent studies in phytochemical-pharmacological research have produced workable treatments for some infections that the manufactured drug industry has been unable to treat. Artemisia annua, Catharanthus roseus, Taxus species, Lantana camara, and Bacopa species are the subjects of the investigation. These plants were once thought to be harmful or ineffective, but research has since proved that they contain extremely useful medicinal particles, and they are now acknowledged as vital spices for healing. The containment of a few novel, possibly significant corrective measure mixes is the sole result of it at this time. A significant amount of prescription medications for rehabilitative Geologists have worked extremely hard and dedicatedly to provide leads and a plethora of novel biochemically bioactive components for organic prescription medications. The modern synthesis of morphine by German scientist E. Merck in 1826 marked the beginning of the organic drug industry's commercialization. About half of the best-selling medications in 1991 were either generic versions of reasonably common product. [6]

1.2. Phytochemistry

The word "plant" in Greek is the source of the name. [7] The study of chemicals generated by plants, especially their secondary metabolites, which are created as a kind of self-defense against diseases, insects, pests, herbivores, UV radiation, and environmental risks, is known as phytochemistry. The structural makeup of these metabolites, their biosynthetic processes, their roles and methods of action within biological systems, as well as their industrial, commercial, and medical uses, are all taken into consideration by phytochemistry. A thorough understanding of phytochemicals is necessary for the creation of innovative therapeutic agents to combat major illnesses and for the search for new drugs..[8]

1.3 Medicinal Plant

Medicinal plants are rich in secondary metabolites and are probably a good source of pharmaceuticals. Steroids, alkaloids, glycosides, coumarins, and flavonoids are among these auxiliary metabolites. The phrase "restorative plant" has been defined in a variety of ways. For example, some of its organs and parts contain compounds that may be utilized for whatever purpose they are intended for, or they may be pioneers in the development of semi-combinations of chemotherapy and drugs. It remedies a few common mistakes with herbs including aloe, tulsi, neem, turmeric, and ginger. These are regarded in many parts of the nation as home remedies. It is well established reality that bunches of buyers are utilizing Tulsi for making meds, dark tea, in pooja and different exercises in their everyday life. In Bangladesh there are around 297 Unani, 204 Ayurvedic and 77 Homeopathic drugs producing enterprises where the restorative plants are widely utilized in both crude and semi– handled types of medication in different drug portion plans. The market worth of drugs delivered by these

enterprises from restorative plants is about Tk. 300 crores. Moreover, town Kobiraj, road Vendors and Tribal individuals likewise utilize a norm number of therapeutic plants for the treatment of different sicknesses. [9]

1.4 Plants in traditional medicinal

The term "traditional medicine" refers to knowledge, skills, and methods based on theories, beliefs, and firsthand experiences that are inherent to different cultures and are applied to promote health as well as identify, locate, treat, or prevent mental and physical instability. Up to 80% of Ethiopians receive vital medical treatment through traditional medicine. Research on the current state of knowledge and network practices throughout the expansion of medical services today is lacking. Traditional medicine administration, one of humanity's oldest and most lasting practices, has long helped people prevent illness and lead healthy lives. Indian, Chinese, and African medical systems are some of the most well-known that Native Americans have long used in conjunction with their innovative approach to their traditional medicinal arrangement. [10]

1.5 Significance of medicinal plants

Medicinal plants are defined as those that are commonly used to cure and prevent specific illnesses and diseases, but are generally thought to be harmful to humans. Medicinal plants have application in the food, medicine, medicinal, and rural industries. Essential metabolites found in plants, such as carbohydrates, lipids, and proteins, meet a variety of requirements necessary for survival and reproduction. Additionally, plants create auxiliary metabolites that protect them against creatures, parasites, and microbes. These metabolites also play a crucial role in the recovery of disorders. Tannins, glycosides, unstable oils, alkaloids, fixed oils, gum gums, nutrients, and minerals are examples of synthetic substances that contribute to a plant's significance. Alkaloids are naturally occurring substances with potent effects that can be poisonous, addictive, or both. Occasionally, they can also be helpful in treating serious conditions. Glycosides are naturally occurring, consistent combinations found in many different plants. It is used as a great laxative and heart energizer. Many different kinds of plants contain tannin chemicals. It is applied to gastrointestinal problems. Unpredictable oil is used to keep germs away. It also improves craving. Saturated oil reduces corrosiveness. Gum-saps have the ability to relieve pain. [10-12]

1.6 Status of medicinal plants in Bangladesh

The 'Present Status and Market Scenario of Medicinal Plants' in Bangladesh was the subject of this investigation. It can be seen from the overview study that 46.6% of firms use more than 60% of imported medicinal plants as raw materials, while 53.3% of businesses use less than 40%. Bangladesh is an Indus valley nation with a diverse diversity of plant species. This region is thought to have 2,000 medicinal plants, of which 449 are registered in Bangladesh. Kavirajs, or healers, have traditionally employed a few well-known herbal crops; however, the precise number of plants used is uncertain.The usage of medicinal herbs has been enhanced by the existence of several communities, such the Chakma, Marma, Rakhine, Tripura, Garo, and Khashia, each with its own oral tradition variation. This conventional treatment approach has triumphed despite the overwhelming dominance of contemporary healthcare systems because to people's confidence in mother nature. As a matter of complying, the expertise of how to use medicinal plants has been passed down from ancestors to fore fathers.

There are no rules, legislation, or government restrictions pertaining to the sale, preservation, or production of therapeutic plants. Approximately 422 businesses produce goods using medicinal plants. [13][14]

1.7 Valuable and Significant characteristics of medicinal plants

Plants have been used to treat illnesses for almost as long as humans have existed. Although the exact composition of restorative plants is not always known, they are frequently recommended due to well-established beliefs about their potential and usefulness, which further highlights their therapeutic qualities. Senna alata, for example, is commonly used to treat bacterial and infectious contaminations. They also shown varying degrees of antifungal and antibacterial activity against microorganisms. .

It's been shown that flavonoids have stronger antifungal and antibacterial effects on a few human pathogenic parasites and microscopic organisms. A restorative plant's usefulness is anticipated given the existence of certain bioactive components. Phytochemical screening techniques, including as dainty layer chromatography and phytochemical assays, are used to identify these beneficial components. A wide range of auxiliary metabolites, including as tannins, terpenoids, alkaloids, and flavonoids, are present in restorative plants. These metabolites, in particular, are responsible for the plants' antimicrobial activities. Similar phytochemical components, such as flavonoids and tannins, have also been shown to be effective against pathogenic bacteria, including Staphylococcus aurous and Bacillus cereus. Because of their tannin content, restorative herbs are useful in the preparation of sterile cleaners, which are often used for cleansing or clearing skin surfaces. Phytochemicals have been shown in literature to have deleterious effects on filamentous organisms, yeasts, and bacteria. They have also been shown to impede viral reverse transcriptase. It has been shown that saponins play a major role as an auxiliary metabolite with antifungal properties. Many physiological activities of tannins, phenols, steroids, and saponins are thought to be more potent and may therefore be responsible for the antibacterial action. [15][16]

1.8 Strategies for finding new product

Herbal medicine texts list several plants that are medicinal but have not been well studied. Pharmacologic screening in accordance with the customary usage of these plants can be used to determine their usefulness. If a notable result is produced, spectroscopic and chromatographic methods can be used to identify the perpetrator. A bioactivity-guided strategy involves three separate stages of research. The crude substance first exhibits biological activity; the inactive fractions are discarded, and a bioassay technique is used to identify the active fractions. The second stage is the fractionation of the raw material.. [17]

1.9 Phytochemical Basis

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 medications, and if their pharmacological activities are established, it makes sense that they are now being used in medications.

substances of these substances in restorative plants. For example, daffodils (daffodils) contain nine alkaloid chunks, including glutamine, which is approved for use in Alzheimer's disease. Alkaloids are pungent, poisonous, and move as stalks of plants that are easy for herbivores to eat. They can also protect against parasites. [18][19]

The extraction of a pharmacologically active or non-active constituent molecule from a medicinal plant that is responsible for its therapeutic efficacy is referred to as phytochemical screening. As a result, photochemical screening has become increasingly important in modern physiology. The phytochemical screening in this study includes a capacity bunch test in the *Terminalia catappa* plant. Some of the useful collections include flavonoids, alkaloids, tannins, gums, steroids, phenol, carbohydrates, saponins, glycosides, and so on. This chemical is mostly found in the tops, leaves, barks, and stems of plants. These mixtures are separated utilizing a different way in today's technology. It means that medically effective compounds found in plants are isolated, screened, and publicly verified. Flavonoids, alkaloids, carotenoids, tannin, cell fortifications, and phenolic compounds are some of the bioactive components extracted from plants [38]. Finding new sources of financial resources such as oils, gums, tannins, and precursors for combining complex compounds is interesting in and of itself, as it can lead to the identification of beneficial experts as well as the discovery of new financial resources such as oils, gums, tannins, and precursors for combining complex compounds. Additionally, understanding the components of plant materials would be helpful in determining their potential worth. [39].

1.9.1 Alkaloids

Any of the nitrogen-containing bases, or alkaloids, found in a variety of living things. The physical effects of alkaloids on humans and other animals are diverse. Alkaloids include things like triclosan, ephedrine, morphine, and vaping. Alkaloids are generally present in nature, and these kinds of blossoming plants are very rich in alkaloids. 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 has just a few distinct kinds of alkaloids; however, the opium poppy (Papaver somniferous) and the ergot parasite (Claviceps) both have over thirty different forms. Alkaloids are prevalent in several plant groups; 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 canadensis) and poison-dart frogs (Phyllo bates). In addition, ergot and a few other parasites create them. [20][21]

1.9.2 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 include 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. [21]

1.9.3 Saponins

Saponins are a varied collection of naturally existing chemicals present in different plant species, known for their capacity to create a soap-like froth when agitated in water. They are glycosides, composed of a sugar molecule linked to a non-sugar moiety, giving them hydrophilic and hydrophobic characteristics. Saponins have the ability to interact with both water and lipids due to their unusual structure, allowing them to exhibit various biological actions. Saponin molecules are often categorized according to their aglycone part, which may be a triterpenoid or a steroid, and the sugar portion, which may vary, leading to a wide range of saponin structures in nature.

Multiple reliable sources enhance our comprehension of saponins. An example of a source is the book "Saponins in Food, Feedstuffs, and Medicinal Plants" by Oleszek and Marston (2000). This book offers detailed information on saponins, including their presence, chemical compositions, biological effects, and uses in food, feed, and medicine. Hostettmann and Marston's book "Saponins" (1995) provides a comprehensive examination of saponins, including their production, plant distribution, isolation and analytical techniques, pharmacological characteristics, and ecological functions.[22,23]

1.9.4 Steroids

Since these hormones Steroids are a broad family of chemical molecules with four cycloalkane rings arranged in a certain way. They are essential to several biological activities, such as immune response, metabolic control, and reproduction. Four linked rings make up the structure of steroids: one cyclopentane ring (ring D) and three cyclohexane rings (identified as rings A, B, and C). Many naturally occurring physiologically active compounds are supported by this unusual structure.

Based on their biological origins and roles, steroids may be generally categorized into many groups. The corticosteroids, which comprise hormones like cortisol and aldosterone and are generated in the adrenal glands, are one of the most well-known categories. For instance, cortisol is essential for controlling the body's immune system, metabolism, and stress levels.

The sex steroids, which include progesterone, estrogen, and testosterone, are another significant group. These hormones, which are mostly produced in the gonads (testes and ovaries), are necessary for both reproductive activity and the growth and maintenance of secondary sexual traits.

The body uses cholesterol, also known as the "parent" steroid molecule, as a building block to synthesize additional steroids. It is an essential part of cell membranes and acts as a starting point for the production of many steroid hormones, vitamin D, and bile acids.

In order to have a biological impact, steroids must interact with certain receptors that are either

within the cell (for intracellular receptors) or on the cell membrane (for steroid hormones). Steroids have the ability to directly impact biological processes, start signal transduction pathways, and modify gene expression when they bind to their specific receptors.[24]

1.9.5 Gums

Gums (gingivae) are the tissues that surround the base of your teeth and help keep them stable. It's essential to protect your gums from periodontal disease, which can erode them and result in the loss of tooth and bone. [25] Another term for gingiva is gums. There are several gingivae. A similar issue is dentistry of the mouth. Gums in good health are stippled, pink, and tough. Additionally, their sensitivity to pain, cold, and pressure is relatively moderate. The red alveolar mucosa and the gums are separated by a scalloped line that roughly resembles the form of the teeth.. [26]

1.9.6 Flavonoids

Plants are rich in flavonoids, a broad class of polyphenolic chemicals that contribute to color and have a variety of biological roles. The chemical structure of flavonoids is comprised of a 15-carbon skeleton that is connected to two aromatic rings (A and B) by a three-carbon chain, forming a heterocyclic ring C. Numerous flavonoid subclasses, such as flavones, flavonols, flavanones, flavanols (catechins), anthocyanins, and isoflavones, may be produced by altering this fundamental structure. Flavonoids are abundant in fruits, vegetables, cereals, tea, wine, and herbs. They are essential for pigmentation, UV radiation protection, and plant defense mechanisms against pathogens. They also have a number of biological actions that are good for human health, including anti-inflammatory, antibacterial, anticancer, cardioprotective, and neuroprotective qualities. Their capacity to bind transition metal ions and scavenge free radicals accounts for their antioxidant action. Through their modulation of immune response and inflammation-related signaling pathways, flavonoids also have anti-inflammatory properties. Additionally, they have been linked to the management and prevention of long-term conditions such as diabetes, cancer, heart disease, and neurological illnesses. Regular intake of foods high in flavonoids has been linked to a lower chance of developing these illnesses, according to studies. However, the human body's capacity to absorb and metabolize flavonoids varies based on a number of variables, including individual characteristics, chemical structure, and the composition of the meal. To maximize the medicinal potential of flavonoids, one must comprehend their pharmacokinetics and impacts on health. [28]

1.9.7 Stress mechanism in human body

It is In the human body, stress produces a complicated reaction involving several physiological systems. The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis are two important pathways implicated in the stress response.

Hypothalamic-Pituitary-Adrenal (HPA) Axis: When the brain recognizes stress, the hypothalamus releases corticotropin-releasing hormone (CRH).

CRH stimulates the pituitary gland to produce adrenocorticotropic hormone (ACTH).

ACTH then travels via the circulation to the adrenal glands, where it promotes the production of cortisol, the chief stress hormone.

Cortisol helps control metabolism, immunological function, and stress response.

Sympathetic-Adrenal-Medullary (SAM) Axis: In reaction to stress, the sympathetic nervous system (SNS) activates, resulting to the production of adrenaline (epinephrine) and noradrenaline (norepinephrine) from the adrenal medulla.

These hormones boost heart rate, blood pressure, and energy mobilization, preparing the body for a fight-or-flight reaction.[44]

1.9.7 Depressants

The process of depression in the human body is complicated and includes different biochemical, genetic, and environmental components. While the actual origin of depression is not completely known, research has discovered numerous critical pathways that contribute to the development and maintenance of depressed symptoms. These mechanisms include:

Neurotransmitter Imbalance: Depression is connected with abnormalities in neurotransmitter levels, notably serotonin, dopamine, and norepinephrine. Dysregulation of these neurotransmitters may impair mood regulation and lead to depressed symptoms.
Neuroendocrine Dysfunction: Dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, which governs the body's stress response, is typically detected in depression. Chronic stress and high levels of the stress hormone cortisol have been related to the development of depressive symptoms.

Inflammatory Processes: Inflammation in the central nervous system has been linked in the pathophysiology of depression. Increased levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), have been seen in persons with depression and may contribute to neurobiological alterations associated with the condition.

Neuroplasticity and Neurogenesis: Depression is related with abnormalities in brain structure and function, including diminished neuroplasticity (the brain's capacity to adapt and change) and impaired neurogenesis (the production of new neurons). These alterations may contribute to the duration of depressed symptoms and the diminished effectiveness of antidepressant therapy.

Genetic and Epigenetic Factors: Genetic susceptibility has a substantial impact in the development of depression, with heritability estimates ranging from 30% to 40%. Additionally, epigenetic alterations, such as DNA methylation and histone acetylation, might impact gene expression and add to the risk of depression.

Environmental Stressors: Environmental variables, such as early childhood adversity, trauma, chronic stress, and social isolation, might raise the likelihood of developing depression. These stresses may combine with genetic vulnerabilities to cause the beginning of depressive episodes. [45]

1.9.8 Stress-Depression Interplay

The link between stress and depression is complicated and bidirectional, with stress frequently functioning as both a cause and result of depressed symptoms. Research has found various routes via which stress might contribute to the development and worsening of depression:

Activation of the HPA Axis: Chronic stress stimulates the hypothalamic-pituitary-adrenal (HPA) axis, resulting to dysregulation of cortisol levels. Prolonged exposure to increased cortisol levels may affect neurotransmitter systems, neuroendocrine function, and neuroplasticity, all of which are involved in the pathophysiology of depression.

Dysregulation of Neurotransmitter Systems: Stress may alter the equilibrium of neurotransmitters, such as serotonin, dopamine, and norepinephrine, which are involved in mood regulation. Alterations in neurotransmitter levels may lead to the development of depressive symptoms.

Inflammatory Processes: Chronic stress induces inflammation in the body, including the central nervous system. Increased levels of pro-inflammatory cytokines have been related to the development of depression. In fact, depression itself may further increase inflammation, producing a vicious cycle.

Impaired Coping systems: Chronic stress may overwhelm an individual's coping systems, leading to feelings of helplessness, hopelessness, and despair—common symptoms of depression. The failure to adequately deal with stresses may raise the likelihood of developing depression or intensify current depressed symptoms.

Environmental Factors: Stressful life experiences, such as trauma, loss, or substantial life transitions, might prompt the development of depression. Individuals who suffer persistent stresses or unfavorable childhood experiences may be at heightened risk for developing depression later in life.[46]

1.9.9 Perspectives on Neuromodulation and Cortisol

Neuromodulation, the process by which neuronal activity is controlled and modulated, plays a critical role in determining the function of the nervous system. It includes the release of neurotransmitters or neuromodulators, which may impact neuronal excitability, synaptic transmission, and total brain activity. Cortisol, a steroid hormone generated in reaction to stress, is closely associated with neuromodulatory processes, exhibiting extensive impacts on brain function and behavior.

One crucial component of the interplay between neuromodulation and cortisol is their bidirectional link. On one side, neuromodulatory systems, such as the serotonergic, dopaminergic, and noradrenergic pathways, may affect the activity of the hypothalamic-pituitary-adrenal (HPA) axis, which governs cortisol secretion. For example, neurotransmitters like serotonin and dopamine may impact the release of corticotropin-releasing hormone (CRH) from the brain, consequently altering cortisol synthesis by the adrenal glands.

Conversely, cortisol may also impact neuromodulatory function in the brain. Cortisol receptors are extensively distributed throughout the central nervous system, enabling cortisol to directly alter neuronal activity. High amounts of cortisol, especially during times of chronic stress, may lead to abnormalities in neurotransmitter systems, synaptic plasticity, and neurogenesis. These alterations may lead to the development of neuropsychiatric illnesses such as depression, anxiety, and cognitive impairment.

Understanding the relationship between neuromodulation and cortisol has crucial implications for both fundamental neuroscience research and therapeutic treatment. Research in this field may give insights into the processes behind stress-related diseases and guide the development of innovative treatment strategies. For example, targeting neuromodulatory systems or cortisol signaling pathways may provide novel options for treating disorders defined by dysregulated stress responses.[47]

1.9.9.1 Neurological Effects of Oxidative Stress

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The damage to the brain generated by oxidative stress has a dramatic influence on the actions of the central nervous system. The brain is especially sensitive to oxidative stress due to its high oxygen needs, high polyunsaturated fatty acid (PUFA) **co**he term reactive oxygen species (ROS) refers to a collection of free radicals that do not strictly conform to the concept of a free radical. This category contains singlet oxygen, superoxide anion, hydroxyl free radical, hydrogen peroxide, and hypochlorous acid. As signaling molecules involved in cell proliferation and death, low levels of reactive oxygen species (ROS) are important for appropriate neuronal development and function in the brain (CNS). In appropriate numbers, they contribute to the battle against infections. However, when their numbers become excessive, they injure cells and cause DNA mutations that eventually result in cell death. Reactive oxygen species are byproducts of oxidation that drive subsequent oxidative chain reactions (ROS). This

chain reaction is dubbed "autoxidation" because it occurs spontaneously and under benign environmental conditions. Peroxidation occurs when peroxidases are formed as byproducts. During the oxidative chain reaction, reactive oxygen species (ROS**)** accumulate owing to an imbalance between their production andntent in nerve cell membranes, and accumulation of transition metals . The high metabolic rate of the brain enables it to use around 20% of the total energy needed by the human body. The production of this fuel is the responsibility of the mitochondria of brain cells. Seventy to eighty percent of the brain's energy is consumed by neurons, while the remainder is used by glial cells [49]. Mitochondria create around 80% of reactive oxygen species (ROS) in brain cells, which assists in appropriate cellular functioning. Due to electron leakage at four complexes (I-IV), the ROS threshold is surpassed, resulting in molecular stress. DNA oxidation, lipid peroxidation, and protein oxidation all occur when ROS levels grow. In addition, the brain has an abundance of polyunsaturated fatty acids, particularly docosahexaenoic acid, which promotes lipid peroxidation. The decomposition of lipids by free radicals is known as lipid peroxidation. When free radicals attack PUFA in the membrane of a brain cell, highly active aldehydes are generated, which subsequently (a) stiffen the membrane, (b) inhibit sodium pump function, (c) alter membrane permeability, and (d) injure the cell. The accumulation of transition metals is the principal cause of oxidative stress in the central nervous system (CNS). Iron, copper, and zinc are all transition metals that play crucial roles in biological processes such as cell division, DNA synthesis, and neurotransmitter creation. In addition, it is well established that iron and copper serve as catalysts for ROS production in cells. These metals produce an increase in reactive oxygen species (ROS), which leads to lipid peroxidation, oxidative stress, and cell damage.

1.10 Herbal Antidepressants Relate to Depression

This Common mental illness that seriously hinders a person's quality of life is major depressive disorder. The majority of synthetic antidepressants have significant downsides, including a lack of alternatives, severe side effects, expensive prices, and the possibility of relapse. Herbal remedies are growing in popularity as individuals seek multi-target antidepressants with minimal adverse effects. The complex etiology of depression is likely related to the observation that a variety of mental disorders share distinct metabolic abnormalities [51]. Decreased levels of 5-hydroxytryptamine (5-HT), norepinephrine (NE), and dopamine (DA) are examples of the damage to monoamine transmission networks that underlies depression [45, 46].

On the basis of this principle, many antidepressants, some of which are herbal, have been produced; they function by preventing the reuptake of certain neurotransmitters by the brain. Numerous medicines operate through this mechanism [51, 52]. Some herbal medications have antidepressant effects through blocking monoamine oxidases or by increasing serotonin receptor sensitivity, whilst others do not. It is commonly known that ginseng, a plant used for thousands of years to enhance mental and physical health in traditional Chinese medicine, has a long history of use in the west. In rodent models, 20(S)-protopanaxadiol, a chemical derived from ginseng, exhibited potential antidepressant qualities by increasing NE and 5-HT levels in the brains of OB rats. Inhibition of monoamine reuptake may contribute to the efficacy of this medication [49]. Peony, often referred to as the root section of the plant Paeonia lactiflora Pall (Ranunculaceaeprocessed), is a key component in several traditional Chinese medicine remedies for the treatment of depression [51]. The ethanol extract and total glycoside fraction of peony (TGP) exhibit antidepressant effects in rats under normal physiological conditions, according to previous investigations . Subsequent study has shown that TGP alleviates depressive symptoms brought on by sustained unexpected stress [54], indicating that the antidepressant-like effect of TGP is mediated through inhibition of monoamine oxidases and the reduction of oxidative stress in mouse brain. At least in part, anxiety, sadness, and the actions of tranquilizers and mood stabilizers may be attributed to 5-HT1A receptors [54]. Ji-Hyun Kim discovered that acute administration with the methylene chloride fraction of Albizia julibrissin (MCAJ) significantly decreased immobility time in forced swimming experiments, suggesting antidepressant potential . Albizia julibrissin, sometimes known as the mimosa tree, is Healthcare Outcomes

A novel therapeutic approach called prehospital thrombolytic therapy has the potential to significantly alter patient outcomes. According to multiple studies, skilled prehospital personnel are able to identify ST-segment elevation on a 12-lead ECG and take appropriate action, such as initiating prehospital thrombolytic treatment or alerting the coronary care institution beforehand. Prehospital fibrinolysis, however, is appropriate and safe when given by trained emergency medical staff. As a result, the most common prehospital fibrinolytic drugs are tissue plasminogen activator, alteplase, or its modified versions, reteplase or Tenecteplase. Because of its handy single or double bolus, Reteplase or Tenecteplase are recommended. To guarantee routine out-of-hospital thrombolytic therapy, appropriate protocols with checklists, 12-lead ECG interpretation and referral, advanced cardiac life

support (ACLS) training, and ongoing medical monitoring are also required. If neurologic findings have changed during or after thrombolytic therapy for acute ischemic stroke, a prospective CT scan of the brain is recommended. Advice from the pharmacy is crucial to guaranteeing the right dosage and avoiding interactions. When used appropriately, thrombolytic therapy is a vital tool in the treatment of some disorders (such as occlusive stroke and AMI). A full professional team, comprising paramedics, physicians, specialists, nurses, and chemists, must collaborate on this task. When all these specialties work together and communicate openly, patients who need thrombolytic therapy can have the best outcomes with the least number of adverse effects.

1.13 Objective of study

This study focused on the phytochemical and certain specific pharmacological examination of the plants. It can be seen in the several place in Bangladesh. It has a wide range of traditional and medicinal uses. The purpose of this research was to investigate the potential activities of Indian almond. For the analysis, the hip extract is used. There are some reasons for taking this plant to exploration. Such as-

✓ To know about Terminalia cattapa

✓ To see the anti-depressents activity of Terminalia cattapa

✓ To identify the antistress activity of Terminalia cattapa

Chapter: Two

Plant profile

2. Plant Profile

2.1 Plant Introduction

The stately and commonly Terminalia catappa tree is native to tropical parts of Asia, Australia, the Pacific, Madagascar, and the Seychelles. It is sometimes referred to as the Indian almond, sea almond, or beach almond.With its broad, glossy, leathery leaves forming a canopy like a pagoda, Terminalia catappa stands between 20 and 35 meters tall. These leaves, which group at the terminals of the branches, change color from a vivid green to striking red, yellow, or purple before dropping, producing an amazing show.In addition to being aesthetically stunning, Terminalia catappa is essential to coastal habitats. Its vast root system functions as a built-in buffer, preventing soil erosion and shielding beaches from wind and tidal waves. Moreover, tannins released by the falling leaves into the water create a mildly acidic microhabitat that supports. As a result, Least Concern is assigned to it. [52]

Figure-1*:* *Terminalia catappa* Plant

2.2 Name of plant [36]

Scientific Name: *Terminalia catappa*
Common Name: *Indian almond*
Bangla Name: *Kath Badam*

2.3 Taxonomical classification [37]

- **Kingdom:** Plante
  - **Phylum:** Magnoliophyta
    - **Class:** Magnoliopsida
      - **Order:** Myrtales
        - **Family**: Combretaceae
          - **Genus:** Terminalia
            - **Species:** Terminalia catappa

2.4 Plant morphology

✓ Large, spreading tree reaching 20-35 meters in height.

- ✓ Large, glossy, leathery, obovate leaves clustered at branch tips. They transition from bright green to stunning red, yellow, or purple before falling.
- ✓ Small, greenish-yellow flowers arranged in spikes
- ✓ Winged drupes with a single edible seed, turning green to brown upon maturity.
- ✓ Extensive root system providing coastal protection

2.5 Composition of chemicals

- ❖ Water = 70% (fresh)
- ❖ Proteins = (15-20)% (dry)
- ❖ Carbohydrates = (40-50)% (dry)
- ❖ Lipids = (2-3)% (dry)

2.6 Plants' medicinal uses:

- ❖ Antimicrobial
- ❖ Wound healing
- ❖ Inflammation
- ❖ Antidiarrheal
- ❖ Antiparasitic
- ❖ Antidiabetic
- ❖ Antioxidan

2.7 Traditional use

This plant is used to treat wound healing traditionally.

Chapter: Three
The goal of the research

3. The goal of the research

The goal of the research is to evaluate the phytochemical investigation, anti-stress and anti-depressants activity of methanol extract of *Terminalia cattapa*

- **Analysis of chemical constituents:** Several phytochemical activities, such as the presence or absence of alkaloids, glycosides, flavonoids, saponin, etc. in those plants, will be assessed based on the results of this study.
- **Pharmacological activity investigation:** An additional goal of this project is to look into the scientific underpinnings of the plant's leaves anti-stress, and anti-depressants activities as potential medicinal uses.

Chapter: Four
Literature review

4. Literature Review

This literature review was completed by me using internet resources from numerous reputable journals, including PubMed, ScienceDirect, ResearchGate, Google Scholar, LinkedIn, and others. Many studies using the plants I chose have been published, but they are completely unrelated to the research I'm doing now.

Antidepressant like effects of hydrolysable tannins of Terminalia catappa leaf extract via modulation of hippocampal plasticity and regulation of monoamine neurotransmitters subjected to chronic mild stress (CMS)

Determining the According to Chandrasekhar et al. (2017), "Antidepressant like effects of hydrolysable tannins of Terminalia catappa leaf extract via modulation of hippocampal plasticity and regulation of monoamine neurotransmitters subjected to chronic mild stress (CMS)" investigates the possibility of hydrolysable tannins from Terminalia catappa leaf extract having antidepressant like effects. Under chronic moderate stress (CMS), the study examines how hippocampal plasticity is modulated and how monoamine neurotransmitter regulation is regulated. This research offers significant understanding of the effects of plant extracts on neurotransmitter modulation and hippocampus plasticity, as well as the possibility of using them as antidepressant medications.

As a dependable model for researching depression, the Chronic Mild Stress (CMS) model has been widely employed in studies on depression because it causes depressive-like symptoms in rats. Monoamine neurotransmitters, which are linked to depressed behaviors, are among the neurobiological processes that this model has been shown to affect. A foundation for evaluating the effectiveness of possible antidepressant medications is provided by the CMS model, which has also been used to analyze how antidepressant medications affect behaviors like sucrose consumption.

To further comprehend depression and the results of antidepressant therapy, research on monoamine neurotransmitters is crucial. One of the main roles that synucleins play in the pathophysiology of depression is in the modulation of monoamine neurotransmitters, namely dopamine, norepinephrine, and serotonin. The evaluation of substances like hydrolysable tannins from Terminalia catappa leaf extract that may have antidepressant effects requires an understanding of how these neurotransmitters are modulated.

It has also been shown that long-term antidepressant therapy increases hippocampus neurogenesis, which is linked to animal models' recovery from depressive-like behaviors, making the study of hippocampal plasticity and neurogenesis important to studies on depression. According to this, substances like the hydrolysable tannins in Terminalia catappa leaf extract that have the ability to alter hippocampus plasticity may have antidepressant properties.

Finally, the research conducted by Chandrasekhar et al. (2017) offers important new information on the possible antidepressant properties of hydrolyzable tannins found in Terminalia catappa leaf extract. Contributing to our knowledge of the processes behind the

antidepressant effects of plant extracts, the study resonates with the body of literature on depression, chronic stress models, neurotransmitter control, and hippocampus plasticity. [32]

Ben EE, Asuquo AE, Owu DU. Possible amelioration of oxidative stress damage via cyclo-oxygenase pathway by aqueous extract of Terminalia catappa leaves in alloxan induced diabetic rats. GSC Biological and Pharmaceutical Sciences. 2021;16(2):038-48.

The article "Possible amelioration of oxidative stress damage via cyclo-oxygenase pathway by aqueous extract of Terminalia catappa leaves in alloxan induced diabetic rats" investigates the potential benefits of Terminalia catappa leaves in mitigating oxidative stress damage through the cyclo-oxygenase pathway in diabetic rats (Ben et al., 2021). The research is significant as it studies the possible pharmacological effects of Terminalia catappa leaves, especially in the context of diabetes and oxidative stress.

Terminalia catappa leaves have been the focus of several research, demonstrating their different pharmacological characteristics. For instance, research has proven the hepatoprotective properties of Terminalia catappa fruit extract, attributing its efficiency to its anti-oxidative and membrane stabilizing actions (Tasduq et al., 2006). Additionally, the antifungal and antibacterial activity of Terminalia catappa leaves have been documented in various research, showing its potential in fighting fungal and microbial illnesses (Webster et al., 2008; Babayi et al., 2005; Terças et al., 2017; Allyn et al., 2018). Furthermore, the inhibitory effects of Terminalia catappa leaf extracts on important enzymes connected to diabetes have been examined, showing its potential in controlling diabetes-related pathways (Iheagwam et al., 2019).

Moreover, the phytochemical characterisation of Terminalia catappa extracts has been studied, revealing insight on the chemicals found in the leaves and their possible carcinogenic activity (Mininel et al., 2014). The research of the effects of Terminalia catappa on UVB-induced photodamage in fibroblast cell lines also reveals its potential in dermatological applications (Wen et al., 2011). Furthermore, the study of the insecticidal ability of Terminalia catappa leaves powder against Acanthoscelides obtectus reveals its various applications outside conventional therapeutic usage (Nta et al., 2019).

the article "Possible amelioration of oxidative stress damage via cyclo-oxygenase pathway by aqueous extract of Terminalia catappa leaves in alloxan induced diabetic rats" is backed by a body of research that illustrates the varied pharmacological activities of Terminalia catappa leaves. These results combined offer a framework for future study of the possible therapeutic effects of Terminalia catappa in ameliorating oxidative stress damage and its implications for diabetic situations.[33]

Ben EE, Asuquo AE, Owu DU. Possible amelioration of oxidative stress damage via cyclo-oxygenase pathway by aqueous extract of Terminalia catappa leaves in alloxan induced diabetic rats. GSC Biological and Pharmaceutical Sciences. 2021;16(2):038-48.

Hyperglycemia, a chronic metabolic disease caused by deficiencies in either insulin action or secretion, or both, is the hallmark of diabetes mellitus. It is linked to elevated oxidative stress, an important factor in the emergence and advancement of diabetes problems. Damage to cells results from oxidative stress, which is caused by an imbalance between the body's antioxidant defense systems and the generation of reactive oxygen species (ROS).

The plant Terminalia catappa, also referred to as tropical almond or Indian almond, has a number of pharmacological qualities, such as anti-inflammatory and antioxidant capabilities. Because Terminalia catappa leaves are rich in flavonoids, polyphenols, and other bioactive substances, its aqueous extract has been shown to have high antioxidant activity.

A major component of the inflammatory response, the cyclo-oxygenase (COX) pathway produces prostaglandins and other pro-inflammatory mediators. The COX pathway may be activated in diabetes, which may result in elevated oxidative stress and inflammation. Thus, by focusing on this system, oxidative stress damage in diabetes circumstances may be reduced.

Numerous research have looked at Terminalia catappa's possible medicinal benefits for diabetes. In a paper published in GSC Biological and Pharmaceutical Sciences in 2021, Ben EE, Asuquo AE, and Owu DU investigated the protective benefits of an aqueous extract of Terminalia catappa leaves against oxidative stress damage in rats induced by alloxan, specifically targeting the COX pathway. A popular diabetogenic drug used to create experimental diabetes in animal models is alloxan.

According to their research, diabetic rats treated with an aqueous extract of Terminalia catappa leaves showed a substantial decrease in oxidative stress indicators and inflammatory mediators. This may be explained by the plant extract's anti-inflammatory and antioxidant qualities, which may work, at least in part, via modifying the COX pathway.

Overall, research suggests that leaves of Terminalia catappa may be a promising natural medicinal agent for the treatment of diabetes-related inflammation and oxidative stress. To clarify the underlying mechanisms of action and assess its potential therapeutic uses in people with diabetes, further study is necessary.

Chapter: Five

Materials and Methods

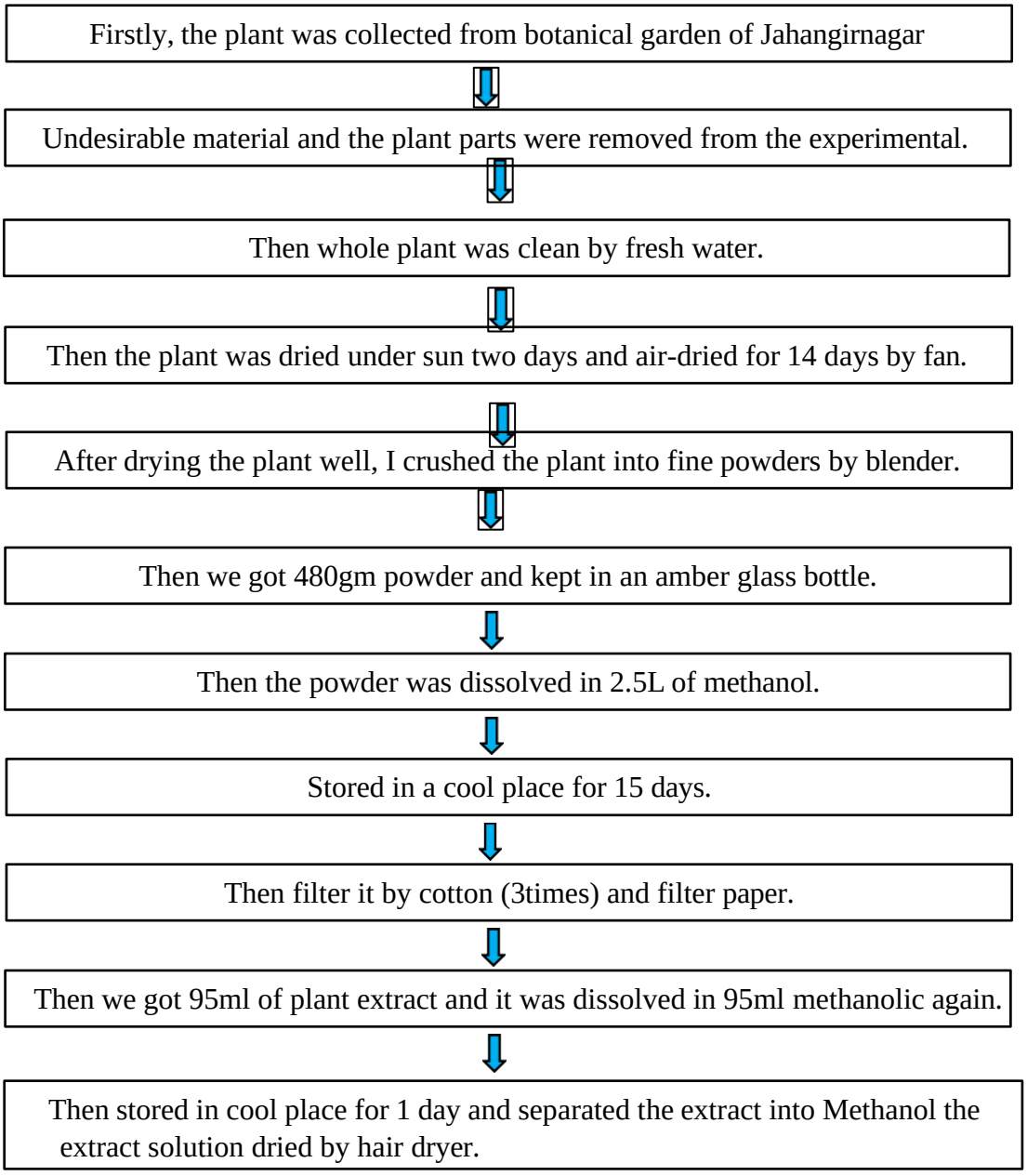
5.1 Preparation of Extraction

5.1.1 Plant Collection

The plant was collected from rural area in Madaripur, Bangladesh.

5.1.2 Preparation of plant material

Unrefined is a medication confined from this plant and the creature and utilize this with no substance change. I arranged unrefined concentrate in a few stages.



5.1.3 Extraction

After giving a bottle jar with a glass a thorough cleaning, methanol was used to rinse it. Subsequently, 2.5L of methanol was added to the jar containing 480gm of dry materials, up to 1 inch above the sample surface, until the sample was adequately covered. The jar was correctly closed using a plastic cover covered in aluminium foil to keep air from getting inside. It looked as though the procedure took fifteen days. Almost two to three times a day, the jar was shaken to improve extraction.

5.1.4 Filtration

Following the extraction procedure, the plant extract was filtered through cotton and filter paper that had been sterilised. The cotton and paper were then rinsed with the necessary solvent and placed within a funnel. The collected filtrate was kept in a glass jar. And carried out the refiltration procedure once again.

Filtration of *Terminalia cattapa*

5.1.5 Evaporation

Using a rotary evaporator, the fluid concentrate was evaporated at a temperature of 64.7 degrees Celsius. A 100 ml container held the dense example. After collecting the pure concentration, measuring glasses were covered with aluminium paper and placed directly under the fan. used a hair drier to remove some of the water that was still in the concentrate in order to disappear the remaining dissolveable that was present.

5.1.6 Different type of test

I did diverse kind of test to distinguish the comprise of synthetic compound at this Terminalia cattapa plant and the actual properties this test is consolidated by-

- ✓ Phytochemical Screening
- ✓ Anti-stress test
- ✓ Anti-depressants test

5.2 Phytochemical Screening

5.2.1 Test Materials

- Extract of the whole plant (*Terminalia cattapa*)

5.2.2 Equipment's list for Phytochemical Screening

Name
Electronic Balance
Vortex Mixture
Test Tube
Test Tube Rack
Water Bath

Table 1: Equipment of Phyto-Chemical Screening Test

5.2.3 Reagent's list for Phytochemical Test

Name
Mayer's reagent
Dragendorff's reagent
Benedict's reagent
DPPH solution
5% of Ferric chloride solution
10% potassium dichromate solution
Dilute sulfuric acid
Distilled water
Aqueous sodium hydroxide
Conc. HCl
Conc. H2SO4

Table 2: Equipment of Phyto-Chemical Screening Test

5.2.4 Apparatus's list for Phytochemical Test

Name
Beaker (100ml & 50ml)
Funnel
Filter paper
Glass rod
Stand with clamp
Cotton
Measuring Cylinder
Test-tube

Spatula
Syringe
Measuring cylinder
Amber glass bottle (2500 ml)

Pipette
Micropipette
Amber reagent bottle
Light-proof box

Table 3: Apparatus of Phyto-Chemical Screening Test

5.2.5 Test for Alkaloids

Mayer's test: In a test tube, 2 ml of the extract solution and 0.2 ml of diluted hydrochloric acid were added. Potassium mercuric iodide, or Mayer's reagent, was then added in a millilitre. Precipitate that forms with a yellow hue suggests the presence of alkaloids.

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 millilitre of the Potassium Bismuth Iodide Solution, or Dragendroff's reagent, was added. Alkaloids are present when an orange-brown precipitate forms.

Wagner's Test: Two millilitres of the extract solution and one millilitre of Wagner's reagent (potassium iodide). Alkaloids are present when a brown or reddish precipitation forms.

5.2.6 Test for Glycosides

A test tube containing a small amount of alcoholic extract and 1 mL of water was filled with a few drops of aqueous NaOH. Glycosides are indicated by a yellow colouring.

5.2.7 Test for Gums

5ml solution of extract was added to Molisch reagent and sulfuric acid. When two liquids combine, a reddish-violet ring forms, signifying the presence of gum.

5.2.8 Test for Steroid

100mg extract was added of 1 ml acetic acid and 1 ml conc. H_2SO_4. Steroids were present when transformation from violet to bluish green.

5.2.9 Test for Saponin

20 ml distilled water solution was diluted with 1 ml of extract solution. After that, give it a good shake for 15 minutes, during which time clear foam will form, signifying the presence of saponin.

5.2.10 Test for Flavonoids

One millilitre of the crude extract was mixed with a few drops of concentrated hydrochloric acid. Instantaneous red colour formation indicates the presence of flavonoids.

5.3 Experimental animals

Swice albino mice (30-36g) were purchased from Jahangirnagar University, Dhaka, Bangladesh and their ages five to six weeks and were housed in animals 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.

5.3.1 Hole cross test

This experiment was carried out as previously described method by Uddin et al. (2006) [35] . A cage was used which have a size of 30 × 20 × 14 cm. A partition was fixed in the middle of the cage. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. Mice were treated with control, standard or extract and were placed in one side of the cage. The number of passage of a mouse through the hole from one chamber to other was then counted for a period of 3 min at 0, 30, 60, 90 and 120 min after the administration of the control, standard and test sample (p.o). Among six different groups of mice, group-I was considered as control and received distilled water (10 ml/b.w., p.o.) and group-II received diazepam (1 mg/kg, b.w., p.o.), which was considered as standard. Group III and IV received methanol fruit peels extract at the dose of 50 and 125 mg/kg body weight respectively. And group V and VI were treated with acetone leaves extract at the dose of 50 and 125 mg/kg body weight respectively.

Movements Inhibition (%)

$$\text{Movements Inhibition (\%)} = \frac{\text{Mean No. of movements (control)} - \text{Mean No. of movements (test)}}{\text{Mean No. of movements (control)}} \times 100$$

5.1.1 Hole board test

The hole-board test was carried out according to the formerly described method by Sheikh et al. (2016)

[36] with slight modifications. For the present experiment, a flat platform of 45 cm × 45 cm in diameter with 16 evenly spaced holes was used. This platform also had a wall of 5 cm height. All animals were divided into 6 groups, control and standard and test groups. Each group was containing five mice. Group-1 was considered as control and received distilled water (10 ml/b.w., p.o.). Group-2 received diazepam (1 mg/kg, b.w., p.o.), which was considered as standard. Group III and IV received methanol leaves extract at the dose of 50 and 125 mg/kg body weight respectively. And. After 30 min oral administration of control, standard and extracts each animal was kept on the center of the platform and allowed to move on the

platform. The number of head dips into the holes by individual mice was counted for 10 min.

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of head dips (control)} - \text{Mean No. of head dips (test)}}{\text{Mean No. of head dips (control)}} \times 100$$

5.1.1.1 Open field test

This experiment was carried out as described by Anisuzzman et al. (2017) [57] . The open field device consisted of a smooth field of half square meter with a series of squares. All squares alternatively painted in black and white. This test board is looks like a chess board. The apparatus also had a wall of 10 cm height. The animals were divided into 6 groups namely control, standard and test groups. Each group was containing 5 mice. Group-I was considered as control and received distilled water (10ml/b.w., p.o.). Group-II received diazepam (1mg/kg, b.w., p.o.), which was considered as standard. Group III and IV received methanol leaves extract at the dose of 50 and 125 mg/kg body weight respectively. The number of squares passed anyway by the animals was counted for 3 min started at 0, 30, 60, 90 and 120 min after oral administration of the test drugs.

$$\text{Movements Inhibition (\%)} = \frac{\text{Mean No. of movements (control)} - \text{Mean No. of movements (test)}}{\text{Mean No. of movements (control)}} \times 100$$

5.1.1.2 Blood collection

The investigational medications and samples were successfully administered to the mice for 7 days before they were killed and blood samples were collected. The cardiac puncture procedure was used to collect a blood sample of 2–3 ml. After centrifuging it, the serum was extracted from the blood and preserved for subsequent study.

5.1.1.3 Biochemical test

The investigation included two biochemical tests. The first is blood glucose, while the second is the Serum cortisol level. Both tests were carried out with spectrophotometry on a Backman Coulter AU-480 (model) machine.

Chapter: Six

Results & Discussions

6. Result & discussion

6.1. Table 4: Result of Phytochemical Test

Tested groups	Methanol extract
Tannins	+
Phenols	+
Flavonoids	+ +
Alkaloids	+ +
Glycosides	-
Tanins	+
Gums	+

(++) indicate more intensity,(+)indicates less intensity,(-) indicates absence

6.2 Result of neuropharmacological potential

Table 5 : CNS depressant activity test of leaves extracts by hole cross method

Group	Dose	Number of movement (% of movements inhibition)				
		0min	30 min	60 min	90 min	120 min
Control	10 ml/kg	4.7±0.82	6.2±0.39	6.2±0.53	6.3±0.32	8.1±0.18
Standard	1	1.6±0.56	1.2±0.37***	1.6±0.48***	1.4±0.68***	0.4±0.30***
Sample-1	50	1.8±0.51	1.7±0.384***	1.6±0.42***	0.7±0.34***	1.7±0.44***

Sample-2	125	1.8±0.78	1.4±0.62***	1.5±0.43***	1.4±0.42	1.8±0.37***

Values are presented as Mean ± SEM (n = 5), ***P < 0.001, which is significant compared with the control group (one-way ANOVA followed by tukey HSD.

Table 6: CNS depressant activity test by hole board method

Group	Dose(mg/kg)	Number of Head dips	% inhibition
Control	10ml/kg	45.6±2.97	0
Standard	1	19.8±2.97	57.01***
Methanol Extract(	50	22±2.97	28.50**
Methanol Extract	125	29.4±2.97	35.52***

Values are presented as Mean ± SEM (n = 5), ***P < 0.001, which is significant compared with the control group (one-way ANOVA followed by tukey HSD.

Table 7 : CNS depressant activity test of leaves extracts by open field method

Group	Dose	Number of movement (% of movements inhibition)				
		0min	30 min	60 min	90 min	120 min
Control	10 ml/kg	28.8±1.90	29±2.48	28.8±2.15	34±0.38	37.6±2.01
Standard	1	13.2±2.33	14.5±3.61*	16.7±3.49**	19±3.11***	19.8±3.48***
Sample-1	50	17.2±1.16	16.8±1.32*	16.4±2.91**	16±1.84***	17.6±2.28***

Sample-2	125	15.4±1.03	17.4±1.21*	18±1.34**	19.8±1.91**	24±2.23**

Values are presented as Mean ± SEM (n = 5), ***P < 0.001, which is significant compared with the control group (one-way ANOVA followed by tukey HSD

6.3 Biochemical Test

6.3.1 Effects of Herbal sample, Standard drugs on blood glucose

Table 8: Effects of Herbal sample, Standard drugs on blood glucose for Chemical induce Stress

Group Name	**Medication & Herbal**	**Blood glucose (mmol/L)**	**Normal Range (mmol/L)**
Control group	Water	4.83±0.450925	4.4-5.6
Group-2 (Anti-depressant)	Clonazepam	5.16.1±0.888819	
Group-3 Sample I	Indian almond Leave extract at a dose 0.2 g/ kg body weight.	5.43±1.047728	
Group-4 Sample II	Indian almond Leave extract at a dose 0.4 g/ kg body weight.	5.12±0.455009	

Table-9: Effects of Herbal sample, Standard drugs on blood glucose (for Heat induced)

Group Name	**Medication & Herbal**	**Blood glucose (mmol/L)**	**Normal Range (mmol/L)**
Control group	Water	10.91± 1.328847	
Group-2 (Anti-depressant)	Clonazepam	4.59± 0.372872	

Group-3 Sample I	Indian almond Leave extract at a dose 0.2 g/ kg body weight.	5.78± 0.517977	4.4-5.6
Group-4 Sample II	Indian almond Leave extract at a dose 0.4 g/ kg body weight.	4.83±0.659874	

Discussion:

Data expressed as mean ± SEM (Standard Error Mean) where n= 3 number of mice for a single group. According to the table, the control group's blood glucose level was 4.83±0.450925 mmol/l, which means that no changes occurred in the level since it was within the normal range. On the other hand, animals in the standard group had glucose levels that were within the usual range (5.16 ±0.888819 level mmol/l), demonstrating that they are quite adept at minimizing stress. However, the blood glucose level of the herbal low dose sample group was 5.78± 0.517977 mmol/l, which was some beyond the normal range of glucose. And blood glucose level of herbal high dose sample group was 4.83±0.659874 that helps to minimizing stress.

6.3.2 Effects of Herbal sample, Standard drugs on serum cortisol

Table 10: Effects of Herbal sample, Standard drugs on serum cortisol (for chemical induction method)

Group Name	**Medication & Herbal**	**Serum cortisol (ug/ dl)**
Control group	Water	2.30±0.110604
Group-2 (Anti-depressant)	Clonazepam	1.32± 0.179536
Group-3 Sample I	Indian almond Leave extract at a dose 0.2 g/ kg body weight.	2.98± 0.135

Group-4 Sample II	Indian almond Leave extract at a dose 0.4 g/ kg body weight.	2.18± 0.29 .

6.3.2 Effects of Herbal sample, Standard drugs on serum cortisol

Table-11: Effects of Herbal sample, Standard drugs on serum cortisol (for heat induction method**)**

Group Name	**Medication & Herbal**	**Serum cortisol (ug/ dl)**
Control group	Water	3.32± 0.543415
Group-2 (Anti-depressant)	Clonazepam	1.47± 0.341955
Group-3 Sample I	Indian almond Leave extract at a dose 0.2 g/ kg body weight.	2.23±0.121244
Group-4 Sample II	Indian almond Leave extract at a dose 0.4 g/ kg body weight.	1.83± 0.075719 .

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

Discussion:

The table expressed that the serum cortisol level for control group in case of chemical induced stress was 1.32± 0.179536 ug/ml and for heat induced it was 3.32± 0.543415
which indicates that stress was induced in the control group animals and it also support that
stress condition can be formed in animals in high temperature and also by chemical imduction.
On the other hand, in standard group animals the cortisol level of Chemical induced mice were 1.32± 0.179536 ug/ml and for heat induced mice it was 1.47± 0.341955 which manifested that they are highly capable in reducing stress and the level was within the normal range. A great finding was observed in herbal sample group where serum cortisol level
1.83± 0.075719 was ug/ml, so this result identified that herbal plants has the capacity in lowering stress in animals.

Chapter: Seven
Conclusion

7. Conclusion:

It's evident that the search for novel medications is placing an increasing emphasis on natural products. *Terminalia Cattapa* is a very helpful species for the discovery and use of natural medications. The research carried out in this study demonstrated that the methanolic extract of *Terminalia Catappa* contained organic components such as carbohydrates, alkaloids, glycosides, phenol, and tannins that may be implicated in pharmacological activity. The current investigation disclosed CNS depressed and antistress behaviors. Further investigation is necessary, but in this analysis, the extract from Terminalia cattapa was useful in identifying several chemicals that are expected to function in a certain way and have a specific effect. It is now possible to conclude that the plant has potential applications as a CNS depressive and an antistress agent based on the findings. But this was merely an experiment. Therefore, more thorough investigation is required to identify active chemical

Chapter: Eight
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