Phytochemical profiling, and neuroendocrine studies of methanolic extract of *Malva parviflora* in experimental wester albino mice

PROJECT WORK

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

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Approval

Phytochemical profiling and neuroendocrine studies of methanolic extract of *Malva parviflora*" was submitted to the Department of Pharmacy, Faculty of Health and Life Science, Daffodil International University and accepted as satisfactory for partial fulfilment of the requirement for the Bachelor of Pharmacy degree and approved in terms of style and content.

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Prof. Dr. Muniruddin Ahmed

Head & Professor

Department of Pharmacy

Faculty of Allied Health Science

Daffodil International University

_____ **Internal examiner-1**

_____ **Internal examiner-2**

_____ **External Examiner**

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First and foremost, I am grateful to Almighty Allah for providing me the ability to finish my project work and the chance to focus on this problem.

A project is not a person's job. It is more than a collection of ideas, suggestions, reviews, contributions, and collaborative efforts. The success and ultimate conclusion of a project needed a lot of direction and support from many individuals, and I feel tremendously blessed to have received this all the way through to the completion of my project.

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The Author,
ABDULLAH ALL RIZON

Dedication

I dedicate this work to my parents first, followed by my esteemed teacher who have always supported and encouraged me.

Declaration

I so declare that the project work title "Phytochemical profiling and neuroendocrine studies of methanol extract of *Malva parviflora*" is required to meet the criteria of the Daffodil International University Faculty of Health and Life Science's Bachelor of Pharmacy (B.Pharm) program.

Supervised By

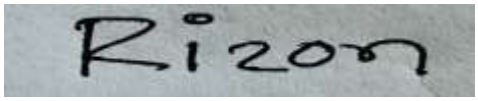
Md. A K Azad

Assistant Professor

Department of Pharmacy

Daffodil International University

Submitted By



ABDULLAH ALL RIZON

ID: 201-29-334

Department of Pharmacy

Faculty of Health & Life Sciences

Daffodil International University

Abstract

In everyday life, we have to deal with stress, sadness, and depression which is very common in that time. Neurodisorders disrupt our personal and social life. The purpose of this research was to investigate the neuro-pharmacological characteristics of *Malva parviflora* leaves. CNS activity were investigated through hole cross, hole board and open field tests at varied extract dosages(50 & 125mg) per kg body weight, also aims to examine how Malva parviflora, a plant, may reduce stress levels by measuring blood glucose and serum cortisol levels. And To determine the impact of antidepressant medications on blood glucose and serum cortisol levels in experimental mice . In case of 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. On the other hand, Blood was collected by a cardiac puncture, and glucose and cortisol levels were measured using a spectrophotometer. The blood glucose level for herbal sample (chemical induction) were respectively 4.75 ±0.27 for high dose and low dose 3.76± 0.41 in compare to control were 4.83±0.88 mmol/L. It was 5.16±0.88 for standard groups. Secondly for Heat Induction The blood glucose level for herbal sample were respectively 4.85±0.37 for high dose and low dose 5.43± 0.04 comparing with control group which was 10.91±1.32 mmol/L. Where standard was 4.59±0.88 . For Serum cortisol level in Chemical Induced test 2.78± 0.10 & 1.51± 0.21 ug/dl for two plant extract and in standard group drug that was 1.32± 0.17 ug/dl & for control group it was 2.30± 0.11 and in heat induction, for control it was3.32± 0.54, for standard 1.47± 0.34 and lastly in terms of sample 200mg level was 2.97± 0.11 and for sample 400mg 2.12± 0.54 . According to this research, herbal plant have ability to reduce stress within a normal range, although further research is required to substantiate this claim .

Key words: Stress, Serum Cortisol, *Malva parviflora*, mice, Antidepressant

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Chapter: one

Introduction

1. Introduction

1.1 General Introduction:

Plants and humans are inherently interconnected and cannot be separated.

Medicinal plants, often known as medicinal herbs, have been identified and used in traditional medical procedures since ancient times. [1] Nature has always supplied humanity with the necessities of life, including drug sources, shelters, food, clothes, flavours, and means of transportation. [2] Stress is described as a condition of anxiety or tension in the mind brought on by a challenging circumstance. [3] The detrimental effects of stress may affect people of all ages, wealth, and poverty. There is a lot of strain in everyday living. To exist in this atmosphere, one must be able to cope with the inevitable stress. For example, psychological tension may make someone more susceptible to physical sickness. stressors related to the environment, psychology (emotional), and biology. [4] There is a complicated and nuanced link between stress and sickness. Everybody handles stress in a different way. It is not a given that anything that sickens one person will also sicken another. A disease may only arise when certain triggers mix with a number of prior conditions. Stress may affect people differently; genetic, coping, personality, and social support factors all play a part. We assess a scenario's seriousness and search our inventory for the required materials. If we believe the problem is serious but are unprepared to address it, we will get stressed. [5]

Depression is a mental condition characterised by chronic sorrow and a lack of interest. Also known as major depressive disorder or clinical depression, it affects how you feel, think, and act and may cause a number of emotional and physical problems. [6] One of the main causes of morbidity and death is disorders associated to the neuroendocrine system[7]. neuroendocrine-related illnesses may lead to general headings such as "cardiovascular disease" or "cancer and in some specific cases this disorder can cause of death.[8]

According to one research, adult populations today have much greater rates of anxiety (33.7%), stress (59.7%), and depression (57.9%) than they had before to the epidemic. According to a different research, tension, anxiety, and depressive symptoms were present in 28.5%, 33.3%, and 46.92% of pupils who were placed in home quarantine [9].

1.2. Bangladesh's current state of medicinal plants

There are an estimated 6500 plants in Bangladesh, of which over 500 species are considered medicinal, and 250 of them are frequently utilised to make medications for medical use. [10]

1.3 Necessities of medicinal plants

Throughout human history, medicinal plants have had a tremendous impact on healthcare. They are vital sources of traditional medicine and the foundation for many contemporary medications. Even with the advances of contemporary medicine [11]. Since ancient times, medicinal plants—also known as medicinal herbs—have been identified and used in conventional medical procedures. For a variety of purposes, including defence and protection against insects, fungus, illnesses, and herbivorous animals, plants synthesise hundreds of chemical compounds [12].

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 used to promote health as well as identify, locate, treat, or prevent mental and physical instability. Up to 80% of Ethiopians get vital medical treatment via 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 traditions, has long helped people prevent illness and lead healthy lives. Native Americans have historically used a variety of well-known medical systems, including those from China, India, and Africa, in addition to their creative interpretation of their customary medical arrangement. [13]

1.5 Strategies for finding new product

A bioactivity-guided strategy involves three 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. Later, the raw material is fractionated. 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.[14]

1.6 Valuable and Significant characteristics of medicinal plants

Nearly as long as people have lived, plants have been utilised as medicine. The fact that restorative plants are often suggested because of long-standing ideas about their potential and utility, even when the precise composition of these plants is not usually known, emphasises their therapeutic capabilities even more. Senna alata, for instance, is often used to address viral and bacterial contaminations. Additionally, they demonstrated varied degrees of antibacterial and antifungal action against microorganisms. Research has shown that flavonoids have more potent antifungal and antibacterial properties against a select group of human pathogenic parasites and microscopic organisms. Because a restorative plant has specific bioactive components, its potential applications are predicted. These advantageous components are found

using phytochemical screening methods such as dainty layer chromatography and phytochemical tests. Restorative plants include a variety of auxiliary metabolites, including as flavonoids, terpenoids, alkaloids, and tannins. The antibacterial properties of the plants are mostly due to these metabolites. Studies have shown the efficacy of flavonoids and tannins as phytochemical components against pathogenic bacteria, such as Bacillus cereus and Staphylococcus aurous.

Restorative herbs are helpful in making sterile cleansers, which are often used for washing or removing skin surfaces, due to their tannin concentration. It has been shown in the literature that bacteria, yeasts, and filamentous organisms are negatively impacted by phytochemicals. Moreover, they have been shown to block viral reverse transcriptase. It has been shown that saponins, an auxiliary metabolite with antifungal effects, are important. The antibacterial effect of tannins, phenols, steroids, and saponins is believed to be attributed to their many physiological actions, many of which are assumed to be more strong.[15][16]

1.7 Phytochemicals

A phytochemical is a substance synthesised by plants. Their roots may be traced back to the Greek terms "phyton" (meaning plant) and "chema" (meaning chemistry). These heterogeneous compounds serve different functions in the plant's physiology [17]. Phytochemicals may provide humans with similar protective benefits. Although certain phytochemicals have similarities with vitamins, they are not considered important for human health in the same way as vitamins and minerals. Instead, they safeguard your cells from harm produced by environmental pollutants and the body’s normal chemical (metabolic) processes[18]. On the basis of their chemical characteristics and structures, phytochemicals are roughly categorised into six categories: carbohydrates, lipids, phenolics, terpenoids, alkaloids, and other nitrogen-containing substances [19].

1.7.1 Alkaloids

Any of the alkaloids, or bases that include nitrogen, that are present in a wide range of organisms. Alkaloids have a variety of physiological impacts on people and other animals. Things like triclosan, ephedrine, morphine, and vape are examples of alkaloids. These sorts of blooming plants are very rich in alkaloids, which are frequently found in nature. To be honest, alkaloids have been detected in several thousand distinct types, and it is believed that up to 25% of higher plants contain them. An animal species typically has just a few different types of alkaloids [20].

1.7.2 Glycosides

Some plants that contain anthraquinone glycosides include rhubarb, cascara, and alexandrin. Plant-based purgatives are made from plants including aloe, rhubarb, and senna. These wonderful medications are called cardiac glycosides, and they are derived from medicinal plants like foxglove and lily of the valley. Among them are digoxin and digitoxin, which induce heart palpitations and act as diuretics. Certain irregular pulses and cardiovascular collapse are treated with drugs called cardiovascular glycosides. They are among the select few classes of medications used to treat cardiac conditions and related illnesses [21].

1.7.3 Flavonoids

Flavonoids are a diverse group of polyphenolic compounds found abundantly in plants, which have a range of biological purposes in addition to contributing to colour. The 15-carbon skeleton that makes up the chemical structure of flavonoids is joined to two aromatic rings (A and B) by a three-carbon chain to create the heterocyclic ring C. By changing this basic structure, a variety of flavonoid subclasses, including flavones, flavonols, flavanones, flavanols (catechins), anthocyanins, and isoflavones, may be generated. Fruits, vegetables, grains, tea, wine, and herbs are rich sources of flavonoids. They are necessary for plant defence mechanisms against infections, pigmentation, and UV radiation protection. Their biological activities also include anti-inflammatory, antibacterial, anticancer, cardioprotective, and neuroprotective properties, all of which are beneficial to human health. Their antioxidant effect is attributed to their ability to bind transition metal ions and scavenge free radicals. Flavonoids also have anti-inflammatory qualities via their regulation of immune response and inflammation-related signalling pathways. They have also been connected to the treatment and avoidance of chronic ailments including diabetes, cancer, heart disease, and neurological disorders. Studies have shown a correlation between regular consumption of foods rich in flavonoids and a decreased risk of getting various disorders. The ability of the human body to absorb and metabolise flavonoids, however, differs depending on a variety of factors, such as personal traits, meal composition, and chemical structure. Understanding the pharmacokinetics and health effects of flavonoids is essential to optimising their therapeutic potential.[22]

1.8 Neuroendocrine Potential

The phrase "neuroendocrine potential" refers to the capacity of a chemical to interact with and impact the neuroendocrine system. This complicated system combines the neurological system with the endocrine system, allowing for precise communication between hormones and nerve cells. Substances having neuroendocrine potential may have large influence on numerous physiological systems, making them attractive instruments for study and possible therapeutic uses [23]. When neuroendocrine confronted with stress, the body triggers the "fight-or-flight"

response, initiated by the Hypothalamic-Pituitary-Adrenal (HPA) axis. This complicated process leads to the production of cortisol, a stress hormone. Cortisol mobilizes energy resources, heightens awareness, and prepares the body for action. Neurotransmitters including serotonin, dopamine, and norepinephrine, affected by numerous hormones, play key roles in regulating mood and motivation. Disruptions in these circuits lead to depressed symptoms [24][25]. Antidepressant are a family of medications which reduces symptoms of depression illnesses by repairing chemical disequilibrium of neurotransmitters in the brain that are responsible for change in mood as well as behavior. Serious side effects and unpleasant effects connected with antidepressant medicine including dry mouth, weariness, anxiety agitation, sleepiness [26][27]

1.8.1 Stress Management

The cholinergic hypothesis of Alzheimer's disease proposes that cholinergic systems in the basal forebrain are destroyed at the outset of the disease, resulting in memory loss and impairment of other cognitive and noncognitive processes, including neuropsychiatric symptoms. It has been suggested that CIs may be employed to delay acetylcholine breakdown across synaptic clefts, hence increasing cholinergic transmission. Currently, three CIs have been licenced to treat moderate to severe Alzheimer's disease. Donepezil, Rivastigmine, and Galantamine [28][29].

1.8.2 Depression Treatment

Antidepressant Products: TCAs antidepressant (tricyclic &tetracyclic)

Nonselective serotonin reuptake inhibitor is less effective than Clomipramine, like Amitriptyline, doxepin, amoxapine is much more effective than NRI. Serotonins action specifically blocked by serotonin reuptake inhibitor

(Reboxetine) is a drug that blocks the reabsorption of dopamine into the brain. [30].

1.9 Objective of study

This research focuses on the phytochemical and some particular pharmacological analysis of the plants. It may be spotted at the numerous sites in Bangladesh. It has a broad variety of traditional and therapeutic applications. The goal of this study was to evaluate the possible actions of winter cabbage. For the analysis, the leave extract is used. There are several grounds for bringing this plant to exploration. Like as-

- To learn about *Malva Parviflora.*
- To identify the Phyto-Chemicals of *Malva Parviflora.*
- To see the Ant-stress activity of *Malva Parviflora.*
- To know the antidepressant activity of *Malva Parviflora.*

Chapter: Two
Plant profile

2. Plant Profile

2.1 Introduction of Plant

Malva parviflora) is a flowering plant from the Malva genus in the Malvaceae family. Its leaves and young plant tips are consumed as greens or leafy vegetables. Malva parviflora is an annual or perennial plant that is native to Northern Africa, Europe, and Asia but is extensively naturalised elsewhere. t is traditionally produced as a winter vegetable in Bangladesh and India, however it may now be grown year-round save except the monsoon season. This vegetable is grown in the Rangpur Division in Northern Bangladesh[31][32].

Figure-1: Malva parviflora Plant

2.1.1 Plant name [33]

Scientific Name: Malva parviflora

Common Name: cheeseweed

2.2 Taxonomical classification [34]

- **Kingdom**: Plante
    - **Class:** Malvales
    - **Order:** Malvales
    - **Family:** Malvaceae
    - **Genus:** Malva
    - **Species:** *Malva parviflora*

2.3 Morphology

In tropical parts of Africa, Malva parviflora L. is an annual or perennial plant with a decumbent or erect habit. That may reach a height of 50 cm. Most people call it cheeseweed. The wide leaves are 8–10 cm in diameter and contain 5-7 lobes. It has tiny, 4-6 mm-long petals on its white or pink blossoms [35].

2.4 Medical benefit [36]:

- Antioxidant .
- Anti-inflammatory.
- Antimicrobial.
- Antiulcer.
- Antidiabetic.
- Anti-irritant.
- Hepatoprotective.
- Analgesic.
- Wound healing properties**.**

2.5 Traditional use

This plant is used to treat inflammation traditionally.

Chapter :Three

The goal of the research

3. The goal of the research

The goal of the research is to Screening of chemical constituents and Pharmacological activity investigation of *Malva Parviflora* .

- **Screening of chemical constituents:** Based on the findings of this research, a number of phytochemical activities, including the presence or absence of alkaloids, glycosides, flavonoids, phenolic compounds, etc. in those plants, will be evaluated.
- **Pharmacological activity investigation:** An additional goal of this research is to examine the scientific foundation of plant leaves' CNS-depressant and anti-stress properties as potential pharmaceutical uses.

Chapter: Four
Literature review

4. Literature Review

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

Malva parviflora, sometimes called cheeseweed, has been historically used in several regions

for its therapeutic qualities. Multiple studies have emphasized Malva parviflora's promise in terms of its pharmacological properties, such as its antidepressant and anti-stress benefits. Malva parviflora has been documented for its anti-inflammatory effects and strong

antioxidant capacity. The plant has shown antiulcerogenic, analgesic, antidiabetic, and

neuroprotective properties as evidenced by studies conducted by Mohammed et al. (2023)

and Mallhi et al. (2014). These characteristics suggest the capability of Malva parviflora in

alleviating stress and associated disorders. The plant has a history of being used as a hepatoprotective agent, and its possible hepatoprotective action may have positive effects on general health, perhaps influencing stress and depressed states. The plant has been noted for its antibacterial and antifungal capabilities, as described by Kalayou et al. (2012), which might enhance its medicinal benefits. The many pharmacological effects of Malva parviflora, including as anti-inflammatory, antioxidant, hepatoprotective, and antibacterial characteristics, indicate its potential for treating disorders associated with stress and depression. Additional study is needed to clarify the precise processes responsible for its antidepressant and anti-stress properties. [37-51]

A Comprehensive Review of Malva parviflora: Ethnobotanical, Medicinal, and Biological Perspectives

Malva parviflora, commonly known as cheeseweed mallow, is a plant species with significant

ethnobotanical, medicinal, and biological importance. This review seeks to cover all aspects of Malva parviflora., including its traditional uses, medicinal properties, biological characteristics, and potential applications. The synthesis of the literature on Malva parviflora encompasses its ethnobotanical significance, medicinal applications, biological attributes, and potential implications in various fields. Malva parviflora holds substantial ethnobotanical significance, as evidenced by its traditional uses in different cultures. It has been reported to be commercialized in the markets of La Paz and El Alto, Bolivia, for its medicinal properties (Macía et al., 2005). Additionally, it has been chosen for study in the context of green roof design and energy-saving initiatives in residential buildings under a semi-arid Mediterranean climate (Abdin et al., 2018). Furthermore, studies have highlighted its distribution and associated species in the Nile Delta,

Egypt, emphasizing its therophytic nature and Mediterranean distribution (Shehata & Galal, 2014). The medicinal properties of Malva parviflora have been a subject of extensive research. It hasbeen investigated for its potential in ameliorating the deleterious effects of a high-fat diet on cognitive function, particularly in the context of Alzheimer's disease (Medrano-Jiménez et al., 2019). Furthermore, the plant has been associated with the elucidation of bioactive compounds with antibacterial activity, such as β-sitosterol from its root bark (Ododo et al., 2016). Moreover, its hypoglycemic activity has been evaluated in streptozotocin-induced diabetic rats, indicating its potential in managing diabetes (Gutierrez, 2012). Additionally,Malva parviflora has been reported to possess anti-inflammatory, free radical-scavenging, and metal-chelating activities, further underlining its medicinal potential (Bouriche et al., 2011). The biological attributes of Malva parviflora encompass its seed dormancy, genetic diversity, and response to environmental factors. Studies have highlighted its hard impermeable seed coat, contributing to its strong dormancy and resistance to ensiling (Piltz et al., 2017; Michael & Steadman, 2006). Furthermore, genetic diversity analyses have provided insights into the relationships among medicinal species of Malva, including Malva parviflora (Ghafoor & Hamarashid, 2022). Additionally, the plant's response to environmental stressors, such as exposure to cadmium, has been investigated, shedding light on its antioxidant bioindicators and growth responses (Zoufan et al., 2018). The potential applications of Malva parviflora span various domains, including agriculture, environmental science, and nanotechnology. It has been identified as a serious weed in Australian farming systems, indicating its impact on agricultural practices (Michael & Steadman, 2006). Moreover, its invasive nature and allelopathic effects have been noted, particularly in the context of no-till farming systems (Abbas et al., 2020). Furthermore, the plant has been utilized for the biosynthesis of silver nanoparticles, showcasing its potential in nanotechnology and material science (Al-Otibi et al., 2021; Oraibi et al., 2022).[52]

Chapter : Five
Materials And Method

5. Materials and Method

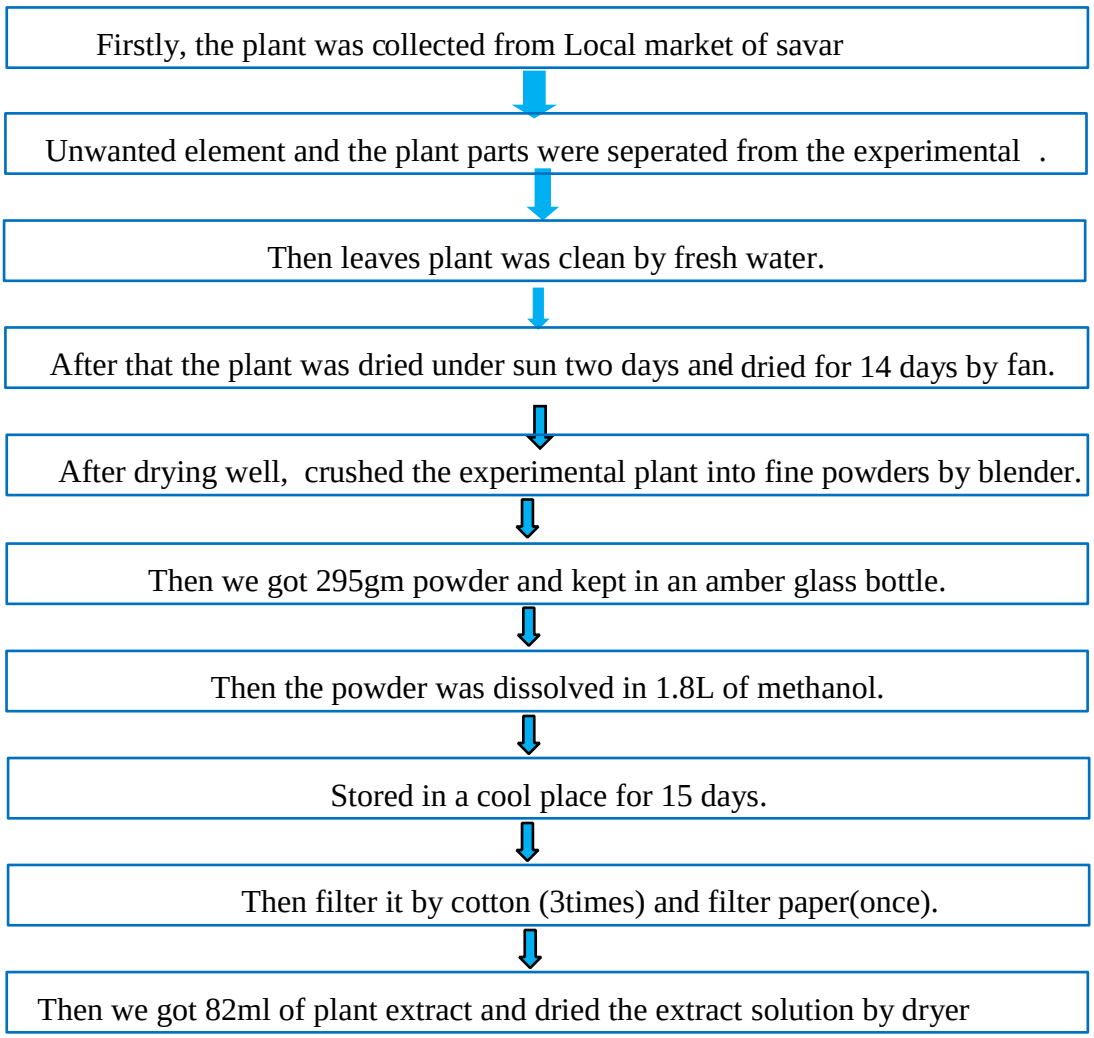
5.1 Extract Preperation

5.1.1 Collection of Plant

This plant was collected from local area of Savar, Dhaka, Bangladesh.

5.1.2 Preparation of plant material

Unrefined is a medicine restricted from this plant and the creature and use this with no substance alteration. I organised unrefined concentrate in a few phases.



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5.1.3 Extraction

After providing a bottle jar with a glass a thorough washing, methanol was used to rinse it. Subsequently, 1.8L of methanol was poured to the jar holding 295gm of dry materials, Approximately one inch above the sample surface., until the sample was sufficiently covered. The jar was appropriately closed using a plastic cover coated in aluminium foil to protect air from getting inside. It seemed as if the process took fifteen days. Almost two to three times a day, the jar was shook to enhance extraction.

5.1.4 Filtration

Following extraction, the plant extract was filtered through sterile cotton and filter paper. The cotton and paper were then rinsed with the appropriate solvent and put into a funnel. The collected filtrate was stored in a glass jar. And performed the refiltration procedure one again.

Figure-2: Filtration of Malva Parviflora

5.1.5 Evaporation

Using a rotary evaporator, the fluid concentration was evaporated at 64.7 degrees Celsius and 30 RPM.. The dense example was stored in a 100 mL jar. After obtaining the pure concentration, measurement glasses were wrapped in aluminium paper and put immediately under the fan. utilised a hair dryer to eliminate part of the water that was left in the concentrate in order to vanish the remaining dissolveable that was there..

Figure-3: Evaporation of Malva Parviflora

5.1.6 Different type of test

I conducted a variety of tests to identify the composition of synthetic compounds in this Malva Parviflora plant from the true qualities. This test is consolidated by-

- Phytochemical Screening
- Antidepressant Test
- Anti-stress Test

5.2 Phytochemical Screening

5.2.1 Test Materials

- Extract of the whole plant (Malva Parviflora)

5.2.2 Equipment's list for Phytochemical Screening

Serial No.	Name
1	Electronic Balance
2	Dropper
3	Test Tube
4	Test Tube Rack
5	Water Bath

Table 1: Equipment of Phyto-Chemical Screening Test

5.2.3 Reagent's list for Phytochemical Test

Serial No.	Name
1	Mayer's reagent
2	Dragendorff's reagent
3	Benedict's reagent
4	DPPH solution
5	5% of Ferric chloride solution
6	10% potassium dichromate solution
7	Dilute sulfuric acid
8	Distilled water
9	Aqueous sodium hydroxide
10	Conc. HCl
11	Conc. H2SO4

Table 2: Equipment of Phyto-Chemical Screening Test

5.2.4 Apparatus's list for Phytochemical Test

Serial No.	Name
1	Beaker (100ml & 50ml)
2	Funnel
3	Filter paper
4	Glass rod
5	Stand with clamp
6	Cotton
7	Measuring Cylinder
8	Test-tube
9	Spatula
10	Syringe
11	Measuring cylinder
12	Amber glass bottle (2500 ml)
13	Pipette
14	Micropipette
15	Amber reagent bottle
16	Light-proof box

Table 3: Apparatus of Phyto-Chemical Screening Test

5.2.5 Test for Alkaloids

Mayer's test: included mixing 2 ml of extract solution with 0.2 ml of diluted hydrochloric acid in a test tube. One millilitre of potassium mercuric iodide, often known as Mayer's reagent, was then added. The yellowish buff precipitate suggests the presence of an alkaloid.

Dragendroff's Test: In a test tube, 2 millilitres of extract solution were combined with 0.2 millilitres of diluted hydrochloric acid. Dragendroff's reagent, potassium bismuth iodide solution, was then added in millilitres. The orange-brown precipitate that develops contains alkaloids.A test tube containing a little amount of alcoholic extract and 1 mL of water was filled with a few drops of aqueous NaOH. Glycosides are identified by their yellow color

5.2.6 Test for Tanin

Two millilitres (2 mL) of the methanol solution of the extract was combined with couple drops of a 10% ferric chloride solution (bright yellow). A greenish-black precipitate showed the presence of tannin.

5.2.7 Test for Glycoside

A little quantity of methanolic extract was mixed with one millilitre of water, and two or three drops of aqueous NAOH were added. The presence of glycoside was shown by the yellow colour.

5.2.8 Test for Phenol

Two millilitres of a 5% neutral ferric chloride solution were combined with one millilitre of the extract; the dark blue colouring indicated the presence of phenolic chemicals.

Benedict's test

After mixing Benedict's reagent with one millilitre of the analyte sample, place the mixture in a boiling water bath and heat it for three to five minutes. The analyte's reducing sugar content is verified by the appearance of a brick-red cuprous oxide precipitate.

Fehling's test (Standard test)

Equal volumes of Fehling's solutions A and B were mixed with two millilitres of the plant material's aqueous extract and one millilitre each. boiled for a little while. Reduced sugar resulted in the formation of a crimson or brick red precipitate.

5.2.9 Test for Flavonoids

A little amount of strong hydrochloric acid was combined with one millilitre of the crude extract. The presence of flavonoids is indicated by the immediate production of a red hue.

5.3 Pharmacological Potential

Chemicals and reagents

Diazepam are used as standard drug which was purchased from Beximco Pharmaceuticals Ltd.

5.4 Experimental design

For this research, Wister Albino mice were procured from the Comilla University Lab. Male albino mice weighing between 30 and 40 g were utilised in the studies. Five days before the experiment, the mice were kept in colony cages in the department's animal room, which was kept at a constant temperature of 25 to 30 degrees Celsius. Every day, the bedding was changed to maintain a clean environment.

Heat induction and chemical induction methods were used for stress induction.

The rats were split up into four groups for the research, with three rats in each group.

The four groups were:

- Group-1 (Control)
- Group-2 (Standard)
- Group-3 (Sample 200mg)
- Group-4 (Sample 400mg)

5.5 Medication and Herbal combination

Table 1: Medications and herbal combinations were used throughout the trial

Group	**Medication & Herbal combination**	**Duration**
Control Group(Group-1)	Normal water	7 days
Standard drug(Group-2)	Clonazepam	7 days
Sample 200 Dose(Group-3)	Cheeseweed extract at a dose 200mg per kg body weight.	7 days
Sample 400mg (Group-4)	Cheeseweed extract at a dose 400mg per kg body weight.	7 days

5.6 Blood Collection

The mice were successfully administered the experimental medications and samples for seven days before being put to death and blood collected. The cardiac piercing procedure was used to collect a blood sample of 1-1.5 ml. The serum was isolated from the blood and preserved for future analysis following centrifugation.

5.7 Biochemical test

The investigation included two biochemical tests. The first measure is blood glucose levels, followed by serum cortisol levels. Spectrophotometry was employed in both studies, utilising a Backman Coulter AU-480 (model).

5.8 CNS depressant activity

Hole cross test

This experiment was conducted using the methodology that Uddin et al. (2006) had previously explained [53]. A cage of 30 × 20 x 14 cm was used. The cage's midsection was fastened with a divider. In the middle of the cage, a 3 cm diameter hole was drilled at a height of 7.5 cm. Mice were housed on one side of the cage and given either control, standard, or extract treatment. Following the injection of the control, standard, and test sample (p.o.), the number of mice passages through the opening from one chamber to the next was then counted for a duration of 3 min at 0, 30, 60, 90, and 120 min. Out of the six mouse groups, group I was given distilled water (10 ml/b.w., p.o.) and was regarded as the control group. Group II, on the other hand, got diazepam (1 mg/kg, b.w., p.o.), which was regarded as the standard. Methanolic leaf extract was administered to Group III at doses of 50 and 125 mg/kg body weight, respectively.

Movements Inhibition (%)

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

Hole board test

The hole-board test was carried out according to the formerly described method by Sheikh et al. (2016) [54] with a few minor adjustments. A 45 cm diameter by 45 cm flat platform with 16 holes spaced equally was employed for the current experiment. This platform also included a five-centimeter-tall wall. Six groups were created out of all the animals: control, standard, test, and group. Every group consisted of five mice. Group 1 was given distilled water (10 ml/b.w., p.o.) and was regarded as the control group. Group 2 was given regular doses of diazepam (1 mg/kg, b.w., p.o.). Methanol fruit peel extract was given to groups III and IV at doses of 50 and 125 mg/kg body weight, respectively. Additionally, acitone fruit peel extract was administered to groups V and VI at doses of 50 and 125 mg/kg body weight, respectively. Each animal was placed in the middle of the platform and given free reign to wander about it after receiving oral administration of the control, standard, and extracts for 30 minutes. For ten minutes, each mouse's head dips into the holes were tallied.

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

Open field test

This experiment was carried out as described by Anisuzzman et al. (2017) [55]. The open field gadget was a half-square-meter smooth field with a grid of squares. Every square was painted in black and white alternately. This test board has a chessboard-like appearance. Additionally, the equipment had a 10-cm-tall wall. Six groups were created from the animals: control, standard, test, and group. Every group consisted of five mice. Group I was given distilled water (10 ml/b.w., p.o.) and served as the control. Diazepam (1 mg/kg, b.w., p.o.) was administered to Group II, which was regarded as standard. Methanol fruit peel extract was given to groups III and IV at doses of 50 and 125 mg/kg body weight, respectively. Additionally, acetone fruit peel extract was administered to groups V and VI at doses of 50 and 125 mg/kg body weight, respectively. After the test medications were given orally to the animals for 0, 30, 60, 90, and 120 minutes, the number of squares that the animals traversed in either case was tallied for three minutes.

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

Chapter: Six

Result & Discussions

6. Result and discussion

6.1 Table 2: Result of phytochemical test

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

(++)indicates high intensity ,(+)indicates low intensity, (-) indicates absence

6.2 Result of neuropharmacological potential

Statistical analysis

Version 20 of the SPSS statistical software was used to analyse the data. Means ± SEM were used to represent the results. One way ANOVA was used to compare the groups, and Tukey HSD was then used for the open field, hole board, and hole cross tests.

Table 3: CNS depressant activity test of leave 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	1.9±0.61	1.7±0.294***	1.5±0.52***	0.6±0.24***	1.5±0.34***
Sample-2	125	1.7±0.64	1.5±0.38***	1.3±0.31***	1±0.22	1.6±0.57***

The data is shown as Mean ± SEM (n = 5), with a significant difference ***P < 0.001, which is significant compared with the control group (one-way ANOVA followed by Tukey HSD).

Effect of leave extracts exposed that the oral administration of leave experimental plant in all doses (50 and 125 mg/kg) caused a significant (***P<0.001) reduction in number of hole crossed.

Table 4: CNS depressant activity test of fruit peel extracts 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	33.88±2.97	25.70**
Methanol Extract	125	27.4±2.97	39.92***

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 5: CNS depressant activity test of fruit 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	19±1.72	17±3.94*	16.2.±2.71**	16±1.78***	17.7±2.18***
Sample-2	125	15±0.64	18.4±1.21*	17±1.34**	19.8±1.91**	22±2.12**

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. experimental extracts at tested doses produced significant (*P< 0.05, **P< 0.01, ***P<0.001) inhibition of locomotion that was brought up from 30 min to 120 min of observation period.

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6.3 Biochemical Test

6.3.1 Effects of standard medications and a herbal sample on blood glucose

Table-6: Effects of Herbal sample, Standard drugs on blood glucose (for Chemical induced)

Group Name	Medication & Herbal	Blood glucose (mmol/L)	Normal Range (mmol/L)
Control group	Water + Dexamethasone	4.83±0.45	4.4-5.6
Group-2 (Anti-depressant)	Clonazepam + Dexamethasone	5.16±0.88***	
Group-3 Sample I(200mg)	Cheeseweed Leave extract at a dose 0.2 g/ kg body weight + Dexamethasone.	3.76±0.41**	
Group-4 Sample II(400mg)	Cheeseweed Leave extract at a dose 0.4 g/ kg body weight + Dexamethasone.	4.75±0.27***	

The data is presented as mean ± SEM (standard error mean), with n = 3 mice in each group. The table shows that the blood glucose level of the control group was 4.83±0.45 mmol/l, which is within the normal range and indicates that no alterations occurred in the level. However, the glucose levels of the animals in the standard group were within the normal range (5.16 ±0.88 level mmol/l), indicating that they are very skilled at reducing stress. The herbal low dosage sample group's blood glucose level, however, was 3.766±0.41 mmol/l, which was a little higher than the usual range for glucose. Additionally, the herbal high dosage sample group's blood glucose level was 4.75±0.2.

Table-7: 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±1.32	4.4-5.6
Group-2 (Anti-depressant)	Clonazepam	4.59±0.37***	
Group-3 Sample 200mg	Cheeseweed Leave extract at a dose 0.2 g/ kg body weight.	5.43± 0.04***	
Group-4 Sample 400mg	Cheeseweed Leave extract at a dose 0.4 g/ kg body weight.	4.85±0.31***	

The data for the Heat Induction technique is shown as mean ± SEM (Standard Error Mean), with n = 3 mice in each group. The blood glucose level of the control group was 10.91±1.32 mmol/l, which indicates that alterations occurred since the level was outside of the normal range, according to the table. Conversely, the rats in the Standard group showed excellent stress-reduction skills since their glucose levels (4.59±0.37 level mmol/l) were within the normal range. Nonetheless, the herbal low dosage sample group's blood glucose level was 5.43± 0.04 mmol/l, which is exactly within the normal glucose range. Additionally, the herbal high dosage sample group's blood glucose level was 4.85±0.31, suggesting that it also helps to minimize.

6.3.2 Effects of Herbal sample, Standard drugs on serum cortisol

Table-8: 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±0.11
Group-2 (Anti-depressant)	Clonazepam	1.32± 0.17***
Group-3 Sample 200mg	Cheeseweed Leave extract at a dose 0.2 g/ kg body weight.	2.78± 0.10***
Group-4 Sample 400mg	Cheeseweed Leave extract at a dose 0.4 g/ kg body weight.	1.51± 0.21** .

6.3.2 Effects of standard medications and a herbal sample on blood cortisol

Table-9: Effects of Herbal sample, Standard drugs on serum cortisol (for heat induction method)

Group	Medication & Herbal	Serum cortisol (ug/ dl)
Control group	Water	3.32± 0.54
Group-2 (Anti-depressant)	Clonazepam	1.47± 0.34***
Group-3 (Sample 200mg)	Cheeseweed Leave extract at a dose 200mg/ kg body weight.	2.97±0.11***
Group-4(Sample 400mg)	Cheeseweed Leave extract at a dose 400mg/ kg body weight.	2.12± 0.54** .

The information was presented as mean ± SEM (standard error mean), with n = 3 for each group. The table shows that the control group's serum cortisol level in the case of chemically induced stress was 1.32± 0.17 ug/ml, and in the case of heat-induced stress, it was 3.32± 0.54 ug/ml. These results support the theory that stress conditions can develop in animals under both chemical and high temperature induction. Conversely, in animals belonging to the standard group, the cortisol levels of mice caused by chemicals were 1.32±0.17 ug/ml, while those of mice induced by heat were 1.47±0.34, indicating their strong capacity to reduce stress while staying within the normal range. Thus, this finding suggests that herbal plants may reduce animal stress.

Chapter : Seven
Conclusion

7. Conclusion:

It's evident that the quest for novel medications is placing an increasing emphasis on natural goods. Malva parviflora is one very helpful species for the discovery and usage of natural medications.

The results of this study's research indicated that the methanolic extract of Malva parviflora included organic components such as tannins, glycosides, phenol, and alkaloids that may have pharmacological action. Additionally, CNS depressive and antistress actions were revealed in the current examination. More thorough research is required, however in this examination, Malva parviflora extract was effectively used to discover certain compounds that have a likely mode of action and are accountable for a particular impact. Based on the results, it is now feasible to draw the conclusion that the plant has prospective uses as a CNS depressant and an antistress agent. However, this was just a test run. As a result, additional in-depth research is needed to find active compounds.

Chapter : Eight
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