# "PHYTOCHEMICAL SCREENING AND ANTISTRESS ACTIVITIES OF THE METHANOLIC EXTRACTS OF *MELOCHIA CORCHORIFOLIA* LEAVES"

A dissertation turned in to the pharmacy department of Daffodil International University in partial fulfillment of the requirements for the Master of Pharmacy (M.Pharm) degree.

## Submitted To

Department of Pharmacy

Faculty of Allied Health Science

Daffodil International University

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# Approval

The thesis work "Phytochemical screening & antistress activities of the methanolic extracts of *Melochia Corchorifolia* leaves" is certified with pleasure. The findings of the research that I oversaw are submitted by ID: 0242220011093006 to the Daffodil International University Department of Pharmacy. Under my supervision, the dissertation was completed, approved for both style and content and acknowledged as meeting a portion of the prerequisites for a master's in pharmacy.. This thesis has not been submitted, nor is it being submitted, in any other venue for the award of a degree.

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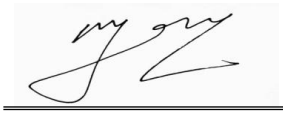

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# DECLARATION

I, Rakiba Akter declare that I worked under the direction of Mr. Md. Mizanur Rahman, Assistant Professor, Department of Pharmacy, and partially finished this assignment toward the requirements Daffodil International University has set for the Bachelor of Pharmacy degree. Not a single other university or institution has ever received the investigation's findings for a degree.

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This confirms that the research findings presented in the dissertation are unique and haven't been fully submitted to be considered for credit toward another degree or certification from an academic institution. This work was completed entirely as a thesis to receive a bachelor's degree in pharmacy from an undergraduate institution.

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Rakiba Akter Bappy
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**DEDICATE**

**“The all-powerful God is the primary recipient of my dedication, followed by my friends, family, and teachers”**

# ABSTRACT

The present study was undertaken to evaluate the possible "phytochemical screening and antistress activities of the methanol extracts of *Melochia Corchorifolia* leaves" (family: *Sterculiaceae*. The leaf's methanol and water extracts have been shown in numerous studies to have antibacterial, antifungal, cytotoxic, and anti-cancer properties. Using conventional procedures, the methanolic extract underwent a phytochemical examination. Numerous phytoconstituents were found, including glycosides, terpenoids, alkaloids, steroids, tannins, phenols, and saponins, according to the analysis. The design research investigates the impacts of spleen anatomy, blood glucose levels, weight fluctuation, and anti stress exercise. The stress group had blood glucose levels of 10.23 ± 1.08 mmol/L and 7.43 ± 0.89 mmol/L, whereas the medicinal sample group had values of 8.9 ± 0.81 mmol/L and 11.03 ± 0.37 mmol/L, respectively. The stress group had a cortisol level of 4.17± 0.19 ug/dl, whereas the medicinal sample group and standard group had levels of 2.13± 0.30 ug/dl and 3.17± 0.18 ug/dl, respectively. Weight fluctuation for the stress group was 26.66 ± 1.20gm, whereas the sample group was 25 ±1 gm. A morphological analysis of the spleen was done in order to evaluate and compare the effectiveness of conventional and herbal medicines against the spleen mass.

Keywords; *Melochia Corchorifolia*, Phytochemical screening, Antistress activity

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# CHAPTER-ONE:

# INTRODUCTION

## 1.1 General Introduction

Decades of intensive study, speculation, and debate have not produced a suitable definition of stress that would capture an organism's response to an unpleasant stimulus and the consequent adaptive alterations to meet environmental needs. Stress is intrinsically subjective, hence the objective way to assess stress in this field is by observing how the organism responds to the stressor.[1] A general precondition for maintaining the stress response, the stable state necessary for effective adaptation. In order to counteract the effects of stress and restore equilibrium to the body, a range of physiological or psychological reactions are included.[2]. The body's overall stress reaction affects every system. In a matter of seconds, several processes become active. The mechanisms involved in the stress response include mobilizing stored energy and blocking subsequent energy storage and gluconeogenesis; raising cerebral perfusion rates and cerebral glucose utilization; enhancing respiration and cardiovascular output; enhancing the transportation of energy substrates to the muscles; repressing reflexive physiology and behavior; altering immunological response; and reducing appetite and feeding. [3] The stress hormones, cortisol in particular, glucagon, catecholamines, and GH, release more in response to stress and emotional stimuli [4–11]. A number of these hormones are diabetogenic, meaning that they may accelerate the onset of diabetes when under stress [8].For example, adrenaline inhibits insulin release in both people and animals [12], which contributes to the development of diabetes. Reference [13]. Stress triggers an adrenaline, hypothalamo-pituitary, and adrenal (HPA) axis reaction [14]. People with long-term medical disorders may see intermittent increases in their weight and adrenal gland activity, which can raise cortisol metabolite levels [15–17]. Diabetes[13] may result from cortisol's permissive effect on the activity of some hormones (catecholamines) and the synthesis of other hormones (GH)[15].

Drug induced stress

Hydrocortisone is one of the synthetic glucocorticoids, or GCs, that are used as an anti-inflammatory and immunosuppressive drug. It is regularly used to treat a range of diseases[18]. Despite its many advantageous applications, hydrocortisone has been associated with a number of extremely dangerous adverse effects, especially when taken long-term and at excessive doses[18]. The rising realization that drugs and chemicals may indirectly control the immune system has not led to a thorough examination of many molecules in this field. Increased

glucocorticoid levels are a sign of stress-like reactions brought on by a number of substances that are significant to the environment. There is a consistent correlation between comparable glucocorticoid levels caused by psychological or physical stressors and the suppression of one or more immunological parameters [19]. Even in the absence of marked immunosuppression, elevated glucocorticoids brought on by

stress can occur[21]. Animals with adrenalectomy may experience immunosuppression from external stressors even in the absence of elevated glucocorticoid levels[22]. After your body releases adrenaline and other "fight or flight" hormones in response to stress, it may produce cortisol, which keeps you on high alert all the time. Additionally, your liver releases sugar, or glucose, for instant energy while you're under stress due to cortisol[23]. ]. Acute stress is an adaptive, transient state. On the other hand, chronic stress is a persistent state that is linked to maladaptive response, suggesting detrimental impacts on physiological processes[24,25]. Extended periods of stress induce the body to secrete specific hormones or chemicals that signal the ongoing stressful state of the body and impact key organs like the heart, brain, or liver in different ways that may not be beneficial to the patient's health. The body has numerous mechanisms that either separately or together control stress levels,[26,27] including the immunological system, the autonomic nervous system (ANS), and the hypothalamic-pituitary-adrenal axis (HPA-Axis)[28-31]. Due to this relationship, the HPA axis is thought of as the mediating system and is employed in the evaluation of the impact of stress on disease processes. Additionally, immune response, behavior, metabolism, and cognition all depend on it[32]. Although the body's cortisol level usually remains high during specific times of the day, like in the morning, it is alarming when this condition lasts the entire day. Biological media that can be used to assess cortisol levels include saliva, blood, urine, and hair samples[26]. As was already mentioned, the ANS is involved in the generation and control of acute and chronic stress. In response to internal (maintaining body homeostasis, including temperature, blood sugar, elimination of excess fluids, obesity, etc.) and exterior stimuli (environment, vision, smell, touch, etc.), it regulates bodily processes through autonomic reactions [33,34–37].

## Effect of stress on cortisol level

The steroid-shaped hormone cortisol has a glucocorticoid impact and is secreted from the suprarenal gland's outer cortex [38,39]. It mainly diffuses passively from capillary arteries to cells

because of its tiny, fat-soluble nature [39,40] It is found in bodily fluids such urine, sweat, hair, saliva, and cerebrospinal fluid [41]. There is a circadian oscillation rhythm in cortisol. Early in the morning is when blood cortisol levels are highest, and midnight is when they are lowest [39-41]. [39, 40]. It has been noted that blood free cortisol levels are correlated with salivary cortisol The majority of cortisol in blood is tightly tied to corticosteroid-binding protein (CBG, also known as transcortin), with just a little amount of cortisol in blood being delivered as bound to albumin. It is shown that only 3–10% of the total cortisol in blood is free cortisol concentrations [39–42]. Because salivary cortisol is easy to get on one's own, doesn't hurt, and can detect free cortisol levels noninvasively, it is an essential metric in psychoneuroendocrinological monitoring [43].

Effect of stress on blood glucose level

stress's impact on body weight
Stress's impact on the anatomy of the spleen
Possibilities for medicinal plants' anti-stress effects
Neutraceuticals or natural plants can help prevent stress.
stress-related biomarkers

The aim of the present study was to evalute phytochemical analysis and antistress activities of the methanol extracts of *Melochia Corchorifolia* leaves .

## 1.2 Plant name [44]

**Scientific Name:** *Melochia corchorifolia L.*

**Botanical Name:** *Melochi corchorifolia*

**English Name**: : *Chocolate Weed*

**Common Name:** Wire bush, Redweed

### 1.2.1 Taxonomical Classification [45-47]

**Kingdom:** *Plantae*

**Sublingdom:** *Tracheobionta*

**Superdivison:** *Spermatophyta*

**Division:** *Magnoliophyta*

**Class:** *Magnoliopsida*

**Subclass:** *Dilleniidae*

**Order:** *Malvales*

**Family:** *Sterculiaceae* Vent.

**Genus:** *Melochia* L.

**Species:** *Melochia corchorifolia* L.

### 1.2.2 General information

## Botany:

This plant is an annual or perennial types of harb reaching up to 150 cm high. Roots are fibrous, white or brown in colour .

## Leaves:

The leaves of *Melochia corchorifolia* are oval in shape, with petioles that are typically 5 cm long and linear stipules that are 6 mm long.

## Flowers:

The flower is purple that are5-7mm long and have five petals.Flowers are bisexual, regular,5-merous of 3mm long.

## Fruits and seeds:

Fruits are contains 5-valved capsule, 5mm long in diameter and have few seedsThe.seeds are 2.0-2.5mm long in length.

## Distribution:

***Melochia Corchorifolia*** is distributed in Bangladesh and tropical areas of Africa, Asia, Australia.

### 1.2.3 Images different parts of *Melochia Corchorifolia*

**Leaves**

**Flowers**

**Fruits**

### 1.2.4 Part of plant use in research

- ✓ Leave

### 1.2.5 Chemical constituents

The leaves of Melochia corchorifolia contain the following compounds: aliphatic compounds; flavonoids (vitexin, robunin), β-D-sitosterol and its stereate β-D-glucoside, alkaloids1, and triterpenes (friedin, friedelinol, and β-amyrin).[48] Proximate analysis of dried ieavea power yiegded ( %). fiber content (23.33%), ash (10.00%), carbohydrate value (30.03%), protein content (23.31%), and lipid value (13.3%), energy value of 275.66
kcal/100 g moisture content of 620.16 percent wet weight.

Menarel analysis of leavea yieled potassium (7.25 mg/100 g DW), followed by calcium (750.37 mg/100 g DW) and then phosphorus (101.89 mg/100 g DW), Sodium content (94.00 mg/100 g DW) , Cu (33.50), Fe (19.91), Mn (9.68) and Zn (6.73)[49]

### 1.2.6 Pharmacological Uses

Coastal East Africa uses leaves for various gastrointestinal ailments. The seed is used to alleviate stomachaches in Benin. The leaves and roots are used to cure a variety of medicinal conditions in Malaysia and India, including sores, diarrhea, abdominal edema, urinary abnormalities, and snakebites. Leaves dissolved in water have insecticidal effects. The storage pest Callosobruchus has been shown to cause less damage and to lay fewer eggs on pulses kept in gunny bags treated with the solution.[64]

## 1.3 Literature Review

Melochia corchorifolia leaves and roots are used to treat bladder issues, stomach It also contains proteins, fatty acids, carbohydrates, and phytoconstituents such as alkaloids, glycosides, terpenoids, steroids, and phenolic compounds. The following pharmacological properties are present in the plant extract: anthelmintic, hepatoprotective, antioxidant, antibacterial, anticancer, diuretic, phytothiatic, and

actions of CNS stimulants.[50]. Melochia corchorifolia extracts (methanol, hydroalcoholic, ethyl acetate, and hexane) were tested for their anti-inflammatory properties using a carrageenan-induced rat paw edema model. The outcomes were compared to those of the common medication, indomethacin. The chosen plant extracts, in conjunction with the conventional medication Indomethacin, greatly reduced the inflammation associated with paw edema. When it comes to paw edema inflammation in rats that have been given carrageenan, methanol extract has the most activity when compared to other extracts. A higher percentage of inhibition, 53.47±2.19, was seen in the methanol extract at a dosage of 500 mg/kg. [51]

As a widespread plant found in many tropical and subtropical locations, Melochia corchorifolia is a member of the Sterculiaceae family. There are various historical uses for M. corchorifolia. M. corchorifolia leaves and roots are used in Indian traditional medicine to cure snakebites, abdominal

edema, kidney problems, and diarrhea. Investigating the antiproliferative and antioxidant qualities of M. corchorifolia aerial parts methanol extract was the goal of this investigation.. The phytochemical screening was carried out by standard methods which revealed the presence of important phytoconstituents such as alkaloids, terpenoids, steroids, phenolic compounds, flavanoids and glycosides. M. corchorifolia methanol extract was subjected to antioxidant assays, including DPPH˙ radical, ABTS˙ + radical cation, OH˙ radical scavenging assays, Phosphomolybdenum reduction, and Fe 3+ reducing power assays. GC-MS analysis and thin layer chromatography were carried out because the results were quite positive. The MTT assay method was used to investigate the MCF7 (breast cancer) cell line's in vitro anticancer efficacy. The assays measuring DPPH˙ radical, ABTS˙+ radical cation, and OH˙ radical scavenging had respective IC 50 values of 35.26, 10.50, and 49.36 µg/mL concentration. Melochia corchorifolia methanol extract contained active volatile chemicals, as shown by GC-MS analysis. Using the MTT assay technique, the MCF7 cell line exhibited 66.84% cytotoxic activity at a dose of 100 µg/mL. The study's findings show that Melochia corchorifolia possesses strong antiproliferative and antioxidant properties..[52] The purpose of the current study was to examine the antidiabetic properties of the leaves of Melochia corchorifolia ethanolic extract. Procedures: Oral EEMC dosages of 250 and 500 mg/kg b.w. were given to diabetic rats produced with Alloxan, and an oral glucose tolerance test was conducted by delivering glucose (2g/kg b.w.) in water to induce hyperglycemia. To assess the hypoglycemic effects, fasting blood glucose levels and biological markers such as serum triglycerides, cholesterol, HDL, LDL, and VLDL were measured. The glucose tolerance test results indicate a noteworthy reduction in blood glucose levels after 90 minutes after consuming an ethanolic extract of Melochia corchorifolia leaves. For a duration of 21 days, ethanolic extract of Melochia corchorifolia leaves was administered orally to diabetic rats induced with alloxan, resulting in a substantial reduction in blood glucose levels. When oral EEMC was administered to diabetic animals, the level of biochemical parameters was altered compared to normal, showing decreased metabolic functions. This improvement was also significant. Conclusion: The findings imply that Melochia corchorifolia leaf ethanolic extract exhibits strong antidiabetic potential...[53]

**Pharmacological activity**

This plant has been the subject of pharmacological research on the following topics: anthelmintic action, hepatoprotective and antioxidant capacity, antibacterial activity, diuretic and antiurolithiatic activity, cytotoxic activity, and anticancer activity.

**Anthelmintic activity**

Palaksha et al. performed studies using Pheitima posthuma (Indian earth worms) to test the anthelmintic activity of plant extracts derived from Melochia corchorifolia.Reference 54 With a concentration of 100 mg/ml, it was discovered that all of the extracts exhibited vermiculite and vermicidal activity. The outcomes were contrasted with those of the common medication albendazole. The anthelmintic activity of Melochia corchorifolia stem extracts in water and ethanol was studied by Palaksha et al. [55] against Pheritima posthma. The anthelmintic activity of both extracts was highly significant at

60 mg/ml as the maximum concentration. Standard reference was piperazine citrate, while the control was regular saline.

**Hepatoprotective and Antioxidant Activity**

Extracts from the aerial parts of Melochia corchorifolia have been shown by Rao et al.[56] and Rao to have hepatoprotective and antioxidant properties.[57] Three free radicals (Superoxide, Hydroxy, and DPPH) were used to measure antioxidant activity, while rats' liver poisoning caused by CCl4 was used to measure hepatoprotective activity. Based on the study's findings, it can be said that M. corchorifolia aerial part extracts include hepatoprotective and antioxidant elements.[56]

**Antibacterial activity**

In an experiment utilizing the cup plate method, Rao et al.[58] and Rao assessed the antibacterial activity of several extracts of Melochia corchorifolia on eight distinct bacterial strains.In [57] A 400µg/cup concentration of methanol, ethanol, and ethyl acetate extract was evaluated against Pseudomonas aeruginosa, Bacillus megaterium, Klebsiella pneumonia, and Staphylococcus aureus. When tested against various bacterial strains, the methanol extract exhibited superior efficacy. In comparison to gram+ve organisms, extracts demonstrated a better zone of inhibition against gram-ve species. Palaksha et al. provided an explanation of the Melochia corchorifolia extracts' in vitro antibacterial activity using an agar cup plate methanol experiment.Reference 54 The 50 mg/ml extracts of petroleum ether, chloroform, and methanol were evaluated against gram positive (Bacillus subtilis and Staphylococcus aureus) and gram negative (Klebsiella pneumonieae, Pseudomonas aeureginosa, and Esherichia coli). The extract shown sensitivity against both gram-

positive and gram-negative bacteria.

**Antioxidant Activity**

By quantifying the DPPH, Nitric oxide, Hydroxyl, and Hydrogen scavenging activities, Palaksha et al.[59] examined the free radical scavenging activity of the Melochia corchorifolia plant extract. Plant components extracts in ethanol, chloroform, and petroleum ether showed robust independent

DDPH, nitric oxide, hydroxyl, and hydrogen peroxide could all be scavenged most effectively at a concentration of 100 µg/ml when tested for radical scavenging activity in all techniques.

**Antioxidant and Anticancer activities**

According to Harini et al.'s research, methanol extract of Melochia corchorifolia L.'s aerial portions has both antioxidant and anticancer properties [60]. The antioxidant tests for the methanol extract of M. corchorifolia were investigated, including DPPH.radical, ABTS.+ radical action, OH+ radical scavenging assays, Phosphomolybdenum reduction, and Fe3+ reducing power assay. The assays for DPPH+ radical, ABTS+ radical cation, and OH+ radical scavenging demonstrated in vitro anticancer activity with IC50 values of 35.26, 10.50, and 49.36 µg/mL concentration, respectively. With a dosage of 100µg/mL, the MTT assay technique demonstrated 66.84% cytotoxic activity against the breast cancer cell line MCF7. Comparing Melochia corchorifolia extract on HCT-116 (Human colon cancer) cell line to MCF-07 (Michigan Cancer Foundation) breast cancer cell line, Palaksha et al.'s evaluation of the extract's in vitro anticancer activity[61] indicates a good proportion of cytotoxicity.

**Diuretic and Antiurolithiatic activity**

Palaksha et al. assessed the diuretic and antiurolithiatic properties of the M. corchorifolia sample extracted in ethanol and chloroform.[62] After five hours, the volumes of urine were 0.44 and 0.16 ml (chloroform) and 0.51 and 0.70 ml (ethanol) due to the diuretic action on both chloroform and ethanol extract at doses of 200 and 400 mg/kg body weight. In contrast to the groups treated with chloroform, the group treated with M. corchorifolia ethanolic extract has strong diuretic efficacy. M. corchorifolia leaf extracts prepared in vitro with ethanolic and chloroform show dose- and time-dependent percentage suppression of antiurolithiatic action. However, the ethanolic extract

displayed the highest level of inhibition at 67.16% when compared to chloroform extracts.

**CNS Stimulant activity**

Using various animal models, Palaksha et al. examined the CNS stimulant effects of M. corchorifolia.In [63] Pharmacological studies have been carried out on the ethanol extracts of M. corchorifolia L. leaves to evaluate their effects on the central nervous system. Multiple methods, such as the photoactometer, rotarod, and tail suspension method, have been used to investigate CNS stimulant activity. A negative control, diazepam, was employed in addition to caffeine as the standard. Strong CNS stimulating properties are present in the ethanolic extract of M. corchorifolia.

# CHAPTER-TWO:

# OBJECTIVES

## 2.1 Objective of the study

Stress is an organism's reaction to a stressor that throws off its equilibrium. Foods or dietary supplements with potential health advantages are known as nutritional supplements. Supplemental nutrition might strengthen immunity and lessen negative reactions to stress.

**General Objective:**

To investigate the phytochemical composition and antistress activities of the methanolic extracts of *Melochia corchorifolia*, aiming to understand the plant's chemical constituents and their potential therapeutic benefits in mitigating stress-related responses.

**Specific Objective:**

1. To identify the bioactive components in *Melochia corchorifolia*
2. To assess the methanolic extracts' antistress effects utilizing standardized stress-related examinations.
3. To examine the neurochemical alterations generated by *Melochia corchorifolia* extracts in the brains of stressed experimental participants, focusing on neurotransmitter levels and stress-related indicators.
4. To assess the effects of methanolic extracts of *Melochia corchorifolia* on blood glucose levels in order to further our knowledge of its role in glucose metabolism under stress.
5. To evaluate the morphological changes that occur in important organs, especially the spleen, after being treated with methanolic extracts *of Melochia corchorifolia* in order to get insight into possible organ-protective actions.

# CHAPTER-THREE: MATERIALS AND METHODS

### 3.1 Collection of plant sample

The leaves of Melochia corchorifolia were collected from Sylhet, and Dhaka district.

### 3.2 preparation of plant sample

The collected materials were thoroughly washed in water, cut into smaller parts and shed dried at 35 - 40º C for a week and pulverized in electric grinder to get extractable powder. The powder materials was stored in a container for further use.

### 3.3 preparation of Extract

Methanol was used to extract finely powdered plant material in a conical flask for 72 hours at a ratio of 1:10 (w/v). To adequately close the jar and prevent air from entering, aluminum foil was wrapped around the plastic cover. For fourteen days, the previously indicated procedure was carried out. To extract the batter, the jar was shook frequently—almost twice a day. After that, the extract was filtered in a different container using filter paper.The above procedures were carried out three times. Through the use of a rotary evaporator, the extracted methanol was concentrated to around 40°C. The extraction volume was then increased to 100 mL by the addition of methanol. For future research, the concentrated extract of *Melochia corchorifolia* leaves was weighed, lyophilized, and stored at 4°C

### 3.4 Phytochemical screening methods

Examination of several chemical groups found in the extract that serve as a representation of the initial phytochemical investigations. The following procedures are followed for the chemical group test:

#### 3.4.1 Test Materials

Extract of leaves of *Melochia corchorifolia*

#### 3.4.2 Test Reagents for Chemical Groups

- Mayer's Reagent
- Fehling's Solution ii
- Dragendroff's Reagent
- Distilled Water
- Fehling's Solution i
- Molish Reagent
- Methanol
- Ferric chloride

#### 3.4.3 Test for Alkaloids

➢ Mayer's testIn a test tube, 2 ml of the extract solution and 1% hydrochloric acid were added. After that, 1 milliliter of potassium mercury iodide, or Mayer's reagent, was added. Alkaloids are present when a yellow-colored or creamy white precipitate forms.

#### 3.4.4 Test for Flavonoids

One milliliter of the crude extract was mixed with a few drops of strong hydrochloric acid. The presence of flavonoids has been demonstrated by the immediate development of red color.

#### 3.4.5 Test for Saponins

There was a 20 ml distilled water to 1 ml extract solution dilution. The saponin is then present when the mixture is violently shaken for 15 minutes, causing clear foam to form.

### 3.4.6 Detection of Phenols

Three or four drops of ferric chloride solution were added to a two milliliter extract solution. Phenols are present when bluish black color begins to form.

### 3.4.7 Test for Carbohydrate

A test tube was filled with two milliliters of the plant material's extract. The test tube was filled with 2 ml of concentrated H2SO4 and 2-3 drops of Molisch's reagent. Carbohydrate content is indicated by a purple ring or a red tint.

### 3.4.8 Test for  Glycosides

➢ (i) A tiny quantity of either dried or fresh plant material was extracted and added to one milliliter of water. Then, some aqueous sodium hydroxide was added in small drops. The presence of yellow was thought to be indicated by between glycosides.

➢ (ii) A reddish brown hue indicates the presence of glycocides when 5 milliliters of extract are added to 2 milliliters of glacial acetic acid, 1-2 drops of FeCl solution, and 1 milliliter of concentrated H2SO4.

➢ (iii) Another quantity of the extract was dissolved in alcohol and water, boiled, and neutralized with sodium hydroxide solution after a few drops of diluted sulfuric acid were added. This process was done to check for the presence of glycoside

### 3.4.9 Test for Tanin

When 2-4 drops of ferric chloride solution and 5 milliliters of extract are added to a test tube, a greenish-black precipitate indicates the presence of tannin.

### 3.4.10 Test for Phytosterol

A test tube containing 2 milliliters of extract, 1 milliliter of chloroform, and 1 milliliter of strong sulfuric acid is added. The test tube's golden red or golden yellow color confirms the presence of phytosterols.

### 3.5 Experimental Design

For this investigation, Swiss albino mice were recruited from the Jahangirnagar University Lab. In the trials, five-month-old female albino mice weighing between 150 and 220 g were used. Before the trial began, the rats were kept in colony cages in the department's temperature-controlled animal room, which was kept between 25 and 30 °C for five days. To ensure hygienic conditions and cleanliness, the bedding was changed daily. Hydrocortisone was given to induce stress. The study involved grouping the mice into four groups, with n = 3 mice in each group. The groups were

- Control Group
- Standard Group
- Stress induced group
- Sample treatment group

### 3.6 Medication & Diet

Table-1 : Medication & sample performed throughout the study

| Group | Medication & Sample | Duration |
|---|---|---|
| Control Group | Normal water | 7 days |
| Stress induced group | Hydrocortisone | 7 days |
| Sample treatment group | Hydrocortisone + extract | 7 days |
| Standard drug Group | Clonazepam | 7 days |

### 3.7 Blood Collection

The mice were successfully given the experimental drugs and materials for 14 days before they were killed and their blood was taken. A blood sample of two to three milliliters was obtained via heart puncture. The blood was centrifuged, and the serum was removed and preserved for further investigation.

### 3.8 Biochemical Test

Two biochemical tests were performed as part of the inquiry. Cortisol is the first, while the blood glucose level is the second. Both tests were performed on a Backman Coulter AU-480 (model) equipment using spectrophotometry

### 3.9 Morphological Study

The mice were slaughtered when the research period finished, and their average body weights were reported. After that, the key organs (spleen) were removed and physical appearance changes such color and size were compared to the control group. The spleen was dissected to look for any symptoms of stress or color change in the layer.

# CHAPTER-FOUR:

# RESULT AND DISCUSSION

**4.1 Results of phytochemical screening.**

Table:1. Results of phytochemical screening of the methanol extract of *Melochia corchorifolia.*

| Phytochemical constituents | Results |
|---|---|
| Carbohydrate | - |
| Glycosides | + |
| Alkaloids | - |
| Saponins | - |
| Tannins | + |
| Phytosterols | + |
| Diterpenes | - |
| Flavonoids | + |
| Steroids | - |

Discussion: Positive (+) denotes the tested group's presence, and negative (−) denotes its absence. The assays determine if the methanol extract of. *Melochia corchorifolia* contains glycosides, tannins, phytosterols, and flavonoids.

### 4.2. Effects on body weight

Table -2: Effects on body weight throughout the study

| Group Name | Medication | Initial weight (gm) | Final weight (gm) | Weight variations (gm) |
|---|---|---|---|---|
| **Group 1(control)** | Normal water | 28 ±1 | 28.66 ± 0.33 | 0.66gm (Slight increased) |
| **Group 2 (Stress Induced)** | Hydrocortisone (2.047mg/kg) | 26.33±1.20 | 26.66±1.20 | 0.33gm (Slight increased) |
| **Group 3 (stress +Drug)** | Clonazepam (0.103mg/kg) + Hydrocortisone | 26.66± 1.20 | 26.66± 0.88 | No change |
| **Group 4 Stress + extract** | MC(0.5g/ kg) + Hydrocortisone (2.047 mg/kg) | 24.66± 1.20 | 25±1 | 0.34gm (Slight increased) |

The data were presented as mean ± SEM (standard error mean), with n = 3 for each group.

The starting and ending weights of the experimental animals, as well as their changes throughout time, were seen in table 02. The control group's weight slightly change , and the only weight gain after the study was 0.66 grams. There was some tiny change in weight in the stress group with a gain of 0.33 gm from the initial weight. On the other hand, it was strikingly discovered that the sample group's weight was 0.34 grams increased and that there was no weight variance in the standard group.

As a result of following a regular, healthy diet for the duration of the trial, there were no significant changes in the weight variation of the any group. Because the sample treatment had a significant quantity of fiber, . According to reports, increasing fiber in the diet can help manage obesity.

### 4.3 Cortisol test Result:

Table 03: Effects of sample (*Melochia Corchorifolia* leaves) on cortisol a biomarker for stress function.

| **Group Name** | **Medication** | **Cortisol level (µg/ dl)** |
|---|---|---|
| **Group 1(control)** | Normal water | 3.09± 0.49 |
| **Group 2**<br>**(Stress Induced)** | Hydrocortisone (2.047mg/kg) | 4.17±0.19 |
| **Group 3**<br>**(stress +Drug)** | Hydrocortisone (2.047mg/kg) +<br>Clonazepam (0.103mg/kg) | 3.17 ±0.18 |
| **Group 4**<br>**Stress + extract** | Hydrocortisone (2.047mg/kg) +<br>MC(0.5g/ kg) | 2.53±0.53 |

The data were presented as mean ± SEM (standard error mean), with n = 3 for each group.

Serum cortisol levels in the normal control stress control, standard and sample groups, as well as the acceptable limits for mice, are shown in Table 04.Since the normal group value is 3.09**(**µg/ dl), the cortisol level of both standard and sample groups are normal,with values of 3.17 **(µg/ dl)** and 2.53(µg/ dl), respectively.The stress group increased as compared to the control group and the value is 4.17( µg/ dl), , according to analysis of the cortisol test result.As can be understood from the above result that this sample group are able to decrease serum cortisol level.

**Table -04: Effects of sample on blood glucose level**

| Group Name | Medication | Initial blood glucose(mmol/L) | Final blood glucose(mmol/L |
|---|---|---|---|
| **Group 1(control)** | Normal water | 5.86±0.49 | 4.46±0.44 |
| **Group 2 (Stress Induced)** | Hydrocortisone (2.047mg/kg) | 7.43±0.89 | 10.23±1.08 |
| **Group 3 (stress +Drug)** | Hydrocortisone (2.047mg/kg) + Clonazepam (0.103mg/kg) | 8.3± 0.26 | 6.86±1.01 |
| **Group 4 Stress + extract** | MC(0.5g/ kg) + Hydrocortisone (2.047mg/kg) | 8.9± 0.81 | 11.03±0.37 |

The data were presented as mean ± SEM (standard error mean), with n = 3 for each group.

In the sample group, total blood sugar levels were 11.03(mmol/L) compared to10.23 (mmol/dl) in the stress group. As a consequence, it is assumed that sample treatment caused the 0.8 mmol/dl level to raise. The glucose levels increased in overall groups by  compared to the control, an outstanding results was marked out in standard group where the standard group values for glucose was 8.3 (mmol/L)in initial and 6.86(mmol/L) .And there was a noticeable difference between the two results.

This is a great opportunity to look into the sample extract even more and a fantastic chance for additional investigation on the sample.

### 4.4 Morphological Investigation of Spleen

In morphological study of spleen ,it was found that the sample ,control & standard group spleen size was smaller than the stress group which indicates medicinal plants and standard drugs are capable in controlling stress.

**Table 5 Size comparison of the spleen in experimental animals**

| Group Name | Medication | Spleen size (cm) |
|---|---|---|
| **Group 1(control)** | Normal water | 1.6± 0.11 |
| **Group 2 (Stress Induced)** | Hydrocortisone (2.047mg/kg) | 1.8 ±0.05 |
| **Group 3 (stress +Drug)** | Hydrocortisone (2.047mg/kg)+Clonazepam (0.103mg/kg) | 1.5 ±0.11 |
| **Group 4 Stress + extract** | Hydrocortisone (2.047mg/kg)+ MC (0.5g/ kg) | 1.7±0.06 |

The data were presented as mean ± SEM (standard error mean), with n = 3 for each group.

Based on the table, we can observe that the mice's spleen measured 1.6±0.11 cm, 1.7±0.06 cm, and 1.5±0.11 cm in the control, sample, and standard groups, and 1.8±0.05 cm in the stress group. Stress induction led to a reduction in spleen size in both the sample group and the standard. On the other hand, the experimental and comparison groups' spleen sizes were noticeably larger.

# CHAPTER-FIVE: CONCLUSION

## 5.1 Conclusion

Naturally occurring bioactive chemicals found in plants can be used to treat a wide range of serious illnesses. *Melochia corchorifolia* has demonstrated the presence of several phytochemicals, indicating that it may be utilized as a medicinal herb The activity of leaf extract may be due to the presence of glycosides, tannins, phytosterols and Flavonoids present in it. The main objective of this study has been to ascertain the effects of hydrocortisone administration on body weight and spleen shape, as well as the effects of *Melochia corchofolia* on glucose and hormone levels (corticosterone). The results of the experiments in this study demonstrated that the methanol extract of *Melochia corchorifolia* contained organic compounds that may be largely responsible for the pharmacological activity. *Melochia corchorifolia* leaf methanolic exhibits antistress activity. dditional research utilizing isolated and purified phytochemical ingredients is required to fully comprehend the anti-stress action mechanism.

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