

Investigation of In-vitro antioxidant potential, Brine shrimp lethality and Thrombolytic activity in *Ficus mollis vahl* Leaves along with Phytochemical Screening

A project submitted

by

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This work is dedicated to my parents for their love and constant support.

## Certification Statement

This is to guarantee that the project titled "Investigation of in-vitro antioxidant potential and Thrombolytic activity in *Ficus mollis vahl* Leaves along with Phytochemical Screening" submitted for the partial fulfillment for the necessities for the Degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own particular work under the supervision of Mr. Ashis Kumar Podder, Senior Lecturer, Department of Pharmacy, BRAC University and this undertaking is the consequence of the creator's unique research and has not beforehand ever been submitted for a degree or certificate in any foundation.

To the best of my insight and conviction, the undertaking contains no material already distributed or composed by someone else aside from reference is made in the thesis itself.

Signed,

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Counter signed by the supervisor

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## **Acknowledgment**

Right off the bat, I am thankful to the Almighty Allah for giving me the quality to finish this thesis. Without his support, I could never have the capacity to finish every one of crafted by my venture.

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## ABSTRACT

In the present study, the methanolic extract of *Ficus molis vahl* from Moraceae family was investigated for various phytochemical and biological properties, Chemical test were performed to check the presence of phytochemicals. The leaf extract proved positive result for the existence of alkaloids, carbohydrates, flavonoids, phytosterols, glycosides, resins, saponins, tannins e.t.c. However, the test results confirmed the absence of Phenols/Phenolic compounds, Glycosides, Resins, Steroids, Saponin and Phlobatannins. In order to investigate the biological property such as antioxidant activity, the DPPH free radical scavenging activity and total phenolic content was determined. In vitro cytotoxic potential of methanolic extract of *Ficus Molis vahl* was also performed utilizing the brine shrimp lethality assay. *Ficus Molis vahl* leaf extract showed antioxidant property, cytotoxicity property and thrombolytic property, as end result showed that the plant extract possess moderate antioxidant activity and cytotoxic potential at higher concentrations.

**Keywords:** Moraceae; leaves; extract; phytochemicals; antioxidant activity, cytotoxic activity.

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## List of Abbreviations

**AA:** Ascorbic Acid

**AAE:** Ascorbic Acid Equivalent

**Abs:** Absorbance

**b.w.** Body Weight

**DPPH:** 1, 1- Diphenyl -2- Picryl Hydrazyl

**FCR:** Folin- Ciocalteu Reagent

**FRS:** Free Radical Scavengers/Scavenging

**GA:** Gallic Acid

**GAE:** Gallic Acid Equivalent

**LAF:** Laminar Air Flow

**Mg:** Milligram

**ND:** Not indicatd

**n.d.** No date

**TPC:** Total Phenolic Content

**VA:** Vanillic Acid

**ug:** Microgram

**VL:** Vanillin

**CHAPTER 1**  
**INTRODUCTION**

## **1.1. Introduction**

Plants are the fundamental piece of our condition and they are exceptionally helpful for human wellbeing. As noted previously, extraordinary sort of illnesses has generally been treated with operators that are gotten from therapeutic plants. The expression "therapeutic plant" comprises of a few sorts of plants utilized as a part of herbology. Here plants use for restorative purposes to treat sicknesses. Home grown plant arrangements that have been utilized to treat infections in the beginning periods of human life have built up the establishment for medicate treatment. In this way, from here up to this point, it is viewed as a standout amongst the most profitable wellsprings of drug. In this way, it is important to identify normally accessible natural medications for the improvement of humankind which have incredible pharmacological impacts.

According to Devi (2015), Our earth is loaded with various sorts of plants and people created around 0.75 million of plant species, among them half million are delegated "higher plants" and 2.5 lakh as "nonvascular plants". Around 60% of all clinically utilized solutions are created from regular items and their normal natural subordinates and items. Around 87% of every single ordered prescription, similar to anti-toxins, antitumor operators, and anticoagulants originate from regular items and their subsidiaries. Synthetic substances that recently accessible on the planet are over 28% of characteristic items (Akter, 2013). As of late, WHO (World Health Organization) said, about 80% individuals around the globe specifically depending on restorative plants of essential treatment. Besides, around 21,000 types of plants have various potential pharmacological importance which could be utilized as nonvascular plants or restorative plants

As they process least symptoms, treatment with therapeutic plants is viewed as innocuous. The real truth is that individuals all things considered and sex can take home grown treatment. The best advantage is cured are tuned in to nature.

## **1.2. The historical backdrop of restorative plants**

It is known to every one of us that utilization of the restorative plant are utilized to treat illnesses began by our predecessor however when it was begun is as yet obscure. While searching for the nourishment they discovered different plants which indicate either the

noxious or capacities like the ability to create immoderate perspiration, decrease agony and aggravation et cetera.

An explanatory history of different therapeutic plants unveils that our predecessors, similar to the Egyptians, the Assyrians, the Babylonians, knew extremely about the restorative properties of different herbs and plants. Since 300 BC many basic medicines were notable to Babylonians and it is expressed that advanced medication still uses certain plants similarly as Babylonians (Ghani, 2003).

The main composition found on the utilization of restorative plants is no less than 400 years of age. The original copy was composed by a gathering of individuals from the antiquated Sumerians composed on little dirt sections. The chunks were found later by Iraqi scientists. The Egyptians in like manner formed a unique duplicate on restorative plants called Ebers Papyrus. More than 700 procedures were discovered, made around 1700 AC. A stunning recorded technique for using remedial plants is the book titled "Pen Tsao" holding the use of more than 300 restorative plants. The Indian helpful structure called Ayurveda, which suggested the use of restorative plants, from 800 A.C (Remedies with Traditional Plants, 2016).

Among old community foundations, India was always celebrated for their use of restorative plants. All through the Indian timberland, the epic measure of restorative and fragrant plants are discovered, shape their creator of arrangements and smell things assembled their rough fixings. Indian medicine structure named AYUSH coded around eight thousand characteristic cures and besides for indigenous pharmaceutical Tribal, Siddha, Unani and Ayurveda arrangement systems are overwhelming. In any case, Indian people exceedingly used Ayurveda and Unani structure, in this way, these systems became most.

Chinese were in like manner having mind boggling learning of remedial plants they use an alternate ordinary treatment, called "Chinese Herbs". It should be seen that the natural sources reinforce the Chinese herbalism. More than 1200 plants are related with the regular treatment structure and for the treatment purposes around 500 remedial plants used to cure a couple of ailments. The Chinese used remedial herbs also they used them beforehand. It should be seen that one-fifth of the Chinese pharmaceutical industry contains around 5,000 standard cures (Li, 2000).

Obsolete specialists accept that various restorative issues and illnesses have only a solitary course of action which is herbs. Old specialists drove an examination on it, try to survey with specific revelations on the amplexness of various herbs that give helpful criticalness to various sicknesses. These arranged medications have no or to a great degree less side effects or hostile reactions. For this quality treatment with home developed plants is winding up continuously surely understood wherever all through the world. These herbs that have restorative qualities offer treatment to various internal diseases that for the most part are seen as hard to cure.

Affiliations like ESCOP (European Organization Cooperative On Phototherapy, 1999), German Commission E (Blumental et.al, 1989) and WHO (World Health Organization) communicated that the use of plants to cure diseases has been seen by the whole world and the use of remedial plants increases a little from than to till date.

### **1.3. Restorative plants accessible in Bangladesh**

A subtropical nation like Bangladesh which encompasses in excess of 5,000 angiosperm plants distributed in 200 families. This region gives the ideal time to develop and feed restorative plants. Dhaka, Sylhet, Chittagong, Rajshahi, and many different regions of tis country are improved around 5000 distinct kinds of restorative plants, as specified in the "'Materia Medica' ". From the earliest starting point of its reality, customary solutions are notable in the nation. The soil of productive advancement of therapeutic plants in Bangladesh likes to treat 500 built up diseases among 2000. What's more, as of late, the utilization of therapeutic plants by monster enterprises and organizations in Bangladesh has expanded extensively. Numerous driving pharmaceutical organizations in Bangladesh presently utilize an assortment of therapeutic plants. Most ancestral and Bangladeshi populaces depend intensely on the restorative plant for essential treatment, maybe they trust that nature won't hurt their wellbeing. As indicated by this confidence and conviction, they utilize different parts of plants: barks, steam, organic products, blossoms and so forth. Following table 1 lists some restorative qualities containing plants which utilized as recuperating in general infection.

**Table 1: List of various Medicinal plants utilized as a part of the planning of customary drug.**

Name of the plant	Medicinal Uses
<i>Allium sativum</i>	Reduction of cholesterol concentration in the blood.
<i>Achillea millefolium</i>	Purported to be a diaphoretic, astringent, tonic, stimulant and mild aromatic.
<i>Aloe vera</i>	To heal burns, wounds and other skin ailments leaves are widely used.
<i>Andrographis paniculata</i>	Treating Fever and liver diseases
<i>Azadirachta indica</i>	All parts are utilized for getting ready a wide range of pharmaceuticals, particularly for skin illness. Some portion of the Neem tree can be utilized as a spermicide.
<i>Bellis perennis</i>	Flowers used in the traditional medicine internally as tea for treatment of disorders of the gastrointestinal and respiratory tract.
<i>Centella asiatica</i>	Curing diarrhea and dysentery
<i>Coccinea indica</i>	Diabetes management
<i>Jasminum officinale</i>	It is used in dermatology as either an antiseptic or anti-inflammatory agent.
<i>Rauvolfia serpentina</i>	The cure for insanity, insomnia and hypertension

#### 1.4. Significance of restorative plants in sedate finding

Restorative plants are utilized as a part of antiquated time for the treatment of various maladies which help to fabricate the historical backdrop of medication revelation. For the most part, a piece of the plant like barks, roots, leaves, blooms or organic products and so on is utilized to make the restorative arrangements. These parts of plants contain compound constituents alongside the different wanted segment. Interestingly, by detaching that coveted segment with the expansion of mass and influencing a measurement to shape prompts sedate disclosure. Despite the fact that loads of new innovations are utilized, for example, combinatorial science and PC based atomic displaying configuration to make engineered particle step by step plant-



inferred medications are having a high rate of acknowledgment by the patients. In addition, the therapeutic plants have a long history of clinical utilize which makes these more dependable (Veeresham, 2012).

World Health Organization (WHO) as of late distributed a measurement that shows roughly 80% individuals of the whole world utilized regular solution for their essential human services to some degree. For example, there are roughly 600-700 plant-based drugs are accessible in Germany and 70% of the German doctors are more intrigued to endorse those medicines. Additionally, the historical backdrop of most recent 20 years in the United States demonstrates that the employments of characteristic solution expanded strongly because of less resistance of engineered medicate by the patient and cost of the medication.

In phytotherapy, therapeutic plants assume an essential part. Vast quantities of the restorative plants may have used as psychedelic drugs stimulants, harms, however there are heaps of therapeutic plants that contains such compound constituents which are fit for creating distinct physiological activity in the human body and give remedial impact and expanded the significance and estimation of that plant as a restorative plant. Some bioactive constituents that make a plant esteemed as restorative plant are -

- Alkaloids
- Glycosides
- Flavonoids
- Phenols
- Steroid
- Saponins
- Tannins

According to Yue-Zhong Shu (1998), The different count demonstrated that around 60% of hostile to microbial, against tumor, against infective regular medications are presently either in the commercial center or in the clinical preliminaries. Most extreme of them are still gotten from wild different therapeutic plants yet a large number of them are not yet orchestrated financially (Taux, 2001).

### **1.5. Medication as a characteristic item got from restorative plants**

The domestic developed plants remained an essential resource of modern blends which are pharmacologically energetic, from these plants effective trade drugs are deduced either direct or alternatively. Generally, 25% of the solutions endorsed on the planet begin from characteristic plants, around 121 plants of which energetic blends are right now being utilized. World Wellbeing Organization (WHO) reported 252 medicines which are enrolled in essential pharmaceutical rundown, around 11% of solutions are starting from the plant source and from common originators an immense sum of designed drugs obtained. Cases of that drugs which obtained from domestic developed plants they are Digoxin from Digitalis species, vincristine and vinblastine gotten from the Catharanthus roseus, Atropa belladonna grant atropine along morphine and moreover codeine from Papaver somniferum (Yue-Zhong Shu, 1998). As of late, utilization of the normal thing as helpful pro growing rapidly, (Goldfrank et al., 1982; Mentz and Schenkel, 1989). Liu et al. (2000) revealed that around half of all promoted drugs were gotten from characteristic items.

All through the past ten a long time, helpful things got from plants, for illustration, Arteether, Conclusion peroxide sesquiterpene lactone and ordinary semi-manufactured things got from Artemisinin utilized for the treatment of intestinal ailment the common alkaloid Galantamine have been utilized as a portion of the treatment of Alzheimer's sickness, characteristic thing Nitisinone got from Leptospermone is utilized as a portion of treatment of antityrosinaemia, Apomorphine a semi-engineered composite which is coming approximately since of morphine utilized as a portion of treatment of Parkinson's ailment, for relentless obstructive aspiratory disease tiotropium an imitative atropine utilized which procured from Atropa belladonna, from Cannabis and Capsaicin plants Dronabinol and Cannabidiol got are utilized as analgesics.(Veeresham C. 2012).

Table 2 gives a few medications which got from the regular plant source.

**Table 2: Some drugs previously isolated from natural plant sources (Meshnick & Dobson, 2001, Serturmer, 1805, Hartunf, 1954, Seader, 2005).**

<b>Drugs Name</b>	<b>Chemical classification</b>	<b>Source</b>	<b>Medicinal use</b>	<b>Mechanism of action</b>
Digoxin	Cardiac glycoside	<i>Digitalis purpurea</i>	Congestive heart failure, Atrial fibrillation	Na <sup>+</sup> /K <sup>+</sup> -ATPase pump inhibition.
Mevastatin	Polyketides	<i>Penicillium citrinum</i>	Cholesterol lowering drug, (Li, 2009, p. 71-96).	Inhibits HMG-coenzyme-A
Morphine	Alkaloids	<i>Opium poppy, Papaver somniferum</i> dried latex.	Analgesic/ Potent Painkiller	Opioid agonist.
Penicillin	Penicillin	<i>Penicillium niger</i>	Antibiotics	Inhibit Peptidoglycan synthesis
Quinine	Alkaloids	<i>Cinchona officinalis</i>	Anti-malarial agent	Inhibit Protein synthesis

### **1.6. Selection of *Ficus mollis vahl* for analyzing in this project**

The chose plant, *Ficus mollis vahl* is an unrevealed plant. In the wake of looking changed diaries and productions on *Ficus mollis vahl* there's no adequate measure of data was found, thus, this plant was chosen to recognize different properties like against oxidant, cytotoxicity, thrombolytic and so forth.

### **1.7. Prologue to the chose plant *Ficus mollis vahl***

*Ficus mollis vahl* is include with the family Moraceae. This sort of plants is found in various bumpy regions of the Asian subcontinent. In Bangladesh they generally accessible in the sloping zones like Moulovibazar, Sylhet, Chittagong, Chittagong Hill Tracts, Cox's Bazar.

*Ficus mollis vahl* is one of the prevailing plant species in Moulovibazar, Sylhet, Chittagong, Chittagong Hill Tracts, Cox's Bazar and Bandarban e.t.c. Additionally, this plant is likewise accessible in the uneven regions of Moulovibazar, Sylhet. (Hossain, Hossain, Alam and Uddin, 2015).

The Moraceae regularly called the mulberry family or fig family are a group of blossoming plants containing around 37 genera and more than 4849 species. Most are across the board in tropical and subtropical districts, less so in calm atmospheres. The leaves are basic and interchange or once in a while inverse. The stipules are little and sidelong or once in a while they frame a top over the bud and leave a round and hollow scar.

The 'blooms' of Moraceae are frequently pseudanthia (lessened inflorescences). They are unisexual and minute and are normally thickly accumulated. These conglomerations as often as possible appear as pendulous aments or catkins. More often than not, the perianth comprises of 4 or 5 undifferentiated tepals, however now and again less or no perianth fragments are available. A run of the mill male bloom has four stamens, one inverse every perianth section. The female blooms have a bicarpellate pistil, for the most part with two styles, albeit one might be stified. The ovary is either unrivaled or sub-par and it likewise contains a solitary pendulous ovule in a single locule.

The natural product created from a solitary female blooming is a meaty drupe or a dry achene. The bloom intertwines as they develop after preparation and a different organic product shapes. The numerous yield comprises of little drupe or achene assembled together in a solitary unit and is generally round or oval formed. Natural product case incorporate drupe and achenes that are regularly mixed or generally totaled into numerous accessories organic products.

The best-known product of the Moraceae Genus is *Ficus carica*, which has been developed for roughly a huge number of years. These developed figs create without fertilization, as this species does not deliver any male blossoms. It is really the syconium that is alluded to as the product of the it. On account of fig assortments, which are pollinated, the genuine organic

product, an achene is created inside the syconium. Figs are pollinated by wasps. The family incorporates understood plants, for example, the Mulberry, Breadfruit, and Jackfruit.

### 1.8. Portrayal of *Ficus mollis vahl* plants

The huge or little tree often with ethereal roots which never form into trunks. Leaves are variable, elliptic-oval, elongated praise, whole or sinuate, tomentose, base cordate or adjusted.

Ancestral Name: Chongralace (Chakma) in Bangladesh.

**Table 3: Plant taxonomy (*Ficus mollis vahl*)**

<b>Kingdom</b>	Plantae
<b>Phylum</b>	Tracheophyta
<b>Class</b>	Magnoliopsida
<b>Order</b>	Rosales
<b>Family</b>	Moraceae
<b>Genus</b>	Ficus
<b>Species</b>	<i>Ficus mollis vahl</i>

### 1.9. Pharmacological properties of genera and species

*Ficus mollis vahl* is the plant of a family Moraceae, which has therapeutic values. Commonly, Moraceae family provides numerous therapeutic properties they particularly utilized for the malady like asthma, anthelmintic, the runs, dysenteryheadache, aggravation, skin inflammation, skin illnesses etc. Here are a few major species in Moracea given underneath:

1. *Ficus atrial*
2. *Ficus Bengalensis* (Banyan)
3. *Ficus elasrica* (Rubber plant)
4. *Ficus religiosa*

### **1.10. Related production on *Ficus mollis vahl***

Pharmacognostic and phytochemical examination and screening of antimicrobial properties have been done. Along these lines, numerous profitable properties and employments of restorative plants are as yet should have been distinguished.

### **1.11. Task legitimization/method of reasoning**

The writing audit of the chose plant called *Ficus mollis vahl* uncovered that, it is noticed that no critical examination has been led till date on *Ficus mollis vahl*. However, prior examinations in a few types of this kind have detailed ground-breaking antimicrobial, against tumor, hostile to diarrheal, anthelmintic, cancer prevention agent, hostile to irritation, kidney malady, skin ailments and cytotoxic exercises.

Along these lines, the primary reason for this investigation is to discover the diverse pharmacological properties from the crude leaf concentrate of the plant. The examination will analyze the obscure properties of the chose plant for the distinguishing proof of new wellspring of medication.

### **1.12. Aims**

A definitive point of the task is to analyze and find the obscure organic capability of the chosen plant.

### **1.13. The purpose of the project**

To satisfy the point of the undertaking, this venture convention contains following advances which finished with methanol concentrate of the *Ficus mollis vahl* clears out.

- a. Chemical screening of *Ficus mollis vahl* Leaves,
- b. Analysis of the antioxidant properties of the methanolic sample of the plant leaves by:
  1. Determining of the aggregate phenolic substance of plant and
  2. Measuring in-vitro DPPH free radical rummaging action
- c. Evaluation of cytotoxic action (Brine shrimp lethality test).
- d. In-vitro assessment of thrombolytic property.

- e. The examination may likewise help to imagine the new properties and unexplored pharmacological activities of this plant, and may present the plant as another wellspring of medication.

#### **1.14. Assessment of cancer prevention agent property of *Ficus mollis vahl* leaves extricate**

Most diseases are principally identified with free radical-incited oxidative worry inside our body. Cancer prevention agent substances have the ability to influence the oxidation procedure by including in response with the reactant metals, chelating operators, free radicals, and furthermore go about as an oxygen safeguard from any source (Buyukokuroglu et al., 2001). The impacts of cancer prevention agents on plant-determined mixes are expanding in intrigue, which might be significant as far as nourishing effect and their part in social insurance and different infection (Steinmetz and Potter, 1996; Couladis et al., 2003). Countless and cancer prevention agent inferred plant test reports have been characterized over the earlier decade. (Velioglu et al., 1998; Pietta et al., 1998). Properties therapeutic plants have been analyzed in ongoing medication science developments around the globe simply because of their powerful cancer prevention agent action, the base measure of symptoms and furthermore money related reasonability (Auudy et al., 2003).

Another engineered cell reinforcement, for example, tertbutyl-1-hydroxytoluene (BHT), propyl gallate (PG), tertiary butyl-hydroquinone, and hydroxystyrene butylate (BHA) utilized as the additional fixing to increment strong with the known impacts not just dangerous and furthermore cancer-causing on human cell (Ito et al., 1986; Wichi, 1988). Thusly, as of late's common cell reinforcement (plant inferred) request increments enormously (Jayaprakasha J. R., 2000). Plant-determined polyphenols have been examined generally due to the likelihood that they might be the reason for the defensive impact of products of the soil utilization against malignancy and other ceaseless ailments (Elena et al., 2006).

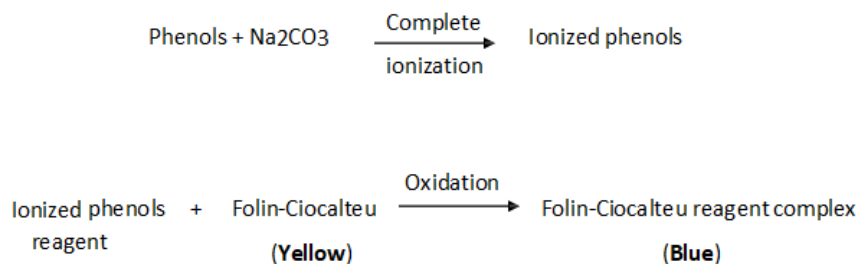
At long last, the motivation behind this analyses were to find and assess *Ficus mollis vahl* leaf remove as potential crisp wellsprings of standard cell reinforcements and phenolic mixes.

Analyzing of antioxidant characteristics can be following manners:

1. Determination of aggregate phenolic content.
2. Determination of cell reinforcement properties: DPPH measure

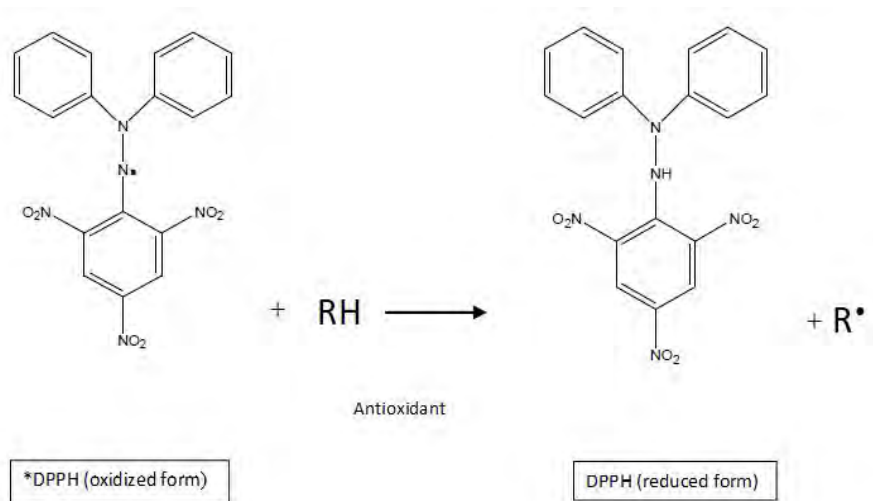
### 1.14.1. Assessment of phenolic content

The phenolic mixes of a plants generally demonstrated their cancer prevention agent property by the redox responses, which has the urgent influence in the assimilation and balance of triplet oxygen, free radicals, and decayed peroxides (Osawa, 1994). Cancer prevention agent impact showed up primarily in light of phenolic segments, for example, flavonoids, phenolic diterpenes and phenolic acids (Shahidi, Janitha and Wanasundara, 1992). Various engineered concoctions procured from plants claim noteworthy cancer avoidance specialist property. They generally associated with a minor rate of mortality in arranged human populaces (Velioglu et al., 1998). Within the stage of fundamental, the phenols are completely ionized. Folin-Ciocalteu substance can without a doubt oxidize the phenols when utilized as a portion of this ionic phenolic course of action, the reagent successfully oxidizes the phenols. At the point when the oxidation technique keeps running within the course of action, a yellow shade of Folin-Ciocalteu manufactured changed into the dim blue. This shading alter quality is assessed by spectrophotometer in 760 nm. Estimation of the absorbance illustrates the total phenolic substance of the substance. (Harbertson and Spayd, 2006).



Antioxidant properties of methanolic plant extricate on stable 1, 1-diphenyl-2-picrylhydrazyl radicals were evaluated (Brand-Williams et al., 1995). At various concentration 2 mL of a methanol arrangement of the plant extract were mixed with 3 mL of DPPH solution (20 $\mu$ g/mL). Accordingly, the antioxidant property was broke down by dissolving the DPPH colored solution with the plant extricate note of the likenesses with ascorbic acid (ASA) by UV spectrophotometer.





Here, \* DPPH = 1, 1-diphenyl-2-picrylhydrazyl

**Figure 1.2: Chemical structure of DPPH**

### 1.15. Brine shrimp lethality assay

Bioactive substances are continuously harmful and hazardous for the living body at higher estimations and legitimize the claim that "Pharmacology is as it were the foremost noteworthy measurements toxicology and fundamentally cut down estimation pharmacology". The brine shrimp test (McLaughlin, 1998) could be a savvy and total common test for the bioactive substances of created, semi-built and normal source. This strategy permits the unmistakable affirmation of bioactivity of the trademark thing, parts of substances and moreover unadulterated substances. In vivo lethality test in a solitary creature particular living being (like shrimp nauplii) continuously utilized for observing and screening within the disclosure of unused bioactive ordinary things. In any case, a characteristic test chooses cytotoxicity and a wide course of activity of pharmacological or normal properties, for occasion, antimicrobials, antivirals, pesticides, and antibodies, et cetera of mixes (Meyer, 1982; McLaughlin, 1998). The characteristic lumbar estimation methodology for salmon shrimp is predominant to anything other cytotoxicity testing reasoning since it is smart, unpretentious and does not require fabulous hardware or aseptic techniques. It employments a distant coming to number of living animals for bona fide support and a respectably small case gage. Moreover, not at all like unmistakable strategies, it doesn't require creature serum. The salted shrimp relatives are conveyed in reiterated seawater to form nauplii. By counting the handled degree

of dimethyl sulfoxide (DMSO), the outline is set up in required fixation by weakening. The nauplii are checked with visual examination and put them in vials which contains reenacted seawater around 5 mL. Thusly, exceptional centralization of tests is included to the tubes with micropipette which was at that point stamped. These tubes are at that point cleared out in a room temperature for 24 hours. At last, survivors are checked unequivocally taking after 24 hours. (Meyer et al., 1982).

#### **1.16. In-vitro examination of thrombolytic properties of *Ficus mollis vahl* leaves**

Since old-fashioned conditions, different plant organizing has been utilized to treat differing illnesses. Clears out, branches, barks, stem, and ground zones are reliably utilized for standard meds. Homegrown things are routinely seen as secured as they show up to be "trademark" (Gesler, 1992). (CVST) Cerebral venous sinus thrombosis is disapproving an bona fide condition which is recognized with bona fide affliction and passing (Watson et al., 2002). Heparin, an anticoagulant energizer, is the critical line of CVST treatment in light of its reasonability, potential comes about, and success. (Biousse and Newman, 2004). Thrombolytic pharmaceutical, for instance, clopidogrel, streptokinase, urokinase, et cetera. They give an indispensable help in controlling of CVST in patients (Baruah, 2006). Thusly, the goal of this test was to perceive another thrombolytic ampleness of methanol focus of *Ficus mollis vahl* takes off.

Thrombolytic drugs are ordinarily used more capably and suitable to upgrade the circulation system and help to reduce or lessen the reactions of various patients with no necessity for additional masters, yet it isn't proposed for everyone. Thrombolytic administrators are by and large used to treat myocardial pollutions. Among of them, streptokinase is for the most part used. These conditions when thrombolytic masters are suggested fuse (Beckerman, 2015): the cerebral drain of cerebral dying, hypertension, dynamic dying, or extreme dying.

**CHAPTER 2**  
**METHODOLOGY**

## 2. Methodology

### 2.1.1 Plant collection

*Ficus mollis vahl* has been targeted for this project as no previous study has been carried out except antimicrobial properties. In September 2017, leaf part of *Ficus mollis vahl* plant was collected from Rangpur government horticulture, Rangpur, Bangladesh.



**Figure 2.1:** *Ficus mollis vahl* leaves

Steps for extracting Medicinal plants include:

It is divided into 2 stage:

1. Preparation and drying of plant material (2 steps)
2. Extraction stage (5 steps)

They are described below.

### **2.1.2. Preparation and drying of plant material**

First of all, the leaves of *Ficus mollis vahl* were plucked off carefully from its plant and washed with clean water to remove all dust particles and the plant debris. Then, clean leaves were dried in shade for around 10 days and then those dried leaves were prepared for the extraction process.

### **2.1.3. Extraction Stage**

#### **2.1.4. Size reduction and weighing**

The crispy dried leaves of *Ficus mollis Vahl* were then converted to a coarse powder using a grinding machine. Later they are packedg into air tight plastic bags with necessary labeling. After that it was left in a cool, dry and dark room until further analysis. To avoid cross-contamination, during the grinding process, necessary measurements were taken.

The total weight of these powdered plant leves was divided into 2 parts. Later, they are stored separately in container.

#### **2.1.5. Extraction Process**

The extraction was applied by maceration process for extraction of the plant materials and here methanol was utilized as an organic solvent. Each beakers containing the powdered plant material was soaked in 800mL of methanol for 3 days at normal room temperature (22-25°C) with occasional agitationing.

The result of maceration process was a 2-layer phase: the lower phase is the sediment and the upper most layer is a methanolic solution of the extract. This was separated later.

#### **2.1.6 Filtration Process**

After 2 days of maceration, the materials of the beaker were transfered. Later filtered them using some Whatman Filter Paper (pore size: 110mm).

### **2.1.7. Concentration Process**

Collected filtrate was concentrated by using a rotary evaporator (Brand Name: Heidolph) at 100 RPM at 30°C until it produce a concentrated methanolic extract. Later, this new mixture was transferred onto a clean and washed Petri dishes for drying under laminar air flow.

### **2.1.8. Drying Process**

Petri dishes were put open beneath Laminar Air Flow (LAF). It'll dissipate the remaining dissolvable still accessible the extricate, and will take off behind a dull colored dry and semi-solid extricate. LAF was utilized for the preventive degree to maintain a strategic distance from any kind of microbial development on the extract layer within the time of drying. After effectively drying of the extricate, Aluminum foil utilized to cover Petri-dishes and refrigerated for assist analysis.

## **2.2. Phytochemical screening Process**

It was analyzed on the crude *Ficus mollis vahl* extracts to access it's all qualitative chemical compositions like: alkaloids, carbohydrates, resins, steroids, tannins, flavonoids, glycosides, etc. In this research, the following tests were also carefully performed:

### **2.2.1. Detection of Alkaloid:**

To determine of alkaloids, 3 tests were performed. First, 0.5g of *Ficus mollis vahl* methanolic extract was dissolved in 5mL of 1% Hydrochloric acid, which later boiled in a water bath. After that filtration was done.

Following tests were performed using the filtrate obtained:

#### **Hager's Test:**

3/4 drops of Hager's reagent were added to 2mL of the filtrate, and the presence of alkaloids was confirmed when the formation of a yellowish precipitate (Waldi, 1965) in the test-tube.

**Mayer's Test:**

Evans (1997) described, 10 mL Mayer's Reagent can be prepared by dissolving 0.5g of Potassium Iodide and 0.1358g of Mercuric (II) Chloride in 10 mL distilled water. Then, a 3 to 4 drops of Mayer's reagent were added along the sides of the test tube to a 2mL of the filtrate. It forms a white or creamy precipitate which indicates the existence of alkaloid substance.

**Wagner's Test:**

As stated by Wagner (1993), a 10 mL Wagner's Reagent can be prepared by 0.6g of Potassium Iodide and 0.2g of Iodine crystals dissolved in 10mL distilled water. There, a few drops of Wagner's reagent were added in 2mL of the filtrate. It forms of a brownish black precipitate. It confirms alkaloid materials existence in the sample.

**2.2.2. Detection of Carbohydrates**

Prasannan, Ramkrishnan and Rajan (1994) said, carbohydrate can be qualitatively detected if 0.5g of methanolic extract of *Ficus mollis vahl* dissolved it in 5mL of distilled water. Then filtered the mixture.

When the filtrate obtained, the following two tests were performed:

**Molisch's Test:**

2mL of the filtrate was taken and treated with 2 drops of Molisch's Reagent which is an alcoholic solution of  $\alpha$ -naphthol to which 2 mL of concentrated sulfuric acid was added along the sides of the test tube and was allowed to stand. New formation of a violet ring indicates the existence of carbohydrate substances.

**Fehling's Test:**

1mL of each of the Fehling's solution A and B were added in a 1:1 ratio in 2 mL of the filtrate, and then boiled for a several minutes. A brick-red precipitate was formed which proves the existence of reducing sugar.

### **2.2.3. Detection of Flavonoids**

#### **Lead Acetate Test:**

Several drops of lead acetate solution were added in the methanolic extract of *Ficus mollis vahl*. The formation of a yellow colored precipitate in the test tube, proves the existence of flavonoid contents.

#### **Zinc Ribbon Test:**

As stated by Manorama, Sindhu, and Uma (2013), the existence of flavonoids can be identified by another method. First, a Zinc small piece and 5-10 drops of concentrated HCl was added to a test-tube which contains 0.5mL of methanolic extract, the newly formed solution was then boiled for a few minutes and later left to stand to make it cool.

A red to crimson color solution was formed which indicates the existence of flavonoid contents.

### **2.2.4. Detection of Phenols/Phenolic compounds**

#### **Ferric Chloride Test:**

As stated by Soni and Sosa (2013), Phenols/Phenolic compounds identification test is done by taking 2mL of extract in a fresh test tube. 15% (w/v) Ferric chloride solution was added 3-4 drops. A bluish-black precipitate forms which indicates existence of phenolic contents.

### **2.2.5. Detection of Phytosterols**

#### **Liebermann Burchard's Test:**

1mL of chloroform was added in the methanolic extract of *Ficus mollis vahl*. and filtered. 2mL of acetic anhydride added to the filtrate. Later boiled and cooled. At the end, 1mL of concentrated sulfuric acid was added to this solution. It forms a brownish ring at the junction which indicates the existence of phytosterol contents (Soni & Sosa, 2013).



### **2.2.6. Detection of Steroids**

#### **Salkowski Test:**

2mL of chloroform, 1 mL of sulfuric acid were added in the methanolic extract of *Ficus mollis vahl*. The aformation of red color indicates the existence of steroid contents (Ghani, 2003).

### **2.2.7. Detection of Tannins**

The following 2 tests were performed to detect tannins:

#### **Lead acetate test:**

Tiwari and Bimlesh (2011) described, few drops of 1% Lead acetate solution were added to 1mL of the extract, and the formation of a yellow-colored precipitate indicates the existence of tannin contents.

#### **Potassium dichromate test:**

According to Ghani, 2003, 10% Potassium Dichromate solution can be formulated by dissolving 1g of Potassium Dichromate in 10 mL distilled water to.

Later, in 1 mL of 5% ferric chloride solution was added to 5 mL aqueous solution of crude extract. A new yellow precipitation forms which indicates the existence of tannin contents.

#### **Ferric Chloride Test**

According to Ghani (2003), 5% Ferric chloride solution can be prepared by dissolving 0.5g of ferric chloride in 10mL distilled water. 1mL of 5% ferric chloride solution was taken and 5 mL aqueous solution of crude extract was added there. The formation of new greenish black precipitation indicates the existence of tannin contents.

### **2.2.8. Detection of Resins**

The existence of resin can be identified if 5-10 drops of acetic anhydride were added to 2mL of the methanolic extract and then heatied the solution gently. Now, add 0.5mL of sulfuric acid to the solution.

The existence of resin can be identified if it forms a bright purple color. (Soni and Sosa, 2013).

### **2.2.9. Detection of Glycosides**

According to Mariappansenthilkumar (2013), before analyzing it to Borntrager's Test, methanolic extract of *Ficus mollis vahl* should be hydrolyzed with dilute Hydrochloric acid.

#### **Modified Borntrager's Test:**

5mL of dilute Hydrochloric acid and 5mL of 5% Ferric (III) chloride were added to the 5mL of the filtrate. Then heat the mixture for 5 minutes in a boiling water bath and later cooling it down. Now, 5mL of benzene was added to the new mixture and thoroughly shaken. Separating funnel used to separate the organic layer and dilute ammonia solution was added there in an equivalent volume. It forms a pinkish-red color in the portion of ammoniac layer which signifies the existence of glycoside contents (Kamalakar, Prabhakar & Shailaja, 2014).

### **2.2.10. Detection of Saponins**

#### **Froth Test:**

According to Kokate (1999), with distilled water, the extract was diluted and the volume was made up to 10mL and the contents shaken in a graduated cylinder for around 10 minutes. A foam layer is formed which is about 2 cm in height. It indicates the presence of saponin contents (Kokate, 1999).

### **2.2.11. Detection of Phlobatannins**

10 mL of extract was taken to a test tube. Adding 2 ml of 10% (w/v) HCl solution to the extract and heated this new solution for 5 minutes. A red precipitate proves the existence of phlobatannins. (Sauwal, Saka, and Mairiga, 2014).

## **2.3. In-vitro antioxidant activities**

There are many procedures available to analyze in vitro methods of determining anti-oxidant activities of a plant extracts. Among these various methods, here 2 methods were chosen to

determine the antioxidant activity of the plant extract of *Ficus mollis vahl*, namely DPPH (1, 1-diphenyl -2-picrylhydrazyl) free radical scavenging assay and the total phenolic content (TPC).

### **2.3.1. Evaluation of free radical scavenging by DPPH assay**

As stated by Choi et al. (2000) and Desmarchelier et al. (1997), To find and assess the impacts of free radicals (antioxidant) DPPH is constantly utilized for various mixes and medicinal plants.

#### **Mechanism of DPPH test**

In arrange to find or gauge the antioxidant action by DPPH, 1 mL of extricated methanol extricated from distinctive sums of concentration was gotten employing a 3 mL methanol DPPH arrangement. Ascorbic corrosive as a rule as standard are by and large utilized to concentrate between 12.5 and 1000 ug / mL. Clear test was too arranged for the ponder. When the test and the dark arrangement are prepared, it was kept within the dull for 30 minutes. Hence, the antioxidant action of the extricate is measured by spectrophotometric investigation beneath UV absorbance at 517 nm wavelength. The DPPH test is primarily utilized to identify a diminish in free DPPH with radicals. As electron produces DPPH free radicals, UV spectrophotometry gives a tall absorbance at 517 nm of nickel. After the response of the steady free radical DPPH, the antioxidant leads to the generation of hydrogen and to the diminish of the DPPHH, which lead. Decolonization comes about because of the way that DPP-H creates a yellow shading regarding the aggregate number of electrons. Higher diminishing capacity demonstrates when decolonization step by step increments. DPPH is viewed as the most ideal approach to examine research to decide the quality of new medications and possibilities. (Brand-Williams et al., 1995).

#### **Materials and reagents**

List of the materials and reagents given below:

1. UV-spectrophotometer
2. Lightproof box
3. Pipette (1mL and 5mL)
4. Ascorbic Acid (ASA)
5. Distilled water
6. Extracts of the experimental plant
7. Methanol
8. Test tubes
9. Volumetric flask
10. DPPH (2,2- Diphenyl-1- Picrylhydrazyl)

### **Control planning for evaluation**

In this test, to induce a prepared standard (positive control) ascorbic acid (ASA) was utilized. The discovered degree of ascorbic acid was broken down in the methanol solution to get a solution which has was 1000 µg/mL. After that to induce unmistakable concentration running from 1000 to 12.5 µg/mL serial weakening was done.

### **Test test planning for evaluation**

To set up the test test, 25 mg of *Ficus mollis vahl* clears out extricate was put in a cleaned test tube at that point 25 mL of methanol was included to the test tube to induce a gathering of 1000 µg/mL. By and by by serial weakening procedure needed concentration running from 1000 to 12.5 µg/mL finished and kept them a dry put with labeled.

DPPH solution for evaluation to set up the DPPH solution, 2 mg of DPPH powder was precisely evaluated and broken up in 50 mL of methanol and a short time later the course of action was put absent in a dim box which is secured by aluminum paper.

### **Assay of DPPH free radical scavenging activity**

Test samples in each test tube having a substitute concentration expanding from 1000 to 12.5 µg/mL were mixed with 2.0 mL of a DPPH arrangement. At that point, this blend is kept in a dim put for 30 minutes to happen reaction. Taking after 30 minutes' blend absorbance was assessed by UV spectrophotometer at 517 nm wavelength. Here, methanol utilized as the blank.

### **Calculation:**

For free radical DPPH, Inhibition in percentage (I %) is:

$$\text{Inhibition (I \%)} = \left( 1 - \frac{A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100$$

Here,  $A_{\text{blank}}$  is the absorbance value for the control reaction.

Afterward, 50% inhibition ( $IC_{50}$ ) was calculated by methanolic plant extract concentration and value acquired from the diagram. Now, plot the inhibition percentage (I%) against the concentration of methanolic plant extract (in µg/mL).

### **2.3.2. Total Phenolic Content**

As indicated the technique, portrayed by Skerget et al., (2005) which included Folin-Ciocalteu as oxidative material and Gallic acid as standard material to analyze the total phenolic content of *Ficus mollis Vahl* leaves extracts.

### **Rreagents and Materials:**

1. UV-spectrophotometer
2. Test tube
3. Vial
4. Micropipette (50-200 µl)
5. Gallic acid
6. Distilled water
7. Folin-Ciocalteu reagent (10 fold diluted)

#### 8. Na<sub>2</sub>CO<sub>3</sub> solution (7.5 %)

#### **Test sample preparation for evaluation**

First, 25 mg of the plant extract was dissolved in the distilled water which gives a sample concentration extending from 1000 to 12.5 µg/mL.

#### **Standard arrangement readiness for gallic acid curve**

Gallic acid is typically utilized worldwide as a standard in the aggregate phenolic content test. The different Gallic acid arrangement was set up with focus running from 1000 to 12.5 µg/mL Concentration by utilizing 2.5 mL of Folin- Ciocalteu solution (diluted 10 times with water) and 2mL of (7.5% w/v) Na<sub>2</sub>CO<sub>3</sub> arrangement was 0.5 mL of Gallic acid included. This solution was set in a dark place at room temperature for 30 minutes. After that by utilizing UV spectrophotometer, the solution was estimated at 760 nm and absorbance was taken. At that point, the absorbance was plotted against the concentration. Thus, a direct relationship was gotten which was utilized get test sample result.

#### **Investigation of Total Phenolic Content**

To acquire solution, 0.5mL of plant extract (2 mg/mL), 2.5 mL of Folin-Ciocalteu substance (Diluted 10 times with water) and 2.0 mL of (7.5% w/v) Na<sub>2</sub>CO<sub>3</sub> were added. At that point, the solution is kept in dim place at room temperature for 20 minutes. After particular time at 760 nm absorbance was checked with UV spectrophotometer alongside by utilizing the standard curve of Gallic acid, the aggregate example was evaluated. samples phenolic content was expressed as mg of GAE (Gallic acid Equivalent)/g of the plant extract.

#### **2.3.3. In-vitro cytotoxicity property analysis**

##### **Brine shrimp lethality assay: Procedure**

##### **Materials for test:**

Materials required for Brine shrimp lethality assay:

1. Glass vials

2. Lamp
3. NaCl
4. Small tank
5. DMSO (Dimethyl sulfoxide)
6. Magnifying glass
7. Test tubes
8. Pipette, Micropipette
9. Plant extract
10. Brine shrimp (*Artemia salina*) egg

### **Formulation of seawater for test**

To get ready seawater arrangement, 38 gm of salt (unadulterated NaCl) was weighted precisely than it was dissolved in distilled water to get 1 L solution and after that sifted the seawater to a clean beaker to secure a clear form.

### **Bring forth of brine shrimps eggs for test**

To perform this examination, eggs of saltwater shrimp (*Artemia salina*) gathered from stores was utilized. Now, a clean little tank or jar has taken. Later, loaded with seawater at that point saline solution shrimp eggs added to it. To get developed nauplii, a ceaseless supply of oxygen was given for the duration of the season of the incubation (48 hours). Lamplight pulls in shrimps in the punctured dam. A clean pasteur pipette was utilized to gather living shrimps ( $10\pm 2$ ) to each test tube; which contains exact 5mL of seawater.

### **The arrangement of test sample for the trial**

Dimethyl sulfoxide (DMSO) was dissolved in the test tube. After that by serial dilution, the particular concentration from  $200\mu\text{g}/\text{mL}$  to  $6.25\mu\text{g}/\text{mL}$  finished. Starting,  $50\mu\text{l}$  of Test which fixation was  $200\mu\text{g}/\text{mL}$  set into test tube holding 5mL of DMSO near by  $10\pm 2$  nauplii. After that, new  $50\mu\text{l}$  DMSO was broken down into the test tube. By this framework arranged fixation was gotten.

**Table 4: Plant sample with various concentration (after serial dilution).**

Test tube serial	Concentration (in $\mu\text{g/mL}$ )
1	200
2	100
3	50
4	25
5	12.5
6	6.25

### **Preparation of control group for the experiment**

To assess the examination comes about and guarantee the outcomes accomplished were proportionate to the execution of the test specialist and the conceivable impacts of other possible stoppages control assemble are extremely basic in cytotoxicity thinks about. As a rule, 2 kinds of control groups are there is practice, positive and negative control.

### **Preparation of positive control**

In cytotoxicity test, positive control is broadly known as a cytotoxic compound. This encourages in test contrasted with the aftereffect of positive control. Here, vincristine sulfate a cytotoxic compound was utilized as standard (positive control). The measurements of vincristine sulfate were dissolved down in DMSO to get the main dosage of  $20\mu\text{g/mL}$  right then and there by serial dilution verity grouping of standard arrangement acquired, for example,  $10\mu\text{g/mL}$  to  $0.0390\mu\text{g/mL}$ . At last, standard (positive control) included the test tubes which holding 5mL seawater alongside  $10\pm 2$  nauplii.

### **Preparation of negative control**

To formulate the negative control, first, 3 clean test tube was taken. Now, 100  $\mu\text{l}$  of DMSO was added in every test tubes. Now, it contains 5mL of seawater along with  $10\pm 2$  nauplii. In case the of rapid death rate for nauplii, which marks the experiment unacceptable and the nauplii died due to some other unexpected reasons.



### **Nauplii counting**

Final result got following 24 hours, with the assistance of an amplifying glass and the quantity of survivors was included precisely every one of the test tubes. From every weakening, (%) level of mortality was computed by direct relapse which is utilized to assess the fixation mortality information. Besides, the focus versus mortality relationship of plant remove is communicated by (LC<sub>50</sub>) esteem which implies middle deadly focus esteem. In this way, the centralization of the substance is in charge of the passing in half of the test nauplii after a particular timeframe.

### **2.3.4. In-vitro thrombolytic property analysis**

In case of thrombolytic property of the plant extract, it can be evaluated by an easy method using Clopidogrel (antiplatelet agent) as standard where for negative control, water was taken.

#### **Materials and reagents**

Used materials in the thrombolytic test: Blood

1. Clopidogrel (Anti-platelet agent)
2. Saline
3. Plant extract
4. Micro centrifuge tube
5. Distilled water

#### **Preparation of test sample**

For the readiness of test, a test tube was taken which containing 10ml of refined water. At that point, 100 mg of plant extricate was dissolved in it. Now, the test tube was kept in dry, dark area for overnight. Then, the Desired dissolvable supernatant was exchanged and filtered legitimately.

### **Preparation of Standard solution**

An antiplatelet agent; Clopidogrel is utilized as standard (positive control) for this examination. 100 mg of Clopidogrel crushed to powder and dissolved in 10ml refined water. This solution put away as a stock standard solution arrangement from 500µl arrangement prepared for the thrombolytic activity test.

### **Blood sample arrangement**

2 sound volunteers, 1 male and 1 female, who have no history of anticoagulant treatment was chosen. Blood was gathered from them guaranteeing aseptic condition. In the wake of gathering blood, first, 1mL of blood was moved into each pre-weighed microcentrifuge tubes. Later, microcentrifuge tubes were kept untouched to form clusters.

### **Thrombolytic property test procedure**

Toward start of the test, first, 5 mL of new blood gathered from each of the volunteer. Then, blood tests were run for five diverse previously weighed sterile organisms and allowed to brood at 37°C for at least 45 minutes. After that, when the coagulation is formed, the upper liquid was altogether expelled from every micro tubes. Now, the coagulation's weight was dictated by comparing the weight of the micro tubes taken earlier after clump development. For this situation, 100µl of Clopidogrel utilized as the standard (positive control). Another 100µl of distilled water were utilized as a non-thrombolytic (negative control). Now, 100µl of each example was included from each test tube. For perception of clump lysis, microtubes were hatched at 37°C temperature for exact 90 minutes. A short time later, while the brooding finished, the fluid was expelled and discharged from the coagulation and once more weighted to get the weight contrast after the coagulation diversion.

At long last, rate (%) of clot lysis appeared as underneath:

$$\text{Percentage (\%)} \text{ of Clot Lysis} = \left( \frac{\text{Released Clot Weight}}{\text{Clot Weight}} \right) \times 100.$$

**CHAPTER 3**  
**OBSERVATION AND RESULT**

### 3.1. Phytochemical screening of *Ficus mollis vahl*

**Table 5: Phytochemical screening of *Ficus mollis vahl***

Serial No	Compounds Type	Result
1	Alkaloid compounds	+++
2	Carbohydrate compounds	++
3	Phenolic compounds	-
4	Phlobatannin compounds	-
5	Phytosterol compounds	+
6	Flavonoid compounds	++
7	Glycoside compounds	-
8	Resins	-
9	Steroid compounds	-
10	Saponin compounds	--
11	Tannin compounds	+++

Here,

- ✓ (+) describes the existence in a one test,
- ✓ (++) describes the existence in two tests,
- ✓ (+++) describes the existence in three tests, and
- ✓ (-) describes absent in the test.

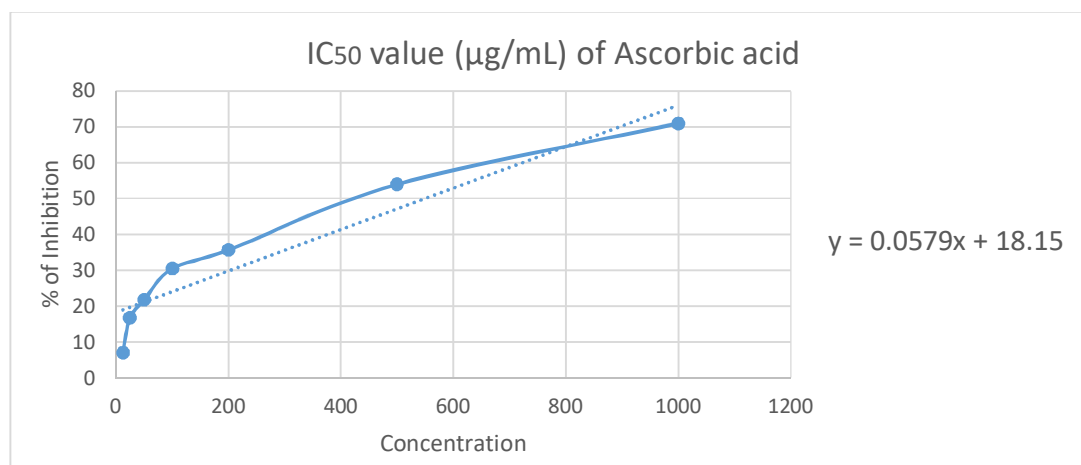
### 3.2. Antioxidant property analysis

#### Evaluation of free radical scavenging activity test by DPPH for *Ficus mollis vahl*

**Table 6: Standard (L-Ascorbic Acid): Absorbance vs. concentration.**

Concentration (ug/mL)	Absorbance at 517 nm	% of Inhibition of L-Ascorbic Acid	IC <sub>50</sub> (ug/mL)
1000	0.181	70.942	550.086
500	0.287	53.932	
200	0.401	35.634	
100	0.433	30.497	
50	0.488	21.669	
25	0.519	16.693	
12.5	0.58	6.902	
Blank	0.623		

**Graph of DPPH % of Inhibition vs. Concentration for L-Ascorbic Acid**



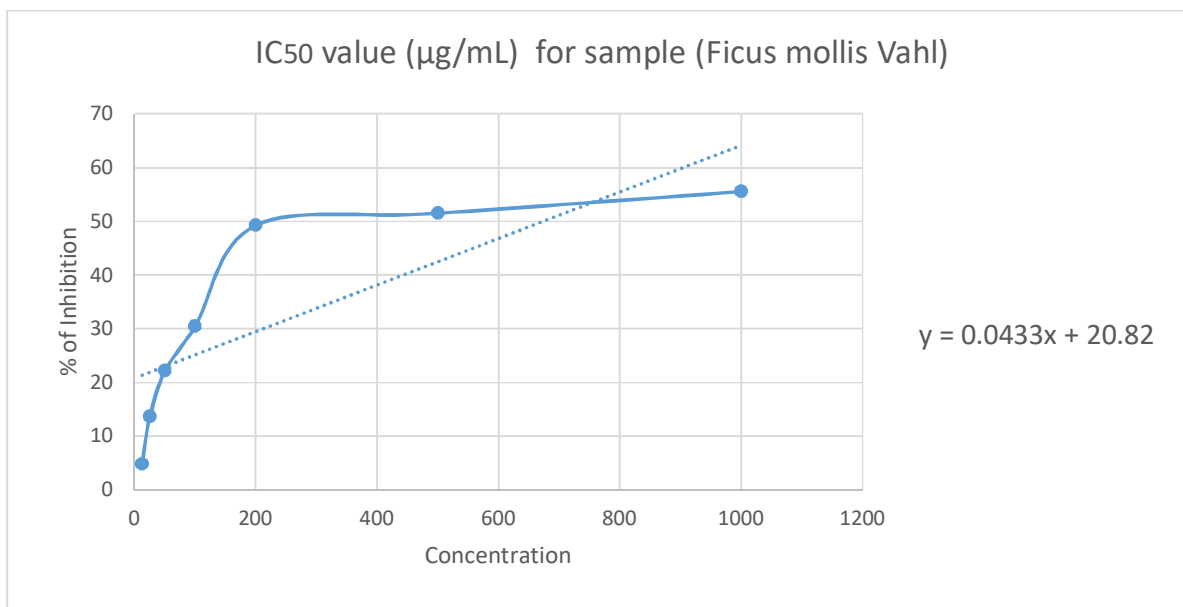
**Fig 3.1.1:** L-Ascorbic acid: % of Inhibition vs. Concentration (in µg /mL).

**Table 7: Free radical scavenging activity test by DPPH:**

**Sample's (*Ficus mollis Vahl*) Absorbance vs. Concentration**

Sample Concentration of <i>Ficus mollis vahl</i> (ug/mL)	Absorbance at 517 nm	% of Inhibition of <i>Ficus mollis vahl</i> (ug/mL)	IC <sub>50</sub> (ug/mL)
1000	0.277	55.537	673.90
500	0.302	51.524	
200	0.316	49.277	
100	0.433	30.497	
50	0.485	22.15	
25	0.538	13.643	
12.5	0.593	4.815	
Blank	0.623		

**Graph of DPPH (% of Inhibition vs. Concentration) for the sample (*Ficus mollis vahl*):**



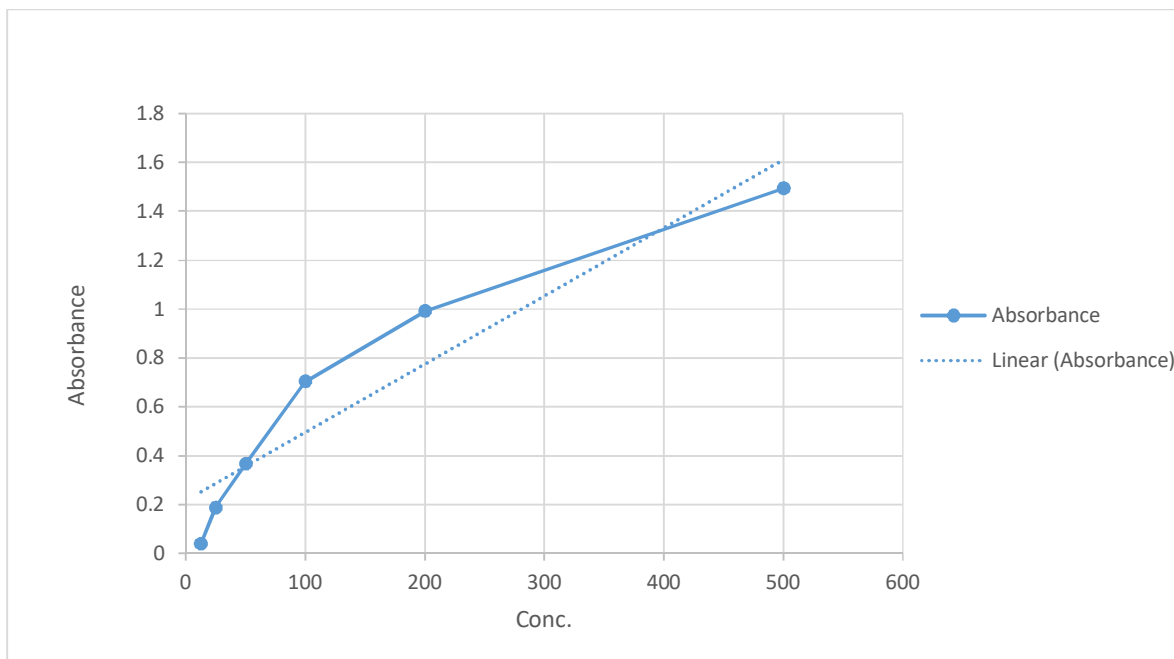
**Fig 3.1.2: Graph of DPPH (% of Inhibition vs. Concentration) (ug/mL) for the sample (*Ficus mollis vahl*)**

### 3.3. Total phenolic content: Evaluation

**Table 8: Gallic acids absorbance**

Concentration ( $\mu\text{g} / \text{mL}$ )	Absorbance	Regression Equation	$R^2$
1000	0.932	$y = 0.0028x + 0.2187$	0.8941
500	0.794		
200	0.511		
100	0.354		
50	0.267		
25	0.188		
12.5	0.041		

**Total phenolic content test: Graph of standard curve for Gallic acid**



**Figure 3.2.1: Standard curve of Gallic acid for the total phenolic content test**

**Table: 9: Result of test sample for the total phenolic content**

Name	Part of the palnt	Absorbance of <i>Ficus mollis vahl</i> plant extract	Total phenolic content (GAE)
<i>Ficus mollis vahl</i> Extract	Leaf	0.488	96.178

So, obtained total phenolic content was 96.178 (mg of GAE /gm of plant extract) for *Ficus mollis vahl* leaves plant extract.



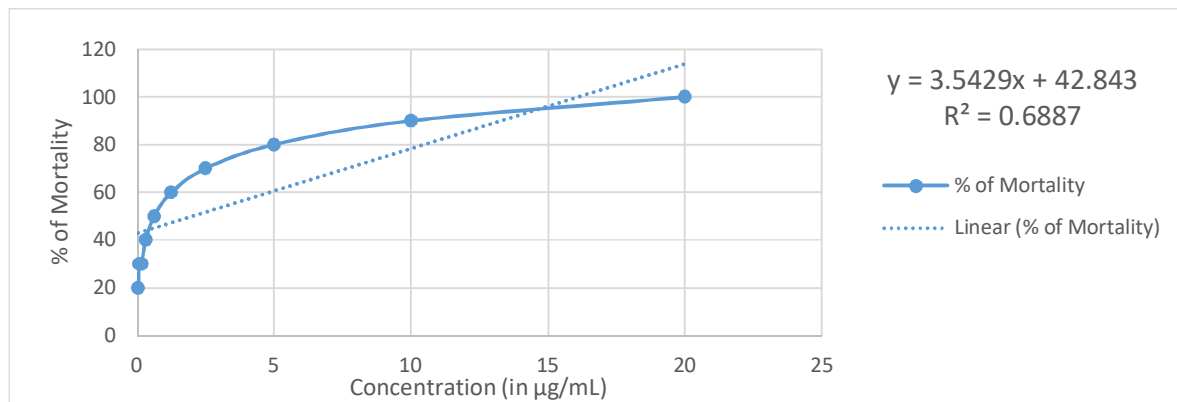
### 3.4. Analysis of In-vitro Cytotoxicity property

#### Brine shrimp lethality assay: Evaluation

**Table 10: Effect of Positive control (vincristine sulfate) on shrimp nauplii**

Concentration of VS ( $\mu\text{g/mL}$ )	Nauplii taken	Dead	Alive	Mortality in Percentage (%)	LC <sub>50</sub> Value ( $\mu\text{g/mL}$ )
0.039	10	2	8	20	2.0203
0.078	10	3	7	30	
0.156	10	3	7	30	
0.312	10	4	6	40	
0.625	10	5	5	50	
1.25	10	6	4	60	
2.5	10	7	3	70	
5	10	8	2	80	
10	10	9	1	90	
20	10	10	0	100	

#### Effects of Vincristine Sulfate on nauplii:

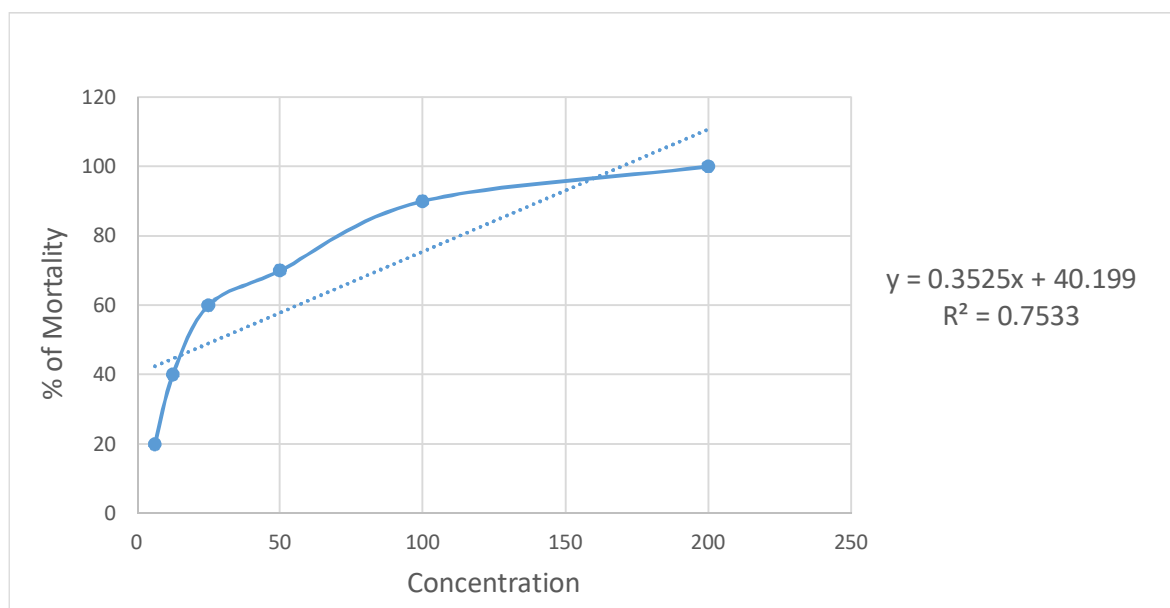


**Figure 3.4.1: Regression and mortality line for vincristine sulfate in Percentage (%)**

**Table 11: Effects of *Ficus mollis vahl* plant extract**

Concentration (µg/ mL)	Nauplii were taken	Dead	Alive	% of Mortality	LC <sub>50</sub> value (µg/ mL)
200	10	10	0	100	27.804
100	10	9	1	90	
50	10	7	3	70	
25	10	6	4	60	
12.5	10	4	6	40	
6.25	10	2	8	20	

**Effects of the plant extract of *Ficus mollis vahl* on nauplii**



**Figure 3.4.2: Percentage (%) mortality and regression line of vincristine sulfate of *Ficus mollis vahl*.**

**Explanation:** LC<sub>50</sub> is 27.804 µg/mL obtained for plant extract from the table 10 and for Vincristine Sulfate LC<sub>50</sub> is 2.0203 µg/mL obtained from Table 11. Which means, compared to Vincristine Sulfate, methanolic extract of *Ficus mollis vahl* requires a higher concentration to provide minimum cytotoxicity effects.

### 3.5. Thrombolytic property analysis

**Table 12: Evaluation of Thrombolytic Activity**

Samples	W1	W2	W3	W4	W5 (W2- W3)	% of clot lysis
The methanol extract of <i>Ficus mollis vahl</i> on Male Subject	0.808	1.917	1.805	0.997	0.112	11.23
The methanol extract of <i>Ficus mollis vahl</i> on Female Subject	0.810	1.936	1.801	0.991	0.135	13.62
Standard (Clopidogrel)	0.799	1.555	1.276	0.477	0.279	58.49
Blank	0.797	1.539	1.511	0.714	0.028	3.92

Where,

$W_1$  = weight of Micro-tube,

$W_2$  = Micro-tube's weight with clot,

$W_3$  = Micro-tube's weight with clot after clot disruption,

$W_4$  = Weight after clot disrupting,

$W_5$  = Released clots weight.

Analyzing this examination, we can say that, plant concentrate of *Ficus mollis vahl* demonstrated a slight impact on clot lysis, however, rate of clot lysis was much lower comparing to Clopidogrel.

**CHAPTER 4**  
**DISCUSSION AND CONCLUSION**

## Discussion

Analysis of Methanol leaf extract of *Ficus mollis vahl*, provided sufficient amount of information which can be helpful in discovering new drugs in medicine world.

The phytochemical screening of *Ficus mollis vahl* leaf showed the existence of Alkaloids, Flavonoids, Tannins, Carbohydrates, Phytosterol whilst it shows the absence of Glycosides, Resins, Steroids, Saponin, Phenols/Phenolic compounds, and Phlobatannins.

In order to verify the antioxidant property of the methanolic extract of *Ficus mollis vahl* leaves was determined properly through DPPH assay. Ascorbic acid was used for the reference standard, where obtained IC<sub>50</sub> value was 550.086 µg/mL and for the methanol extract of *Ficus mollis vahl* obtained IC<sub>50</sub> value of was 673.90 µg/mL.

However, the total phenolic content analyzing test of *Ficus mollis vahl* leaf plant extract showed a satisfactory result, the obtained value was 96.178 (mg of GAE/ gm of plant extract). Thus, this study suggests that, antioxidant agents can be collected. Moreover, the presence of such antioxidant substances in methanolic leaf extract of *Ficus mollis vahl* may justify its use in heart complaints, the treatment of throat and oral diseases and ailing scabies in folkloric remedies.

The brine shrimp lethality bioassay was performed to assess the exact cytotoxic properties of methanolic extract of *Ficus mollis vahl* leaves. In the graphical representation, plotting the % of mortality against the test sample concentration, LC<sub>50</sub> value of the examined sample was determined. From the data, Regression analysis was used to determine the fitted line from the standard curve. In this experiment, as a standard Vincristine sulfate (positive control) was used. Analogize to the standard product, methanoluc extract of the plant *Ficus mollis vahl*, gave LC<sub>50</sub> value 27.804 µg/mL, where LC<sub>50</sub> value was obtained 2.0203µg/mL for vincristine sulfate, Therefore, *Ficus mollis vahl*, showed the cytotoxicity property. To ensure its cytotoxicity property furthermore research is required.

On thrombolytic activity test, the methanolic extract of *Ficus mollis vahl* also showed a significance effect. In this experiment, as a standard Clopidogrel was used. For which clot lysis was found 58.49%. As a negative control, distilled water was used. It proved 3.92% lysis of the blood clot. It was found 11.23% clot lysis on Male Subject and 13.62% clot lysis on Female Subject. Comparing with the positive control value, the clots lysis value of methanol extract discovered thrombolytic activity.

## **Conclusion**

The methanolic extract of the *Ficus mollis vahl* leaf was investigated to identify and evaluate various biochemical properties and characteristics. Throughout the whole process, the plant showed various biological properties. This plant has a moderate level of antioxidant property, slight thrombolytic property with a high level of cytotoxic property in research study.

Further pharmacological studies of this plant are should be carried out to find out unidentified biological properties. Such research activities into these exercises may prompt the medication disclosure, tranquilize detachment and may fill in as a characteristic hotspot for the advancement of novel medication mixes.

**CHAPTER 5**  
**FUTURE DIRECTIONS**

## **Future Directions**

1. The knowledge of the in-vitro antioxidant activity of *Ficus mollis vahl* opens up another possibility to carry out the in-vivo antioxidant study to understand its pharmacological effect on laboratory animal models.
2. Further pharmacological studies on anti-atherosclerotic, anti-cancer, anti-diabetic anti-hyperlipidemia and anti-inflammatory activities of this plant are yet to be carried out.



## **CHAPTER 6**

## **REFERENCE**

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