In-vitro Biological Screening of Methanol Extract of *Syzygium tetragonum* (Myrtaceae) Leaves

By

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A thesis submitted to the Department of pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled "*In-vitro* Biological Screening of Methanol Extract of *Syzygium tetragonum* (Myrtaceae) Leaves" submitted by Sadia Yeasmin (15146086) of spring 2015, has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor in Pharmacy on 22 August 2019.

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Ethics Statement

No trial animal was harmed during the work.

Abstract

This examination was led to discover and assess potential natural properties of the medicinal plant called *Syzygium tetragonum* which has a place with family Myrtaceae. To satisfy the exploration a few investigations were done like anti-oxidant property test which incorporate DPPH test and complete phenolic substance test, brine shrimp lethality test, thrombolytic property test and antimicrobial property test. Subsequent to finishing these tests clear from different perception that, this plant demonstrated great degree of anti-oxidant property, moderate degree of thrombolytic property and critical degree of cytotoxicity property. Furthermore, antimicrobial property test did not demonstrate wanted outcome for this plant, which shows it may not contain any anti-microbial property. In any case, this exploration is beginning to the finding of pharmacological property of this plant. Based on this examination it may be asserted that this plant *Syzygium tetragonum* can be considered to give great effect in therapeutic world and advancement of worldwide preventive medicine.

Dedication

This work is devoted to my family for their unlimited love and abutment

Acknowledgement

This project turns into a reality with the benevolent support and help of numerous people. I might want to stretch out my earnest to every one of them.

At the leading edge, I need to offer this endeavor to Almighty for the wisdom he gave to me, the strength, tranquility of my mind and great wellbeing so as to complete this project. I might want to offer my thanks towards my family for the support which help me to finish this undertaking.

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List of Acronyms

mg	Milligram
ml	Milliliter
µg	Microgram
IC50	Median Inhibitory Concentration
LC50	Median Lethal Concentration
DPPH	2,2-Diphenyl-2-picrylhydrazyl
UV	Ultraviolet
DMSO	Dimethyl Sulfoxide
HCl	Hydrochloric Acid
FRS	Free Radical Scavengers
R_2	Regression Coefficient
WHO	World Health organization
m	Meter
cm	Centimeter
gm	Gram
Conc.	Concentration
ME	Methanol Extract
S. Tetragonm	*Syzygium tetragonum*

Chapter 1

Introduction

1.1 Background

Nature is consistently a brilliant sign to demonstrate the wonders of concurrence. Characteristic items from plants, creatures and minerals are the reason for treating human ailments. The appeal and acknowledgment of therapeutic plants are expanding dynamically. Without a doubt, plants assume a significant job by giving basic administrations in environments. Without plants, people and other living beings can't live in a manner living ought to be. From the very beginning of life ancient people mostly depended on natural plants to alleviate their sufferings. Discovery of medicinal plants are serendipity of human beings when they are in quest of something which can prevent illness. Plants and person are interconnected with one another from the beginning of human development whether to fulfil their yearning or to treat disease, human vigorously subject to plants around them. Plants those have pharmacological or therapeutic effect on human body are designated as medicinal plants which has active ingredients mostly needed to prepare medicine. Medicinal plants has vast history since people use them as the primary source of health care. Bangladesh being an Indian subcontinent nation and biogeographically there is a transition between the Indus-Genetic fields and the eastern Himalayan region, just as Bangladesh is a piece of the Indo-Chinese sub district. In light of these there is a mix of pyriform freshwater and a huge ocean – Bangladesh is offered with a colossal decent variety of plant species (Chowdhury, 2001; Hossain, 2001; Nishat et al., 2002). An estimation of 70-80% people who completely rely on traditional medicine to diagnose themselves (Farnsworth & Soejarto, 1991; Shengji, 2001). The subsistence of different dynasty people like Chakma, marma, garo, shaotal, Khashia with cultural assortment has also fortified the use of medicinal plant. This ancestral exertion to treatment now take over to the modern

medicine due to the belief of folk in Mother Nature. The pieces of therapeutic plants that might be utilized are various sorts of seeds, root, leaf, natural product, skin, blossoms or indeed, even the entire plant. The dynamic mixes in many parts of the restorative plants have immediate or aberrant helpful impacts. The advancement of science and modern technology reveals the magical bioactive constituents of plants like alkaloids, flavonoids, glycosides, tannins, terpenes, resin etc. are used for new drug development. Furthermore these plants have nutritional value so as recommended for therapeutic use (Rasool, 2012). As reported by World Health Organization (WHO) therapeutic plants are easily accessible affordable and socially proper wellspring of essential medicinal services for over 80% of Asia's populace (Sharmin, 2004). Most of the individuals in Asian nations depend on plant-based conventional medicine for social insurance.

1.2 Past history of medicinal plant

It is known to all that utilization of medicinal plant to treat illnesses begun by our precursor however when they began is as yet obscure. While searching for foodstuff they discovered various plants which shows toxic impact and shows various capacities like ability to deliver sweat, diminish agony and irritation. Explanatory history of restorative plants reveals that our progenitors, similar to the Assyrians, the Babylonians, and the Egyptians have well thought about the therapeutic properties of herbs and trees. A major measure of significant medications was notable to Babylonians (around 300 BC) and expressed that contemporary medicine still uses certain plants similarly as Babylonians (Ghani, 2003).

The primary composition on the utilization of restorative plants is right around 400 years of age. The original copy was composed by a gathering of individuals from the old Sumerian culture living on the Euphrates River and the Tigris. They composed on little dirt chunks. The sections were then found by later Iraqi analysts. The Egyptians additionally composed an

original copy on restorative plants called Ebers Papyrus. In excess of 700 strategies were found, expounded on 1700 AC. A brilliant reported method for utilizing restorative plants is the book titled "Pen Tsao" holding the utilization of in excess of 300 therapeutic plants. The Indian therapeutic framework called Ayurveda, which alluded to the utilization of restorative plants, from 800 A.C (Remedies with Traditional Plants, 2016).

Old Greek individuals were likewise acquainted with the therapeutic properties of some therapeutic plants, and Hippocrates, the originator of Greek prescription and Aristotle, student of Hippocrates, utilized therapeutic plants for the treatment of maladies. From that point forward, Theophrastus, a Greek researcher, established the School of Therapeutic Plants. At that point, Pedanius Dioscorides (He lived in the main century A.D), a doctor and specialist in the a long time 75-45 BC, composed a reference book, called De Materia Medica, to depict 600 helpful therapeutic plants as a progression of logical examinations on therapeutic plants (Jamshidi-kia et al., 2018)

In India, the historical backdrop of conventional medications and its human services record returns to 5000 years BCE, when human services need and ailments were noted in old writing, for example, "Rigveda" (1700–1100 BCE), "Yajurveda" (1400–1000 BCE), and "Atharva Veda" (1200 BCE). Afterward, the compositions such as "Charaka Samhita" (990 BCE), "Sushruta Samhita" (660 BCE), and "Dhanwantari Nighantu" (1800 CE), where the utilization of plants was accentuated and generally rehearsed (Adhikari & Paul, 2018)

Mid nineteenth centenary remained a defining moment for learning and utilization of restorative plants. The disclosure, confirmation, and segregation of alkaloids from quinine (1820), poppy (1806), pomegranate (1878), strychnos (1817), ipecacuanha (1817) and different plants, at that point the detachment of glycosides, denoted the start of logical drug store. While updating of the concoction techniques, alternative dynamic textures were likewise found from

therapeutic seedlings, for example, tannins, saponosides, etheric oils, nutrients, hormones, and so forth. In nineteenth century glycosides and alkaloids sequestered in unadulterated structure were progressively supersede the medication in distinction to that segregated (Petrovska, 2012).

1.3 Medicinal plant in Bangladesh

Bangladesh, a nation of an exceptionally ripe land, has a rich flora of medicinal plants. A sum of 4939 angiosperm plant species is dispersed all through the woods, wildernesses, slopes, fields, harvest fields, street sides, gardens, boggy grounds and watery spots of Bangladesh, out of which 750 species are utilized in conventional medication (Pasha and Uddin, 2013; Uddin, 2010). Tropical woods contain more than half of the world's evaluated 500,000 plant species and under 1% of these plants have been explored for therapeutic action (Conte, 1996). The earth of fruitful improvement of medicinal plants in Bangladesh likes to treat 500 old style infections among 2000. Furthermore, the utilization of medicinal plants by commercial enterprises and organizations in Bangladesh has expanded extensively. Many driving pharmaceutical organizations in Bangladesh presently utilize an assortment of therapeutic plants. Most ancestral and Bangladeshi populaces depend vigorously on the therapeutic plant for essential treatment, maybe they accept that nature won't hurt their wellbeing. As per this confidence and conviction, they utilize different pieces of plants: barks, steam, organic products, blooms and so on.

Table 1: List of Medicinal plant used in preparation of traditional medicine

Scientific Name	Common Name	Therapeutic Use
Andrographis paniculata	Kala megh	Hepatitis, anthelminthic dysentery; fevers & stomach trouble
Asparagus racemosus	Shatamuli	Diuretic; measles; pox and diarrhea; energetic (tonic).
Adhatoda vasica	Vasak	Expectorant, coughs asthma

Scientific Name	Common Name	Therapeutic Use
Centella asiatica	Thankuni	Energetic (tonic); nerve diseases; (fairer)
Phyllanthus emblica	Amloki	Rich in vitamins (components of triphala)
Terminalia arjuna	Arjun	Heart diseases (component of triphala)
Ocimum sanctum	Tulsi	Cough, fever, dysentery, stomach diseases, mosquito repellent

Source: (Khan & Rasid,2006)

1.4 Selection of *Syzygium tetragonum* for this project

Chosen plant is an unrevealed plant in the wake of looking through journals and publication on Syzygium *tetragonum* as there is no adequate measure of data was found, thus this plant is chosen to recognize different properties like antioxidant, cytotoxicity, antimicrobial, thrombolytic and so on. Along these lines, this activity to decide these properties of *Syzygium tetragonum* is the objective of this present venture.

1.4.1 Introduction to the selected plant *Syzygium tetragonum*

Myrtaceae, the myrtle group of trees and bushes, in the request Myrtaceae, containing around 3,300 species as well as 150 genera that are broadly circulated around tropical zone. They have rather weathered evergreen leaves with oil organs. A few individuals from financial significance are rose apple, guava Surinam cherry, eucalyptus and feijoa. Among spices clove oil flavors gotten from herbs of this Myrtaceae group. Different individuals from the Myrtaceae incorporate Brisbane box, Callistemon, Eugenia, Leptospermum, myrtle, and jaboticaba.

1.4.2 Characteristic morphological features of *Syzygium tetragonum*

Trees, to 20 m tall. Branches forceful, 4-angled, edges prominent. Petiole 1-1.6 cm, forceful; leaf edge weathered, elliptic to obovate, 12-18 × 6-8 cm, ab-axially somewhat pale when dry, ad-axially dull dark colored and not gleaming when dry, optional veins 9-13 on each side of median and 7-10 mm separated, reticulate veins prominent, central veins 2-3 mm from edge, base extensively cuneate to adjusted. Bloom buds 6-7 mm. Hypanthium short, obconic. Calyx portion thick and short. Petals intelligible, white. Stamens ca. 3 mm. Natural product tinged yellow, globose, ca. 1 cm in diam.

Origin

The plants are commonly found in Africa, Madagascar, Australia and other regions. E. Asia - southern China, Nepal, Bangladesh, northeast India, Myanmar, Thailand.

1.4.3 Plant taxonomy of *Syzygium tetragonum*

Table 2: Taxonomy hierarchy of Syzygium tetragonum

Rank	**Scientific Name**
Kingdom	Plantae
Phylum	Angiosperms (Flowering plant)
Class	Eudicots
Order	Myrtales
Family	Myrtaceae
Genus	*Syzygium*
Species	*Syzygium tetragonum*

1.4.4 Pharmacological properties of other genera and species

Syzygium tetragonum is the evergreen tropical tree in the family of Myrtaceae has immense medicinal value and furnished with a broad range of therapeutic properties. Some Myrcia

species have been utilized as people drug, for the most part as implantation for quite a while. There are in excess of 1,000 species recorded under Syzygium sort of which S. aromaticum, S. cumini and S. jambos are among the couple of well-known for their therapeutic properties. S. aromaticum is notable for its antibacterial, anti-fungal and anti-oxidant property while S. cumini is known to treat looseness of bowl, stomach, related issues and diabetes. S. cumini additionally answered to have anti-oxidant and anti-microbial properties. Then again S. jambo has been accounted for cancer prevention agent, antibacterial and hostile to anti-inflammatory properties (Chew et al., 2017). The most referred to conventional utilization of Myrcia species is identified with a little gathering of Myrtaceae M. salicifolia is useful for mouth blisters and mouth ulcers. Other Myrcia species have customary uses. Myrcia bacteata DC is used to treat dyspepsia, M ovata cambess is utilized on the treatment of gastric ailment, gastritis, looseness of bowl (Cascaes et al., 2015).

Myrtus communis L. (Myrtaceae) is known as True Myrtle. M. communis concentrates and basic oil are significant in medication advancement with some pharmacological exercises in the Middle East particularly in Iran. For quite a while M. communis has been utilized in conventional drugs for the treatment of lung issue and as a germicide, anti-inflammatory, mucolytic, carminative and astringent cure. M. communis has been appeared to have cancer prevention agent, pain relieving, antibacterial and antifungal exercises and larvicide, and repellency impacts (Asgarpanah & Ariamanesh. 2015).

1.4.5 Related publication on *Syzygium tetragonum*

Syzygium tetragonum is as yet an unpublished plant in light of the fact that no examinations have yet been done on its synthetic properties and pharmacological usage. Along these lines, numerous important properties and employments of therapeutic plants still should be distinguished**.**

1.4.6 Project justification / rationale

Writing audit of the chose plant called *Syzygium tetragonum* it is noticed that no huge investigation has been led on this plant. However, prior investigations in a few types of this thoughtful have detailed incredible antimicrobial, against diarrheal, anthelmintic, cancer prevention agent of antioxidant, anti-inflammation, kidney illness, skin ailments and cytotoxic exercises. In this way, the fundamental reason for this examination is to discover the diverse pharmacological properties from the crude leaf concentrate of the plant. Research will likewise try to inspect the obscure properties of the chose plant in the improvement of pharmaceutical world.

1.5 Aim of the project

A definitive point of the task is to look at and find obscure natural capability of the chose plant, *Syzygium tetragonum* (Family*:* Myrtaceae).

1.6 Objective of the project

This undertaking convention contain following advances which finished with methanol concentrate of the *Syzygium tetragonum* leaves.

1) Evaluation of the anti-oxidant property of the methanol concentrate of the plant leaves by applying *in-vitro* DPPH free radical scavenging technique and assurance of the complete phenolic substance of plant.

2) Evaluation and screening of the plant's antimicrobial action.

3) Evaluation of cytotoxic action.

4) Determination of thrombolytic activity.

1.7 *In-vitro* evaluation of antioxidant property of *Syzygium tetragonum* leaves extract

As of late anti-oxidant research has increased much significance in the medicinal field. Inquiry of significant biologically active segments being in numerous species and nourishment ingredients have gotten plenty consideration. Various human issue along with infections seems as the result of oxidative damage. At the point when a reactive molecule, for example, oxygen, and nitrogen, chlorine species contains at least one additional valence electrons, atom like this is named as a free radical. Here oxidation brought about by responsive oxygen species (ROS) are in charge of maturing and causes cell membrane breaking down, impairment in membrane protein, DNA transformations and different ailments (Rotundifolia et al., 2016).

Anti-oxidant eventuates normally in vegetables and seeds, for example, ascorbic acid, vitamin E and phenolic mixes have the capacity to diminish the oxidative harm related with numerous sicknesses. That is the reason numerous specialists have concentrated on natural anti-oxidant and, in the plant kingdom, various crude extracts and unadulterated common mixes have been accounted for to have anti-oxidant properties. Countless medicinal plants have been explored for their anti-oxidant properties. Natural anti-oxidant whether it is in the form of crude concentrates or their chemical constituents are exceptionally successful to counter the damaging procedures brought about by oxidative stress (Braca et al., 2002).

The point of this examination was to screen plant material concentrates with analogous to complete phenolic substance together with cell reinforcement action so as to discover advance potentiality of regular antioxidant activity.

1.7.1 Evaluation of phenolic content

Phenolic mixes are generally spot in one and the other consumable and non-succulent plants, furthermore those mixes have been accounted for numerous organic impacts, encompassing cancer prevention agent action. The powder form of organic products like herbs, legumes, grains, and auxiliary plant object rich in phenolic, hold progressive enthusiasm in the pharmaceutical business since those hinder oxidative debasement of lipids what's more, along these lines reform the aspect and health benefit of drug. The significance of anti-oxidant fractions of plant in the conservation for wellbeing as well as safeguard from congestive heart failure and other syndrome is additionally rear enthusiasm in the midst of researchers, sustenance producers (Lo¨-liger, 1991).

Potential derivation of anti-oxidant has been looked in a few kinds of plant materials, for example, vegetables, organic products, leaves, grain, barks furthermore, flavours, herbs, also unrefined plant drugs. Pant phenolic contents, for example, phenolic acids, tannins, stilbene and lignin, are particularly basic in plant leaves, plant tissue woody parts, for example, stems and barks (Larson, 1988).

Folin-Ciocalteu's phenol reagent is mostly use to identify the phenolic content in plant. Folin-Ciocalteu have the ability to oxidize the phenols effectively when these synthetic substances utilized in phenolic solution, the reagent effectively oxidizes the phenols. At the point when the oxidation technique finished, yellow shade of Folin-Ciocalteu compound transformed into blue color. This shading change quality is estimated in spectrophotometer at 760 nm range. Absorbance of different concentration show the sum phenolic content of the substance. (Harbertson and Spayd, 2006).

1.7.2 Evaluation of antioxidant property by DPPH examine

Plant extracts of *S. tetragonum* and the standard was surveyed based on the radical scavenging impact of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical action through altered strategy. Methanol is utilized where diluted aqueous solution of the sample extricates were dissolved in. As standard solution Ascorbic acid was utilized (Khalaf et al., 2008). In this way, antioxidant property was investigated by fading the DPPH solution with the methanol plant concentrate and note the likenesses with ascorbic corrosive (ASA) by UV spectrophotometer.

1.8 *In-vitro* evaluation and discover of cytotoxic property of *Syzygium tetragonum*

The brine shrimp (Artemia salina) lethality test is considered to be a helpful instrument for primary estimation of cytotoxicity (Solis et al., 1993). Brine shrimp assays have likewise been utilized to detect fungal toxins and their metabolites (Harwig and Scott, 1971), active plant constituents (Meyer et al., 1982), for the examination of pesticide deposits (Grosch, 1967), and to screen the harmfulness of natural waste to marine organisms (Hood et al., 1960).

The brine shrimp examine is helpful tool for the segregation of bioactive mixes from plant extracts (Sam, 1993). The technique is appealing in light of the fact that it is extremely basic, cheap and low toxin are adequate to execute the test in the micro well scale.

Methanol extract of *Syzygium tetragonum* are tried in vitro for their cytotoxic impact against the brine shrimp nauplii and relate toxoid results with their known ethno-pharmacological activities. In vivo lethality test has been effectively utilized as a primer investigation of cytotoxic and antitumor agents (Ramachandran et al., 2010).

Brine shrimp eggs were acquired from the Department of Pharmacy, BRAC University as a blessing test for the examination work. Sifted, artificial seawater was processed by dissolving 38 g of ocean salt in 1 liter of refined water for bring forth the shrimp eggs. The seawater was

placed in a round glass jar (hatching chamber) with a lamp. Shrimp eggs were included into the chamber while the light will assist to hatch (Olowa & Nuñeza, 2013)

1.9 *In-vitro* evaluation of thrombolytic property of *Syzygium tetragonum leaves extract*

A blood coagulation (thrombus) created in the circulatory system because of breakdown of hemostasis causes vascular blockage and results in atherothrombotic illnesses, for example, myocardial or cerebral infarction. Thrombolytic operators that incorporate tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) and so on are utilized everywhere throughout the world for the treatment of these disease. In India, Bangladesh, mostly developing countries however Streptokinase and Urokinase are generally utilized because of lower cost, when contrasted with other thrombolytic drugs. However, natural medications are wide-spoken as accepted medication for their safe and reliable medicinal services standards. The conventional natural medications expanded an uprising enthusiasm since couple of decades because of their extraordinary pharmacological activities, financial reasonability and less symptoms. Accordingly, immense attempt has also been lead towards the revelation and advancement of natural item with an (Demrow et al., 1995) (Brigs et al., 2011), anticoagulant, antithrombotic and thrombolytic activity of the plants.

This examination plans to explore the aqueous concentration of *S. tetragonum* for their coagulation lysis property (thrombolytic action) by utilizing an in-vitro system. An in vitro thrombolytic assay was used to check the clot lysis effect of *Syzygium tetragonum with* clopidegrol as a positive control and distilled water as a negative control (Prasad et al., 2007).

1.10 Antimicrobial property evaluation

Antimicrobial agents are basically significant in decreasing the worldwide weight of irresistible illnesses. However, development and propagation of multi drug resistant (MDR) strain in pathogenic microbes have turned into a huge risk as there are less, or even now and again no,

powerful antimicrobial specialists accessible for the disease brought about by pathogenic microscopic organisms. Countless therapeutic plants have been perceived as profitable assets of regular antimicrobial compounds as an alternative that can possibly be compelling in the treatment of these hazardous bacterial diseases. As indicated by the World Health Organization (WHO), medicinal plants would be the best source to acquire an assortment of medications.

Numerous plants have been utilized on account of their antimicrobial characteristics, which are because of phytochemicals combined in the secondary metabolism of the plant. Plants are wealthy in a wide collection of optional metabolites, for example, tannins, alkaloids, phenolic mixes, and flavonoids, which have been found in vitro to have antimicrobial properties. Various phytotherapy manuals have referenced different medicinal plants for regarding contagious disorder as urinary tract infections, gastrointestinal clutters, respiratory illness, and cutaneous diseases (Bardhan et al., 2018).

Thinking about the huge probability of plants as hotspots for antimicrobial medications, this examination planned to explore in vitro antibacterial and antifungal action of concentrates from *Syzygium tetragonum.*

Chapter 2

Methodology

2.1 Plant collection

Syzygium tetragonum has been chosen as a plant for this exploration as no past examination on its natural properties has been completed. Intensive bibliographic investigation of this plant and its accessibility, the plant was picked for examination. The leaf portion of *Syzygium tetragonum* plant was gathered in April 2019 from Bangladesh National Herbarium.

Table 3: Research of Syzygium tetragonum

Name of the Plant	**Scientific Name**	**Family**	**Part**
Khudy jam (In Bangladesh)	*Syzygium tetragonum*	*Myrtaceae*	*Leaves*

2.2 Process of extraction

Extraction of the Medicinal plant included different advances:

The whole extraction technique can be isolated into two sections also,

A. Plant material preparation from crude extract- 2 stages

1. Washing of plant leaves.
2. Air drying

B. Extraction process (5 stages)

1. Size reduction by crushing
2. Extraction (maceration)
3. Filtration
4. Concentration
5. Drying of the plant concentrate
6. Plant extract.

2.2.1 Preparation of plant material for crude extract

The leaves were tearing from the stem of the plant and washed with clean water to expel the plant scrap and residue particles. The spotless leaf was then permitted to dry at room temperature for multi week after that when the leaves were totally dried, dry leaves were then stored for the subsequent stage.

2.2.2 Plant Extraction procedure

- **Size reduction and weighing**

The dry and hard leaves were then crushed with coarse residue utilizing a high limit blending machine. Around, 950g of powder gathered and this was trailed by pressing in hermetically sealed plastic holders with the fundamental mark that was at last left in a cool, dry, and dim spot until further examination, basic advances were taken to keep away from cross-contamination.

- **Extraction of plant by solvent**

Based on the types of solvents used, the extraction methods can be divided into two part.

1. Extraction with aqueous solvents
2. Extraction with organic solvents

For the point of this investigation, the extraction maceration procedure was utilized for extraction of Plant materials and methanol as organic solvent. Measuring glass containing plant material of *Syzygium tetragonum* powder was absorbed 1.5L of methanol for a time of 2 days at room temperature (22-25°C) with occasional stirring.

- **Filtration**

Following two days of maceration, the substance of the measuring glass was separated by utilizing a clean cotton cloth and Whatman filter paper (pore size: 110 mm).

➢ Concentration

The gathered filtrate of plant extraction was concentrated utilizing a rotational evaporator (Heidolph) at 100 rpm at 30°C, until the methanol concentrate is created. At that point thick concentrated concoction gathered in a petri-dish.

➢ Drying

At last, the petri-dish was put under laminar airflow (LAF) to vaporize the solvent. LAF was utilized as a preventive measure, estimated to stay away from any probability of microbial development in the concentrate while drying. After the effective drying of the extract kept the petri dish in refrigerator.

Table 4: The weight of Syzygium tetragonum methanol leaf extract obtained as a result of complete extraction procedure

Initial weight/g (Petri-dish)	67.45
Final weight/g (Petri-dish + extract)	103.55
Weight of extract/g	36.1

Total load of plant extricate after methanol extraction was 36.1g.

2.3 In-vitro antioxidant property analysis

2.3.1. Evaluation of free radical scavenging DPPH assay

DPPH Activity

The anti-oxidant activity of the plant extricates and the standard was evaluated based on the radical scavenging impact of the 1, 1-diphenyl-2-picryl hydroxyl (DPPH) free radical activity technique with minor adjustments. The test extract was diluted as serial dilution in methanol. Ascorbic acid was utilized as reference compound (100 µg/ml arrangement). DPPH of 0.004% was dissolved in methanol and a 3 ml of this arrangement was mixed with 2ml of test solution

and standard solution separately. The mixtures were kept in dark for 30 min at room temperature. After incubating, the absorbance was spectrophotometrically estimated at 517 nm against blank solution which is basically methanol. In DPPH test, DPPH radical has been utilized widely as a stable free radical to decide the decreasing substances or anti-oxidant activity of plant extricates. The anti-oxidant activity of plant extract containing poly phenol compound is because of their ability to be donors of hydrogen particles or electrons and to catch the free radicals. The purple colored 1, 1-diphenyl 2-picrylhhydrazyl (DPPH) will diminish to yellow shaded complex. This DPPH test is viewed as most significant system for the *In vitro* evaluation of antioxidant action.

➢ Materials and Reagents

List of the materials and reagents given below

Table 5: Materials and reagents

Materials	**Reagents**
Test tubes	DPPH (2,2-Diphenyl-2-Picrylhydrazyl)
Volumetric flask	Ascorbic Acid (ASA)
UV-spectrophotometer	Methanol
Pipette (1mL and 5mL)	Distilled water
Beaker	Extracts of the experimental plant

➢ Control preparation for evaluation

In this experiment, ascorbic acid (ASA) was used as positive control. Calculated amount of ascorbic acid were dissolved in the methanol solvent to acquire a solution which concentration was 1200 µg/mL. After that to get different concentration ranging from 1200 to 200 µg /mL serial dilution was done.

Table 6: Amount used in preparation of control

Name of Chemicals	Calculated Amount
Methanol	10ml
Ascorbic acid	120mg

➢ Test sample preparation for evaluation

120mg of *Syzygium tetragonum* leaves concentrate was set in a cleaned test tube to prepare the test sample after that 10mL of methanol added to the test tube to get concentration of 12mg/ml. it is the sample solution for test. Soon by serial dilution method 1200µg/mL, 800, 400, 200µg/mL concentration accomplished and kept them dry spot with imprint.

➢ Preparation of DPPH solution for evaluation

So as to formulate the DPPH solution, 2 mg of DPPH powder were effectively estimated and dissolved in 50 mL of methanol to gather 20µg/mL concentration. At that point the solution was put away in a dark place which is secured by aluminum foil paper.

Table 7: Amount used in DPPH solution preparation

Name	Calculated amount
DPPH (2,2-Diphenyl-1-Picrylhydrazyl)	2mg
Methanol	50ml

➢ Assay of DPPH free radical scavenging activity

Test solution in each test tube having distinctive concentration ranging from 1200µg/mL, 800, 400, 200µg/mL were mixed with 2.0 mL of a DPPH solution which concentration is 20µg/mL. At that point this blend is kept in a dim spot for 30 minutes to occur response. Following 30 minutes' absorbance of mixtures were estimated by UV spectrophotometer at 517 nm wavelength. Here, methanol utilized as blank.

➢ Calculation

Inhibition in percentage *(*I%) of Free radical DPPH was calculated as follows:

Inhibition (I%) = (1 – A sample/A blank) x 100

Where, A blank stands for absorbance of the control reaction

Afterwards, 50% inhibition (IC50) was provided by methanol plant extract concentration and value obtained from the graph where plotted inhibition percentage (I%) against the concentration of plant extract (µg/mL).

2.3.2 Evaluation of total phenolic content

➢ Materials and reagents

Table 8: Materials and reagents for phenolic content measurement

Materials	Reagents
Test tube	Folin-Ciocalteu reagent (10 fold diluted)
UV-spectrophotometer	Gallic acid
Vial	Na2CO3 solution (7.5 %)
Micropipette (50-200 µl)	Distilled water

➢ Test sample preparation for evaluation

120mg of *Syzygium tetragonum* leaves concentrate was set in a cleaned test tube to prepare the test sample after that 10mL of methanol added to the test tube to get concentration of 12mg/ml. it is the sample solution for test. Soon by serial dilution method 1200µg/mL, 800, 400, 200µg/mL concentration accomplished and kept them dry spot with imprint.

➢ Standard solution preparation for gallic acid curve

Gallic acid is typically utilized worldwide as a standard in absolute phenolic substance test. Different Gallic acid concentration was prepared by utilizing 2 mL of Folin-Ciocalteu compound dissolved in 18ml distilled water (multiple times diluted with water) and 2mL of (7.5% w/v) Na2CO3 were added furthermore 0.12g of Gallic acid included. The arrangement

solution was put in a dark space at room temperature for 20 minutes. After that by utilizing UV spectrophotometer solution was estimated at 760 nm and absorbance was taken. At that point the absorbance was plotted against the concentration subsequently a direct relationship was gotten which was utilized to get test result.

- **Analysis of total phenolic content**

To get solution, 0.5mL of plant extract (2 mg/mL), 2.5 mL of Folin-Ciocalteu synthetic (multiple times blended with water) and 2.0 mL of (7.5% w/v) Na2CO3 were included. At that point the solution is kept in dark space at room temperature for 20 minutes. After explicit time 760 nm absorbance was assessed with UV spectrophotometer alongside by utilizing standard solution of Gallic acid, the complete example was evaluated. Test's phenolic substance was expressed as mg of GAE (Gallic acid equivalent)/g of concentration

2.4 *In-vitro* cytotoxicity property analysis

2.4.1 Experimental Procedure of Brine shrimp lethality assay

- **Materials for test**

Table 9: List of materials required for Brine shrimp lethality assay

Sl. No.	Name of materials
1	Brine shrimp (*Artemia salina)* egg
2	NaCl
3	Round glass jar
4	Micropipette, pipette
5	Test tube
6	Dimethyl sulfoxide (DMSO)
7	Lamp to attract shrimp
8	Plant extract
9	Magnifying glass
10	Glass vial

➢ Preparation of seawater for test

To prepare seawater, 38g of salt (unadulterated NaCl) was weighted precisely then it diffused in refined water (2L) and afterward sifted the water to maintain clear solution.

➢ Hatching of brine shrimp's eggs for test

For assessment, brine shrimp (Artemia salina) eggs were given from BRAC University, Department of Pharmacy was utilized for assessment. A round glass tank taken and loaded up with seawater at that point shrimp eggs included into it. To get mature nauplii the tank was incubated for 2 days with nonstop supply of oxygen for the duration until hatching was completed. Light pulls in shrimps through the punctured dam. Pasteur pipette used to gather 20±2 living shrimps which included into each test tube which containing 5mL of seawater.

➢ Preparation of test solution for experiment

Test solution was taken in a test tube and disintegrated with dimethyl sulfoxide (DMSO). After that by serial dilution distinctive concentration extending from 1200µg/mL to 200µg/mL accomplished. First 50µl of Sample which concentration was 400 µg/mL put into test tube holding 5mL of DMSO alongside 20±2 nauplii. After that 50µl DMSO was added to test tube to dilute the solution by this technique diverse concentration acquired.

Table 10: Plant sample with different concentration after serial dilution

Test tube no	Concentration (µg/mL)
1	1200
2	800
3	400
4	200

➢ Preparation of control group for experiment

In cytotoxicity study to affirm the examination procedure and guarantee that the outcomes accomplished were equal to the performance of the test operator and the potential impacts of other possible stoppages control group is fundamental. Normally 2 sorts of control groups are practice, they are positive6 and negative control.

1) Preparation of positive control

In cytotoxicity test positive control is generally known as a cytotoxic compound, which help in test contrasted with the consequence of positive control. Here, vincristine sulfate a cytotoxic compound was utilized as standard (positive control). The dose of vincristine sulfate was mixed in DMSO to acquire the principal portion of 20µg/mL right then and there by serial dilution verity concentration of standard solution got, for example, 10µg/mL, 5µg/mL, 2.5µg/mL, and 1.25µg/mL. At long last, standard (positive control) included the test tube holding 5mL seawater alongside 10±2 nauplii.

2) Preparation of negative control

So as to prepare negative control, 3 test tube were taken and 100 µl of DMSO was added to the every one of the test tube which contain 5mL of seawater alongside 20±2 nauplii. In the event that if the demise of nauplii is quick, which shows the test is unsatisfactory and the nauplii expired because of some undesirable reason.

➢ Nauplii counting

Result got following 24 hours, by the assistance of an amplifying glass and the quantity of survivors was measured precisely in the every one of the test tube. From every dilution, (%) level of mortality was determined by direct relapse of IBM-PC program which is utilized to assess the mortality information. Moreover, the concentration versus mortality relationship of plant concentrate is communicated by (LC50) esteem which means middle deadly focus

esteem. In this way, the concentration of the synthetic chemical is accountable for the demise in half of the test nauplii after a particular timeframe.

2.5 In-vitro thrombolytic property analysis

Thrombolytic property can be assessed by a simple strategy where plant extract as test sample, Clopidogrel (anti- platelet specialist) as positive standard and water as negative standard.

➢ Materials and reagents

Table 11: Used materials in thrombolytic test

Sl. No.	Name of materials
1	Blood
2	Clopidogrel (anti-platelet agent)
3	Micro centrifuge tube
4	Distilled water
5	Plant extract

➢ Test sample preparation

For arrangement of test sample, a test tube was taken which containing refined water (10mL) at that point 400mg of plant concentrate was suspended in it, after that test tube was kept in dry, dim spot for overnight and accordingly wanted solvent supernatant was moved in solution and afterward solution separated by filtration.

➢ Standard solution preparation

Clopidogrel an antiplatelet specialist utilized as a standard for this test. 75mg of clopidogrel dissolved in refined water (10mL) and blended suitably at this point this suspension put away as a stock standard solution from which 100µl arrangement was used in thrombolytic action test.

➢ Blood sample preparation

5 sound volunteers (n=5) have no history of anticoagulant treatment chose from them blood sample were gathered by maintaining aseptic condition. In the wake of gathering blood 1mL of blood was moved into pre-weight micro centrifuge tube. After that small scale tube kept for incubation at 37-degree c. for 45 minutes which exaggerate clump formation.

➢ Thrombolytic property test process

At start of the test, 5mL of new blood was gathered from each humanitarian worker. Blood tests were taken from five diverse pre-weighed sterile organisms and permitted to incubate at 37°C for 45 minutes. At the point when the coagulation is formed, the upper liquid was totally released from all smaller scale tube. The weight of the coagulation was measured by the weight of the tube taken before the coagulation is shaped. For this situation, 100µl of clopidogrel utilized as positive control and 100µl of water (distilled) were utilized as a non-thrombolytic negative control with 100µl of each example was included from each test tube. For perception of clump lysis, micro centrifuge tube was incubated at 37°C for 90minutes. A short time later when the incubation was finished, the fluid was expelled which was discharged from cluster and again weighted the tube to watch the weight contrast after the coagulation diversion. Finally, percentage (%) of clot lysis as shown underneath:

Percentage (%) of clot lysis = (released clot weight /clot weight) × 100

2.6 Antimicrobial property analysis

Methanol extract of *Syzygium tetragonum* leaves used as test sample.

Apparatus and reagents used for antimicrobial analysis

Table 12: List of apparatus used

Sl. No.	Apparatus
1	Filter paper discs
2	Nutrient Agar Medium
3	Petri-dishes
4	Micropipette
5	Sterile forceps
6	M.H. Agar
7	Screw cap test tubes
8	Autoclave
9	Spirit burner
10	Refrigerator
11	Nose mask and Hand gloves
12	Incubator
13	Laminar air flow hood

Micro-organism used in the test

Bacterial strains gathered from unadulterated culture and here both gram positive and gram negative creatures were taken for the trial and list of the microorganism are given beneath.

Table 13: List of micro-organism used in antimicrobial analysis

Gram positive	Gram negative
Streptococcus pyogene	*Escherichia coli*
Staphylococcus aureus	*Pseudomonas aeruginosa*
Bacillus subtilis	*Shigella dysentariae*

➢ Sterilization procedure of test

Before directing test all device incorporates measuring glasses, conical flask, petri-dishes, cottons and forceps disinfected and kept in aseptic spot. This disinfection done to stay away from any sorts of cross contamination or microbial pollution during the procedure. To keep up control condition all work done in under Laminar Air Stream Hood, before beginning analysis UV light was turned on for 1 hour in the Laminar Air stream Hood. Micropipette tips, cotton, forceps, blank disc were additionally treated by UV light. Then via autoclave machine petri-dishes and different mechanical assembly were sanitized at a temperature of 121ºC and a weight of 15-lbs/sq. inch for 60 minutes. After trial all the device cleaned and utilized bacterial strains were demolished to maintain all sorts of contamination.

➢ Anti-microbial test procedure

Firstly, for culture preparation 2.5g of nutrient broth dissolved in 100mL distilled water. At that point 6 conical flasks were taken every conical flask contains 10mL of stock solution there 6 distinctive bacterial strains included into it and kept these cone like jars at shaking motion into incubator at temperature 37 ºC for 24 hours. Following 24 hour these conical flasks were expelled from incubator and kept in controlled condition. From that point onward, agar medium arranged by utilizing 7.6g of Mular Hinton agar which dissolve in 100mL of refined water. Following readiness Mular Hinton agar set into petri-dishes and kept these petri-dishes for chill off at room temperature. In the meantime, test solution of plant extricates ranging from 1200mg/mL to 200mg/mL were arranged and let them socked into the filter paper disc. Whenever Mular Hinton Agar in petri-dishes ended up strong then bacterial strains skewered into it by utilizing cotton bars. Streptomycin utilized as standard plates and test plant concentrate circles put in the petri-dishes. At that point these petri-dishes kept into incubator at 37ºC for 24 hours to give ideal condition to bacterial development. Again after 24hours later

petri-dishes containing distinctive bacterial strains alongside standard and test plates gathered and watched restraint zone created by standard and test circles.

- **Determination of inhibition zone for test**

Antimicrobial property of *Syzygium tetragonum* is assessed by the ability to stop the microorganism development around the plates in the petri-dishes. Counteractive action of microorganism development showed when plates gives clear locale of restraint. At the point when the incubation is finished antimicrobial property of the sample were assessed by computing the distance across of the restraint territory with a clear zone.

Chapter 3

Observation and result

3.1 Antioxidant property analysis

3.1.1 Evaluation of free radical scavenging assay of *Syzygium tetragonum*

Table 14: IC50 value (µg/mL) of Ascorbic acid (ASA)

Conc. (µg/mL)	Absorbance of Standard (ASA)	% of inhibition	IC50 (µg/mL)
1200	0.067	99.38	81.99
800	0.010	97.02	
400	0.017	89.95	
200	0.015	34.24	
Blank	0.617		

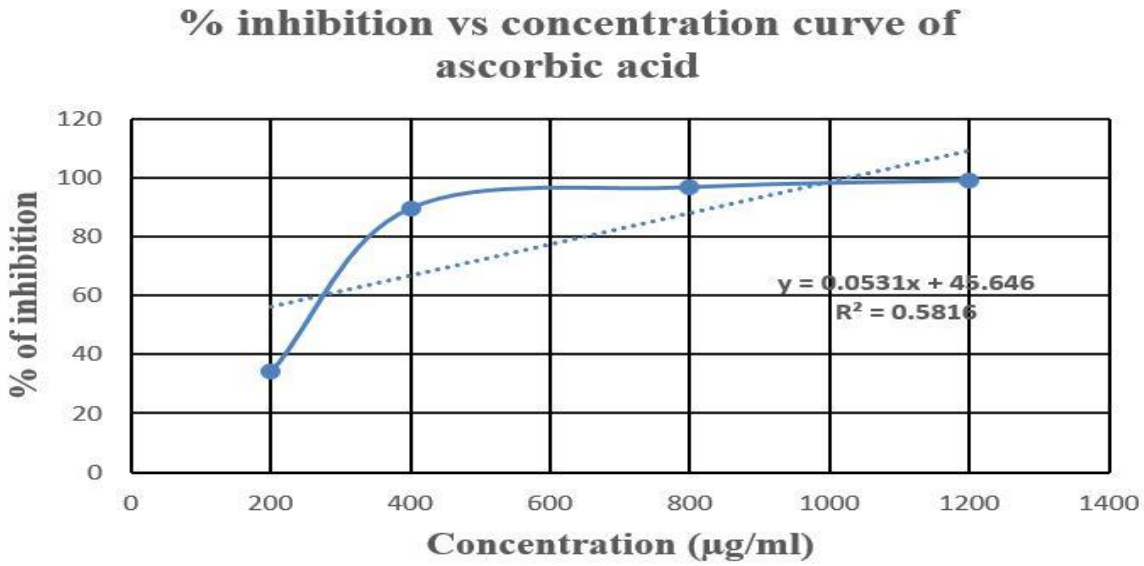

Figure 1: % Inhibition vs. Concentration curve of ASA

Table 15: IC50 value (µg/mL) of Methanol plant extract of Syzygium tetragonum

Conc. (µg/mL)	Absorbance of Methanol plant extract	(%) of inhibition	IC50 (µg/mL)
1200	0.094	96.79	35.25
800	0.071	93.78	
400	0.053	88.58	
200	0.042	37.93	
Blank	0.617		

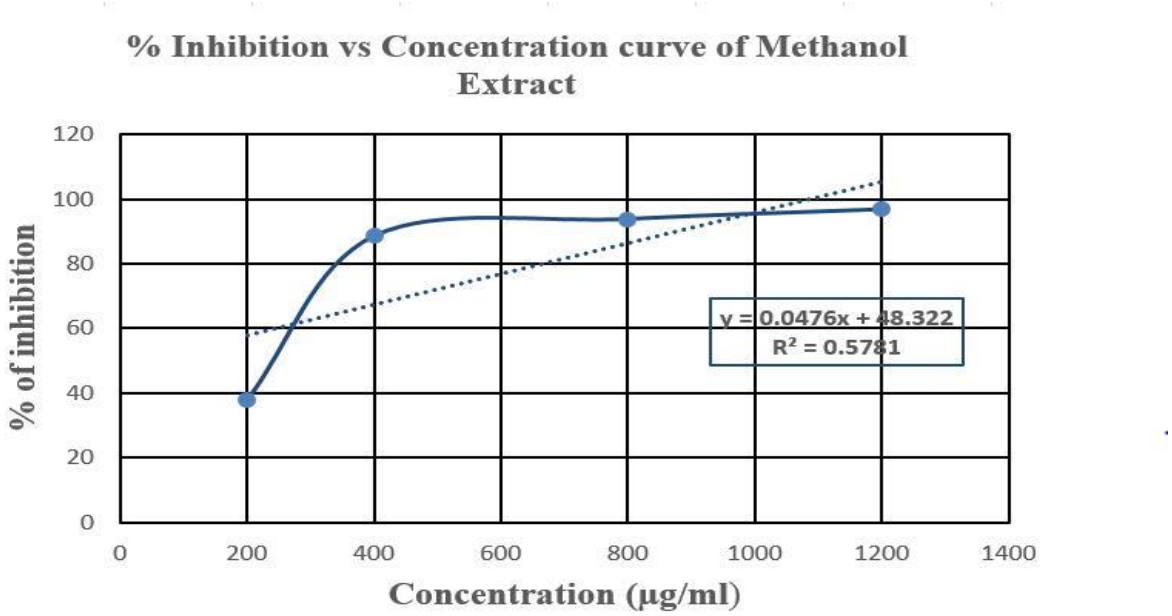

Figure 2: % Inhibition vs. Concentration curve of Methanol extract of Syzygium tetragonum

Clarification

After perception, the table (3.1 and 3.2) demonstrated that rate (%) restraint of free radical DPPH scavenging of the concentrate of *S. Tetragonum* was somewhat lower at certain focuses however in certain focuses *S. Tetragonum* have higher % of inhibition than the equal centralization of ascorbic acid. And furthermore IC50 µg/mL estimation of methanol concentrate of *S. Tetragonum* was lower than ascorbic acid.

3.1.2 Evaluation of total phenolic content

Table 16: Absorbance of Gallic acid

Conc. (µg / mL)	Absorbance	Regression line	R_2
12	0.019		0.9795
8	0.011	Y=0.0015x+0.0003	
4	0.006		
2	0.004		

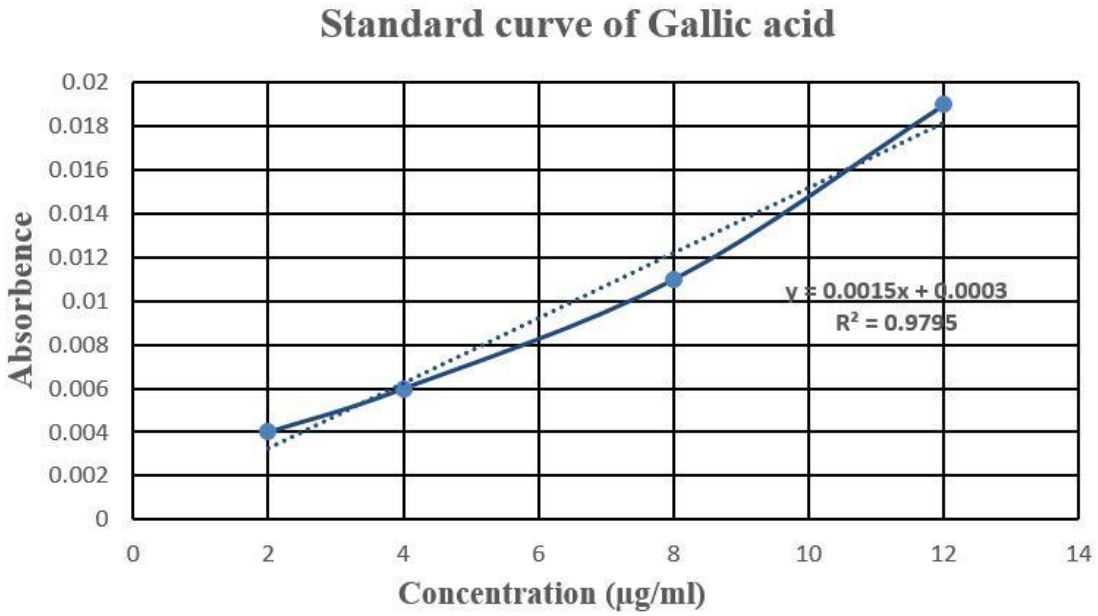

Figure 3: Gallic acid's standard curve for total phenolic content test.

Table 17: Result of total phenolic content of test sample

Sample code	Name of extract	Plant part	Absorbance of methanol plant extract	Total phenolic content (mg of GAE / gm of extract)
ME	Methanol extract	Leaves of *Syzygium tetragonum*	3.886	647.47

So, Total phenolic content was obtained 647.47 (mg of GAE / gm of extract) of the methanol extract of *Syzygium tetragonum* leaves.

3.2 *In-vitro* Cytotoxicity property analysis

3.2.1. Evaluation of Brine shrimp lethality assay

Table 18: Positive control (vincristine sulphate) effect on shrimp nauplii

Concentration (µg/mL)	Log Concentration	Nauplii taken	Nauplii Dead	Nauplii alive	% of Mortality	LC50 (µg/mL)
10	1	10	8	2	80	3.065
5	0.70	10	6	4	60	
2.5	0.40	10	5	5	50	
1.5	0.18	10	4	6	40	

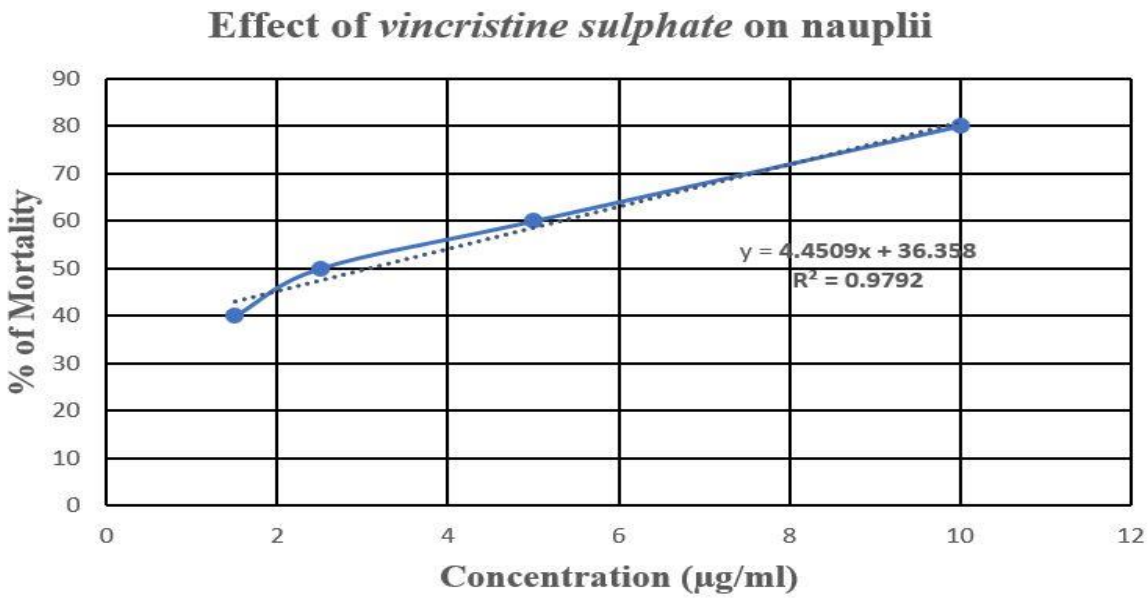

Figure 4: Percentage (%) mortality and predicted regression line of vincristine sulphate

Table 19: Effects of the methanol extract of Syzygium tetragonum

Concentration (µg/mL)	Log Concentration	Nauplii taken	Nauplii Dead	Nauplii alive	% of Mortality	LC50 (µg/mL)
12	3.08	20	5	15	75	7.196
8	2.90	20	11	9	55	

Concentration (µg/mL)	Log Concentration	Nauplii taken	Nauplii Dead	Nauplii alive	% of Mortality	LC50 (µg/mL)
4	2.60	20	7	13	35	
2	2.30	20	4	16	20	

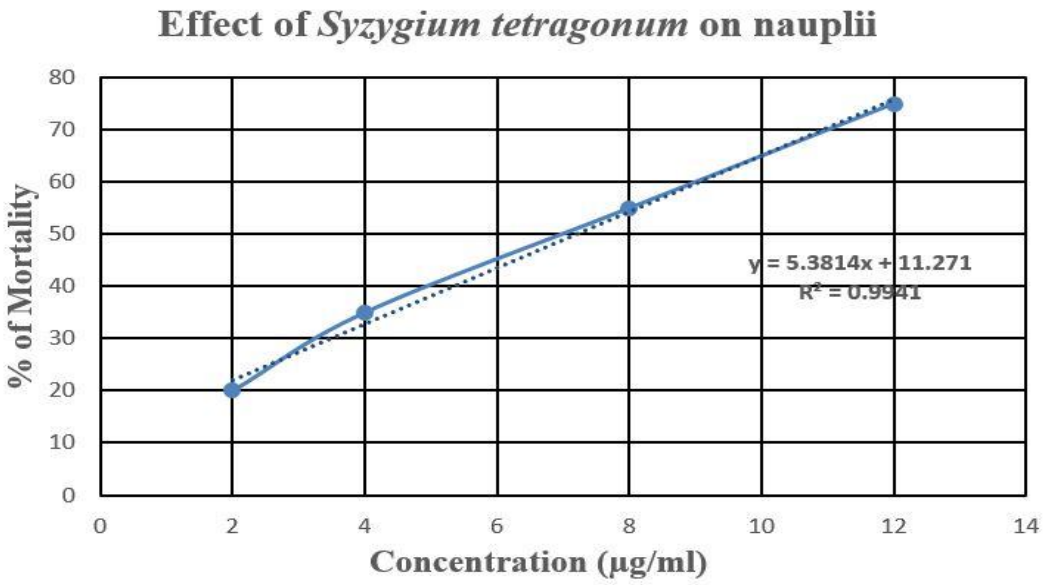

Figure 5: Percentage (%) mortality and predicted regression line of Methanol extract of Syzygium tetragonum

3.3. Thrombolytic property analysis

Table 20: Evaluation and result of Thrombolytic Activity

Name of Samples	Weight of Empty ependroph W1	Clot with weight of ependroph W2	Weight of clot W3=W2-W1	Weight of ependroph after clot lysis W4	Weight of released clot W5=W2-W3	%of clot lysis
Methanol extract of	0.837	1.798	0.961	1.614	0.184	19.14

Syzygium tetragonum						
Clopidogrel (Anti-platelet agent) as standard	0.837	1.805	0.968	1.569	0.236	24
Blank	0.837	1.741	0.904	1.578	0.163	18

From this analysis it very well may be expressed that, Methanol concentrate of *Syzygium tetragonum* indicated symbolic impact on clot lysis, yet contrast with clopidogrel cluster lysis rate was lower.

3.4 Anti-microbial property analysis

3.4.1 *Syzygium tetragonum* leaves antimicrobial activity evaluation

Methanol concentrate of *Syzygium tetragonum* leaves was taken to perform against microbial activity investigation, distinctive concentration was readied going from 1200µg/mL to 2005µg/mL. With these concentration every one of the bacterial strain inspected however no huge outcome was found. There are little potential outcomes of some concentration to demonstrated exceptionally mellow impact yet the vast majority of the focus did not demonstrate any antimicrobial property. Explanation for this negative outcome may be test mistakes and others. However, during this examination streptomycin which is utilized as standard indicated antimicrobial action appropriately on both gram positive and gram negative microscopic organisms.

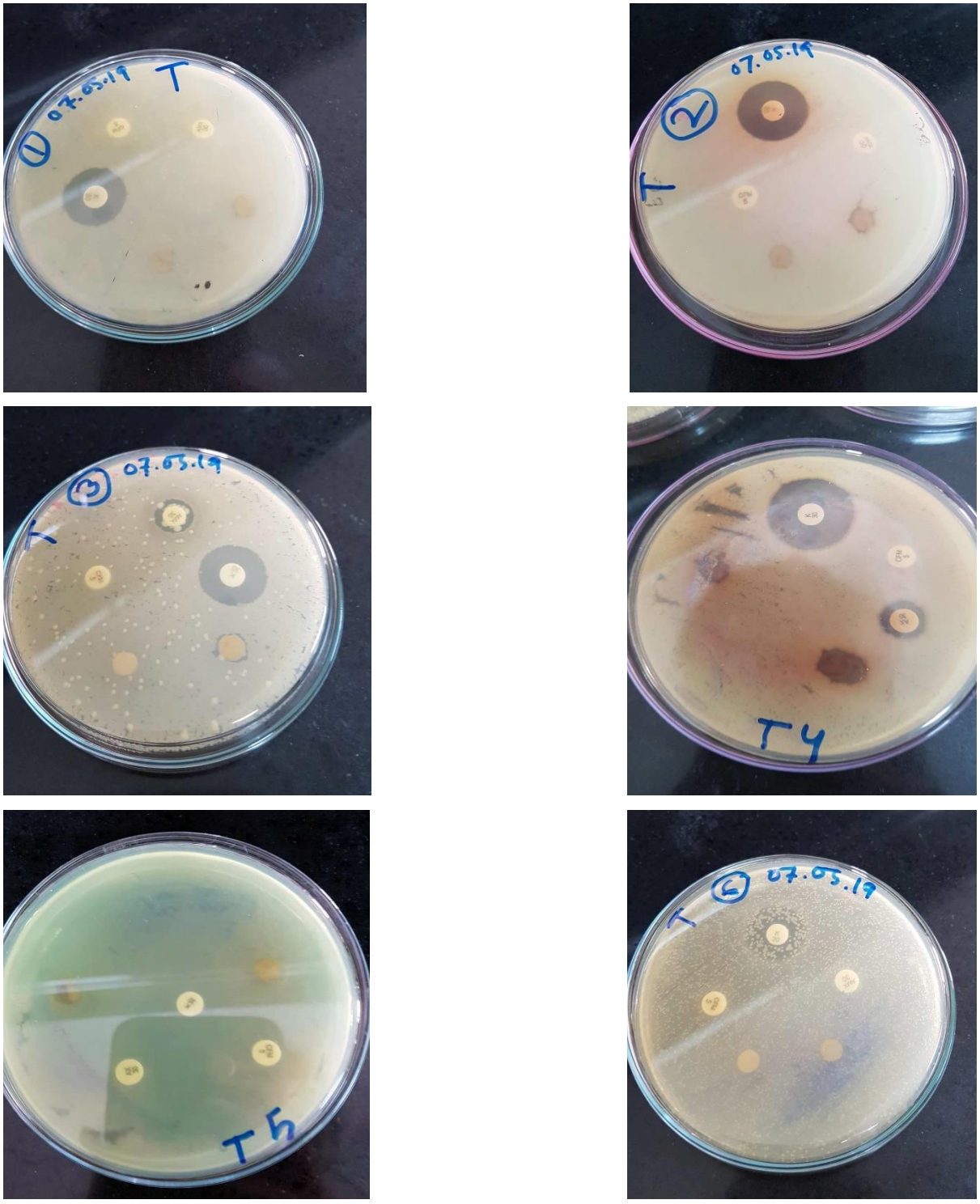

Figure 6: Antimicrobial property of plant Syzygium tetragonum leaves evaluation on petri-dishes

Chapter 4

Discussion

Natural screening techniques for the methanol leaf concentrate of the picked plant *Syzygium tetragonum*, provides adequate measure of data which can be used in medication world. The methanol concentrate of *Syzygium tetragonum* leaves was checked appropriately through DPPH measure to decide the anti-oxidant property of this plant. As the reference standard, ascorbic acid was utilized in this examination for which IC50 worth was acquired 81.996µg/mL and the methanol concentrate of *Syzygium tetragonum* which IC50 estimation of was gotten 35.252µg/mL. Also, absolute phenolic substance trial of this plant indicated palatable outcome, esteem got 647.466 (mg of GAE/gm of concentrate). Subsequently, the present investigation may propose that this plant can be utilized as antioxidant agent.

The brine shrimp lethality assay was achieved to survey the cytotoxicity property of methanol concentrate of S*yzygium tetragonum* leaves. LC50 estimation of the analyzed example was resolved from the graph where plotting the level of mortality (nauplii) against the plant concentration. To decide the best fitted line from the curve achieved from the information, regression analysis was utilized. Vincristine sulfate was utilized in this investigation as a standard (positive control), which LC50 was gotten 3.065µg/mL contrasted with the standard methanol concentrate of the plant *Syzygium tetragonum* gave LC50 esteem 7.196µg/mL. Thus, it is raised that this *Syzygium tetragonum*, demonstrated the cytotoxicity property that's why more research required to guarantee its property for the improvement of the worldwide human services.

Methanol concentrate of S*yzygium tetragonum*, indicated noteworthy outcome on thrombolytic action test. Here, clopidogrel utilized as a positive control for which 24% cluster lysis was seen. Refined water was utilized as a negative control, which demonstrated 18% lysis of the blood

coagulation. The methanol concentrate of S*yzygium tetragonum*, demonstrated 19.14% cluster lysis. Looking at the coagulations lysis estimation of methanol extract with the positive control esteem, plant uncovered viable thrombolytic action.

Antimicrobial property test was additionally done to assess methanol concentrate of S*yzygium tetragonum* leaves. From this investigation no noteworthy outcome was acquired, which means plant may not contain any antimicrobial property. This can be happened because of some trial mistake. In this case, more examination is required affirming about the plant's antimicrobial property present or not.

Chapter 5

Conclusion

Methanol concentrate of the *Syzygium tetragonum* leaf was examined to assess the organic properties. Afterwards leading this examination, it has been cleared that the plant demonstrated distinctive organic properties. This examination study demonstrated that plant has moderate degree of antioxidantion property and thrombolytic property alongside noteworthy degree of cytotoxicity property. Although, this plant did not demonstrate any antimicrobial property, yet it requires further examination on antimicrobial property of this plant. Moreover, research recommends increasingly definite examination of this plant *Syzygium tetragonum* to discover unidentified organic properties which will help in the improvement of the world medical management and may present any new pharmacological property in field of prescription.

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