Investigation of In-vitro Anticancer and Pesticidal Activity of *Bougainvillea spectabilis* Leaves Methanolic Extract

M. Pharm (Masters) Thesis Report

In the partial fulfillment of the requirements for the degree of Masters of Pharmacy

Submitted To

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Submitted By

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APPROVAL

This document confirms that the thesis titled **"Investigation of In-vitro Anticancer and Pesticidal Activity of *Bougainvillea spectabilis* Leaves Methanolic Extract"** has been submitted to the Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University. It fulfills the partial requirements for the degree of Masters of Pharmacy and has been approved for its style and content.

BOARD OF EXAMINERS

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DECLARATION

I, Saila Kabir Maeesa, affirm that the thesis titled **"Investigation of In-vitro Anticancer and Pesticidal Activity of *Bougainvillea spectabilis* Leaves Methanolic Extract"** was conducted under my effort, and the observations and conclusions presented in this work are based on my observations. This work was conducted under the supervision of **Mr. Md. A.K. Azad**, Assistant Professor & Coordinator M. Pharm, Department of Pharmacy, Daffodil International University, to partially fulfill the requirements for the Masters of Pharmacy degree. I confirm that this work has not been previously submitted, either in full or in part, to any other university or institution, to obtain any other degree or professional qualification, except as explicitly stated.

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I am forever thankful to my **Parents** for their spiritual support throughout my life. I am also thankful for the constant encouragement, support, and guidance of all my teachers, lab assistants, and friends from the Department of Pharmacy at Daffodil International University.

Written by

Author

DEDICATED
TO MY
PARENTS

Abstract

Background: This thesis contains cytotoxicity, lethality, and pesticidal tests to investigate in-vitro anticancer and pesticidal properties of the methanolic extract of *Bougainvillaea spectabilis* leaves.

Methods: The CellTiter 96 Non-Radioactive Cell Proliferation Assay was used to assess the extract's cytotoxic activity against HeLa cells (human cervical carcinoma). The brine shrimp lethality test (BSLT) was used to conduct preliminary toxicity screening. Using Rice weevils, pesticidal activity was evaluated in a lab setting.

Results: With an LD50 value of ~8.7mg/ml indicating anticancer activity and a concentration-dependent reduction in cell viability, the extract demonstrated notable cytotoxicity in HeLa cells. Strong overall toxicity was shown by the extract's 100% death rate in the BSLT. The extract's potential as a natural pesticide was demonstrated by the pesticidal assay, which showed 100% mortality.

Conclusion: The results show that bioactive chemicals with significant anticancer and pesticidal effects are present in the extract of *Bougainvillaea spectabilis* leaves. These findings provide the groundwork for additional bioassay-guided fractionation and characterization to create new agrochemical and medicinal compounds.

Keywords: *Bougainvillea spectabilis*, anticancer activity, pesticidal activity, HeLa cells, brine shrimp lethality, natural bioactive compounds.

CONTENTS

Chapter Five: Materials & Methods

Chapter Six: Total Results & Discussions

Chapter Seven: References

Chapter One: Introduction

1.1: Introduction

Millions of people die from cancer every year, making it one of the biggest worldwide health issues. According to the most recent global cancer statistics for 2024, with an estimated 10 million cancer-related fatalities per year, cancer is still one of the major causes of death globally [1]. The urgent need to find new, efficient, and reasonably priced therapeutic agents is highlighted by the drawbacks of current treatments, such as drug resistance, adverse effects, and high expenses [2]. Plants have long been a mainstay of drug development because they are a natural source of bioactive secondary metabolites that offer a variety of molecules with different modes of action [3]. Similarly, interest in plant-based pesticides as sustainable and environmentally friendly substitutes has increased due to growing worries about insect resistance and environmental damage linked to synthetic agrochemicals [4].

The tropical plant *Bougainvillea spectabilis* is well-known for both its healing qualities and vivid blossoms. Studies demonstrate that it is a rich source of bioactive substances with anti-inflammatory and antioxidant properties, such as phenolics and flavonoids [5]. These substances can stop tumor growth and cause cancer cells to die. This suggests that they may have anticancer effects. Also, has been used as a traditional medicine to treat microbial infections and illnesses including diabetes, cardiovascular problems, wound healing [6], sore throat, and asthma [7]. *B. spectabilis* is a multipurpose decorative and medicinal plant that has fascinated scientists for years due to its many health advantages and pharmacological promise in treating a wide range of illnesses. Although *B. spectabilis* has a well-established pharmacological potential, more studies are needed to identify its potential uses in both the medical and agricultural fields.

Plant extracts are frequently tested for their anticancer properties using cancer cell line tests, such as HeLa cells (human cervical carcinoma) cytotoxicity test [8]. The capacity of bioactive substances to stop the proliferation of cancer cells and cause cell death is revealed by these in-vitro systems. At the same time, the brine shrimp lethality test (BSLT), which is used as a first screening

instrument, is a dependable and economical way to evaluate general toxicity and forecast bioactive potential [9]. In the agricultural setting, evaluating plant extract's pesticidal effectiveness has two benefits: it may help with pest control issues and lessen dependency on dangerous synthetic pesticides [10].

The potential of the methanolic leaf extract of *Bougainvillea spectabilis* as a dual-purpose bioresource is examined in this work. The three goals are to: (1) screen its toxicity profile using BSLT; (2) analyze its in-vitro cytotoxic activity against HeLa cells; and (3) determine its pesticidal performance against pest species. By combining these methods, this study hopes to offer a thorough grasp of the extract's bioactivity and establish the foundation for the separation and characterization of its active ingredients under the guidance of bioassay. These discoveries might aid in the creation of sustainable agrochemicals and new medicinal compounds for cancer treatment from *B. spectabilis.*

1.2: Aim of The Study

1. To find out a potential bioactive compound that might be a potential treatment for Cancer.

2. A potential medicine from a natural source, which has great availability, as well as low in cost.

3. Find out a nature-friendly pesticide, which is as well not harmful for human consumption.

Chapter Two: Literature Review

2.1. I conducted my literature research using internet resources from many reputable publications, including ResearchGate, LinkedIn, and others. Numerous studies on the plants I chose have been published, but they are completely different from the study I am doing now.

The following is a summary of the main therapeutic effects of *B. spectabilis* extracts:

1. **Anti-Inflammatory Activity**

   By reducing inflammation and nociception, *B. spectabilis* leaves have strong anti-inflammatory effects. This suggests possible uses in the management of pain and inflammatory diseases [11].

2. **Antimicrobial Activity**

   A variety of bacterial species, including Escherichia coli, Staphylococcus aureus, *Klebsiella pneumoniae*, *Vibrio cholerae*, and *Streptomyces*, are susceptible to the plant's strong antibacterial action. These results imply that it can be used as a natural antibacterial [6].

3. **Antioxidant Activity**

   B. spectabilis aqueous extracts have shown potent antioxidant properties. This characteristic may be crucial in shielding cells from oxidative stress and associated illnesses [6].

4. **Antidiabetic Activity**

   Research shows that *B. spectabilis's* aqueous extract increases superoxide dismutase (SOD) activity and glutathione (GSH) levels, suggesting that it is useful in reducing oxidative stress in diabetes animals. This implies that it may be used as a natural antidiabetic [12].

5. **Antifertility Activity**

   Native people have historically used *B. spectabilis* decoction and aqueous extract to manage fertility. This demonstrates its importance for reproductive health and the necessity of more studies to confirm its processes [13].

6. **Anticancer Activity**

   HeLa cells are cytotoxically affected by extracts from similar species including *Bougainvillea shubhra* and *Bougainvillea peruviana*, indicating that the genus may contain anticancer chemicals. This calls for more research on *B. spectabilis* to look for comparable effects [14].

7. **Antihepatotoxic Activity**

   Extracts from *B. spectabilis's* leaves, stems, and flowers have cytotoxic and hepatoprotective properties, suggesting that they may be used to treat liver-related conditions [15].

8. **Antihyperlipidemic Activity**

   It has been discovered that *B. spectabilis* leaf extract in methanol lowers lipid levels, suggesting that it may be used to treat hyperlipidemia and related cardiovascular risks [16].

9. **Antiulcer Activity**

   B. spectabilis leaf ethanol extracts have antiulcer properties, indicating that they may be used therapeutically to treat gastrointestinal and stomach issues [17].

The potential of *Bougainvillea spectabilis* as a rich source of bioactive chemicals is highlighted by its pharmacological flexibility. It has potential for pharmacological development and has uses in diabetic care, cancer treatment, antibacterial drugs, and anti-inflammatory medicines. To identify and confirm the active ingredients causing these effects, more research is required.

Chapter Three: Plant Profile

3.1: Introduction of *Bougainvillea spectabilis*

A colorful plant from South America, *Bougainvillea spectabilis*, also known as the paper flower, is prized for its colorful bracts that encircle tiny white blooms that are sometimes confused for petals [18]. It is widely accessible in Bangladesh as well. Adapting well to poor soils and needing little water, this member of the Nyctaginaceae family grows well in tropical and subtropical climates. It is perfect for natural fences, hedges, and gardens because of its thorny stems and quick growth. It has therapeutic value in addition to its aesthetic appeal; historically, it has been used to treat inflammation, respiratory conditions, and diarrhea. It has antibacterial and antioxidant qualities and is rich in bioactive substances including alkaloids and flavonoids, which makes it a topic of research study.

Botanical name: *Bougainvillea spectabilis*

Local name: Bagan Bilash (Bangladesh)

3.2: Taxonomical Classification

Domain: *Eukaryota*

Kingdom: *Plantae*

Phylum: *Tracheophyta*

Subphylum: *Angiospermae*

Class: *Magnoliopsida*

Order: *Caryophyllales*

Family: *Nyctaginaceae*

Genus: *Bougainvillea*

Species: *Bougainvillea spectabilis* [18]

3.3: Plant type

Bougainvillea spectabilis is an evergreen, woody vine or climber. It is a member of the fast-growing plant group and is commonly grown as an ornamental plant because of its flexibility and colorful bracts. It may potentially develop into a large climber under the right circumstances [19].

3.4: History

Native to South America (Brazil, Peru, and Argentina), *Bougainvillea spectabilis* was initially described by French botanist Philibert Commerçon during the mission led by Admiral Louis Antoine de Bougainville in the 1760s. In the late 1700s, the plant was brought to Europe as an ornamental species, valued for its hardiness and colorful bracts. It had spread extensively over tropical and subtropical countries, including Asia, Africa, and the Americas, by the 19th century. Due to its ability to withstand harsh conditions, *Bougainvillea spectabilis* has gained popularity as a representation of tropical settings. It is a favorite plant all over the world because of its therapeutic applications, which further enhance its value [20].

3.5: Plant Morphology

The plant morphology of *Bougainvillea spectabilis* is characterized by several distinct features:

1. Size and Growth Habit

The woody climber *Bougainvillea spectabilis* grows quickly and can grow to a height of 3–12 meters (10–40 feet). If left unsupported, it can potentially spread out into a bush. It can climb and spread because of its woody stem and prickly branches.

2. Leaves

The simple, elliptic to ovate leaves of *Bougainvillea spectabilis* have whole edges. Usually, they are 1.5–4 cm broad and 3–8 cm long. The dark green, glossy leaves are grouped in alternating patterns on the stems.

3. Bracts

Among *Bougainvillea spectabilis's* most remarkable characteristics are its vibrant bracts. Frequently confused for petals, these are modified leaves rather than actual petals. Around the tiny, unnoticeable white blooms are papery bracts that are vividly colored in pink, purple, crimson, orange, or white. Bracts typically measure 2 to 3 cm in length.

4. Flowers

The actual blooms are tiny, white, and tripartite, meaning they have three separate petals. They are encircled by the vibrant bracts in clusters that resemble stems. The stamens are noticeable and the blooms range in diameter from 5 to 10 mm.

5. Roots

The plant's wide and deep root system, characteristic of vines, allows it to cling to surfaces and draw nutrients and water from a range of soil types.

6. Thorns

The sharp, straight thorns that adorn the stems of *Bougainvillea spectabilis* aid in the plant's ascent and offer defense against animals [21].

3.6: Phytochemical Screening [22]

Alkaloids	Triterpenoids
Flavonoids	Glycosides
Phenolic compounds	Steroids
Saponins	Essential oils

3.7: Nutritional values [23]

Macronutrients	Essential vitamins	Minerals
Carbohydrates Proteins Dietary fiber	Vitamin C Vitamin E	Calcium Magnesium Potassium

3.8: Traditional Use

Traditionally, this herb has been used to treat a variety of illnesses. Decoctions of its flowers and leaves are often applied to ease respiratory disorders, including coughs, colds, bronchitis, and asthma. Leaf extracts are used as a febrifuge to lower fever, while leaf infusions are used to treat diarrhea and stomach issues. Its leaves or petals are used to make poultices that are applied to burns, wounds, and infections to help them recover. Its components also help relieve inflammation and swelling. The plant is used in certain traditional practices to treat menstruation issues, urinary tract infections, and blood sugar regulation [24].

3.9: Health benefits

Because of its abundance of bioactive chemicals, the plant has several health advantages. Its phenolic components and flavonoids have strong antioxidant qualities that lessen oxidative stress. Anti-inflammatory properties found in extracts from its leaves and blossoms help with skin problems and arthritis. It helps cure infections and encourage wound healing because of its antimicrobial action, which includes antibacterial and antifungal qualities. According to tradition, the plant helps to maintain respiratory health by easing the symptoms of bronchitis, coughs, and colds. Leaf extracts may help control blood sugar, which is advantageous for managing diabetes. Infusions alleviate diarrhea, lessen gastrointestinal problems, and enhance digestion. It also helps lower fever, improves skin health, and increases immunity [24].

3.10: Side Effects

When the plant's thorns or sap come into touch with the skin, it can cause rashes, irritation, or itching. An allergic response may cause edema or breathing problems in certain people. Vomiting, diarrhea, or nausea may result from overconsumption. In addition, excessive dosages or during pregnancy may enhance photosensitivity and present dangers [25].

3.11: Plant Preview

Fig1: *B. spectabilis* leaves and thrones

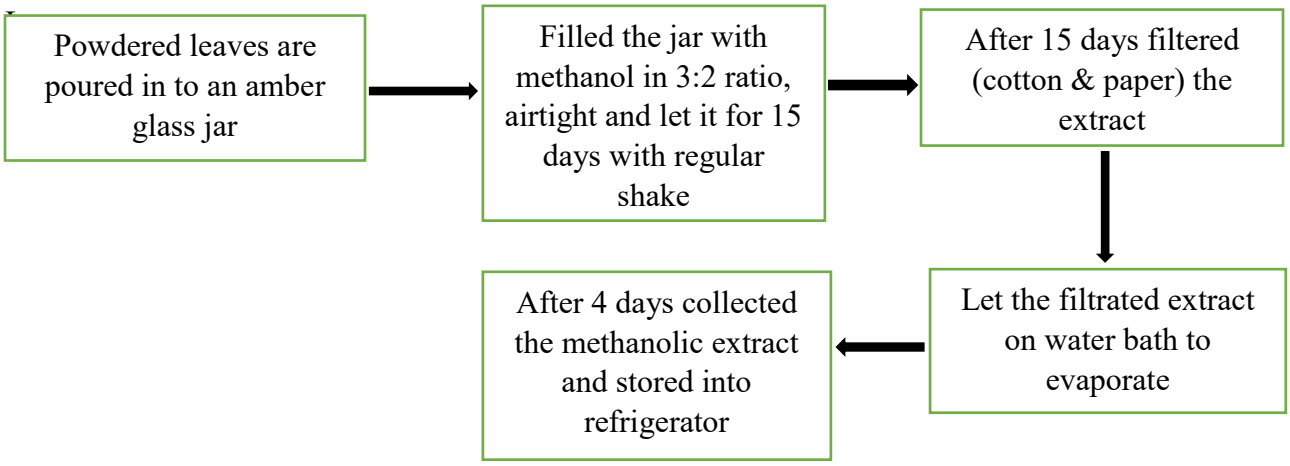
Fig 2: *B. spectabilis* Bracts

Chapter Four: Sample Preparation & Total Tests Design

4.1: Collection of *Bougainvillea spectabilis*

Fresh leaves of *Bougainvillea spectabilis* were collected from flowering plants in the local area of Mirpur, Dhaka, Bangladesh, during the flowering season. The collected leaves were air-dried under shade to retain their phytochemical properties and subsequently ground into a fine powder, yielding 300.53 g of powdered material.

4.2: Preparation of Methanolic Extract



4.3. Total Experimental Design

There are 3 types of tests designed for this finding.

Points	Lethality test [26]	Cytotoxicity test [27]	Pesticidal activity test [28]
1. Organism	Brine shrimps	HeLa cells	Rice weevils
2. Measurements	% Mortality	LD50	% Mortality
3. Treatment Period	24h	24h	48h
4. Standard	Potassium dichromate [29]	Medium with cells	Permethrin
5. Sample	Methanolic extract of *B. spectabilis*	Methanolic extract of *B. spectabilis*	Methanolic extract of *B. spectabilis*

Table 1: Contains the Total experimental design

Chapter Five: Materials & Methods

5.1: Brine Shrimp Lethality Test

5.1.1: Materials Required

1. **Brine shrimp eggs** (*Artemia salina*)
2. Artificial **Seawater** solution (35 g/L NaCl in distilled water)
3. **Light source & Oxygen Pump** (for hatching brine shrimp)
4. **Methanolic extract of Bougainvillea spectabilis** (sample)
5. **Dimethyl sulfoxide (DMSO)** (co-solvent)
6. **Potassium Dichromate** (as standard)
7. **Pipettes, Micro Pipettes & Graduated cylinders** (for measurements)
8. **Test tubes** (for serial dilution)
9. **Beakers** (for stock solution preparation)
10. **Compound microscope** (for counting nauplii)

5.1.2: Experimental Organism (Brine Shrimp)

Hatching of Brine Shrimp Nauplii

The brine shrimp (Artemia salina) eggs were bought from a nearby aquarium store and kept in a hatching chamber with fake seawater (made by dissolving 35 grams of sodium chloride in one liter of distilled water). The hatching container was kept at a temperature of 25 to 30°C and continuously aerated using an oxygen pump to guarantee adequate oxygenation. These conditions were provided for 24 hours to stimulate the hatching

Fig 3: Hatching of Brine Shrimp

process. After the incubation period, free-swimming nauplii were collected for subsequent toxicity testing.

5.1.3: Experimental Design

A total of 3 groups were prepared in 17 test tubes, where each test tube was containing 10 nauplii.

Group	Treatment	Concentration (µg/mL)	Number of Nauplii per test tube	Comments
Negative Control	DMSO + Seawater	1%	10	Ensures no mortality is caused by the solvent.
Positive Control	Potassium dichromate in seawater	5 10 20 40 80 160 320 640	10	Verifies the reliability of the experimental setup.
Test Group	Methanolic extract of *Bougainvillea spectabilis*	5 10 20 40 80 160 320 640	10	Assesses the toxicity of the plant extract at different concentrations.

Table 2: Contains the experimental design for the Brine Shrimp Lethality Test.

5.1.4: Experimental procedures

5.1.4.1. Preparation of Test Solutions

To create a stock solution of 100 mg/ml, the methanolic plant extract was dissolved in dimethyl sulfoxide (DMSO). Seawater was used to serially dilute the stock solution, yielding final concentrations of 5, 10, 20, 40, 80, 160, 320, and 640 µg/ml. To prevent solvent toxicity, the final DMSO content in all test solutions including controls was kept below 1% (v/v).

5.1.4.2. Experimental Setup

Test tubes were used for the experiment. Ten nauplii and ten milliliters of the corresponding test solution were placed in each test tube. Positive controls were made with potassium dichromate at the same quantities as the sample, whereas negative controls were made with seawater containing 1% DMSO and no plant extract.

Fig 4: Experimental setup

5.1.4.3. Exposure and Observation

For 24 hours, the test tubes were incubated at 25 to 30°C. The number of dead nauplii in each well was counted using a compound microscope and magnifying glass following the exposure period. Nauplii were considered dead if they remained motionless even after being gently shaken.

Fig 5: Observation of nauplii

5.1.4.4. Data Analysis

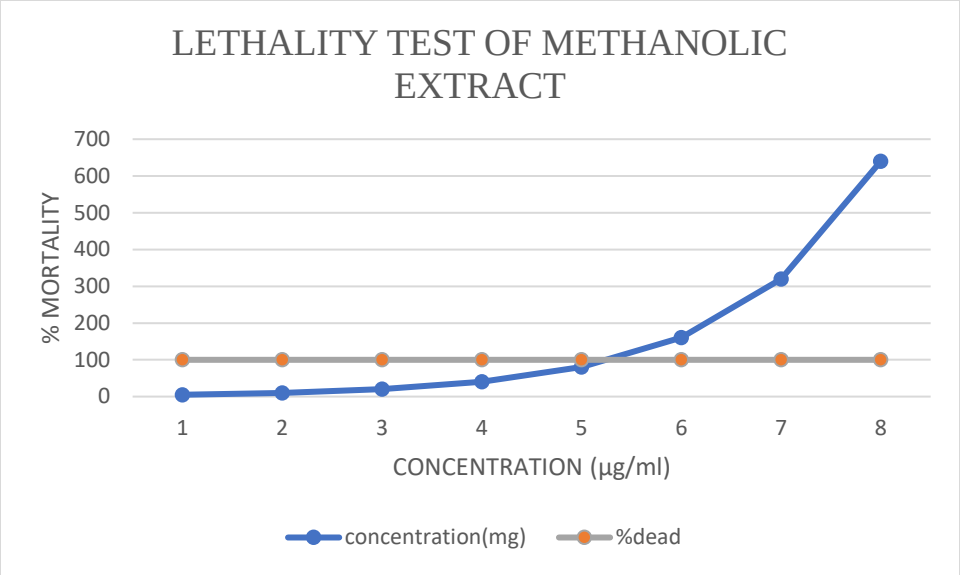
The percentage of mortality was calculated for each concentration.

5.1.5: Results & Discussion

After exposing brine shrimp to different concentrations of the methanolic plant extract, positive and negative control, the number of dead nauplii in each test tube was calculated.

Treatment	Conc. (µg/ ml)	No. of nauplii taken	No. of dead nauplii	% Mortality
Methanol extract	640	10	10	100
	320	10	10	100
	160	10	10	100
	80	10	10	100
	40	10	10	100
	20	10	10	100
	10	10	10	100
	5	10	10	100
Potassium Dichromate (positive control)	640	10	10	100
	320	10	10	100
	160	10	10	100
	80	10	10	100
	40	10	10	100
	20	10	10	100
	10	10	10	100
	5	10	10	100
DMSO (negative control)	100	10	3	30

Table 3: Contains the data of Mortality.



Graph 1: Contains the data of Mortality for Brine Shrimp Lethality test.

All tested concentrations (5–640 µg/mL) of *Bougainvillea spectabilis* methanolic extract resulted in 100% mortality in brine shrimp. This suggests that the extract is extremely poisonous and probably has cytotoxic properties. Its toxicity profile should be better understood by conducting additional trials at lower dosages.

6.1: Cytotoxicity Test on HeLa Cell

6.1.1: Materials Required

1. **Biological Bio Safety Cabinet** (Model NU-400E, Nuaire, USA)

2. **CO_2 Incubator** (Nuaire, USA)

3. **Trinocular microscope with a camera** (Optika, Italy)

4. **Hemocytometer**

5. **Microplate Spectrophotometer** (EPOCH, Winooski, VT, USA)

6. **96-well plate**

7. **15-ml tubes**

8. **Tips**

9. **Gloves**

10. **Culture flask**

11. **Cell culture media**

12. **Antibiotics (P+S)**

13. **Gentamycin**

14, **Serological pipette**

15. **Trypsin**

6.1.2: Experimental Organism (HeLa Cell)

Human cervical cancer cell line HeLa was cultivated in Dulbecco's Modified Eagle's Medium (DMEM), a popular nutrient-rich medium intended to promote mammalian cell proliferation. To prevent bacterial contamination, 1% penicillin-streptomycin (1:1 ratio) and 0.2% gentamycin were added to the medium. This gave the medium extra defense against a wider range of

microorganisms. The medium also included 10% Fetal Bovine Serum (FBS), a vital source of growth factors, hormones, and nutrients required to sustain cell viability and health in vitro, to guarantee ideal cell growth and proliferation.

6.1.3: Experimental Design

Groups were prepared in 96-well plates, where each plate contained 100 HeLa cells.

Group	Treatment	Concentration	Comments
Negative Control	DMSO	3%	Ensures no mortality is caused by the solvent.
Positive Control	Medium with HeLa cells	2.0 ×10^4 cells per 100 µl medium	Verifies the reliability of the experimental setup.
Test Group	Methanolic extract of *Bougainvillea spectabilis*	1.25 mg/ml 2.5 mg/ml 5 mg/ml 10 mg/mL	Assesses the toxicity of the plant extract at different concentrations.

Table 4: Contains the experimental design for the Cytotoxicity Test on HeLa Cell.

6.1.4: Experimental procedures

The cytotoxic effect of the sample was evaluated at the Center for Advanced Research in Sciences **(CARS)** using their commercial laboratory services. HeLa cells were seeded at a density of 2.0 ×10^4 cells per 100 µl of culture medium into each well of a sterile 96-well plate. The plates were then incubated overnight at 37°C in a humidified atmosphere containing 5% CO_2 to allow the cells to adhere and stabilize.

The next day, 25 µl of the filtered test sample was carefully added to each well. The treated plates were then returned to the incubator for a further 24 hours to assess the effect of the sample on cell viability. The viability of the cells was determined using the **CellTiter 96 Non-Radioactive Cell**

Proliferation Assay Kit (Promega, USA), which measures the metabolic activity of viable cells as an indicator of cell health. Each sample was tested in duplicate wells to ensure the reproducibility and reliability of the results.

6.1.5: Results & Discussion

After exposing HeLa cells to different concentrations of the methanolic plant extract, positive and negative control, the % of survival cells were calculated.

Treatment	Conc. (mg/ ml)	Absorbance at 570nm		% of Survival cells	LD50
Methanol extract	1.25	1.78	1.807	90.74	~8.7mg/ml
	2.5	1.711	1.873	90.66	
	5	1.485	1.602	78.09	
	10	0.807	0.837	41.58	
Positive control	2.0 ×10^4 cells per 100 µl medium	2.028	1.925	100	---
Negative control	3%	-	-	100	---

Table 5: Contains the data of Survival cells

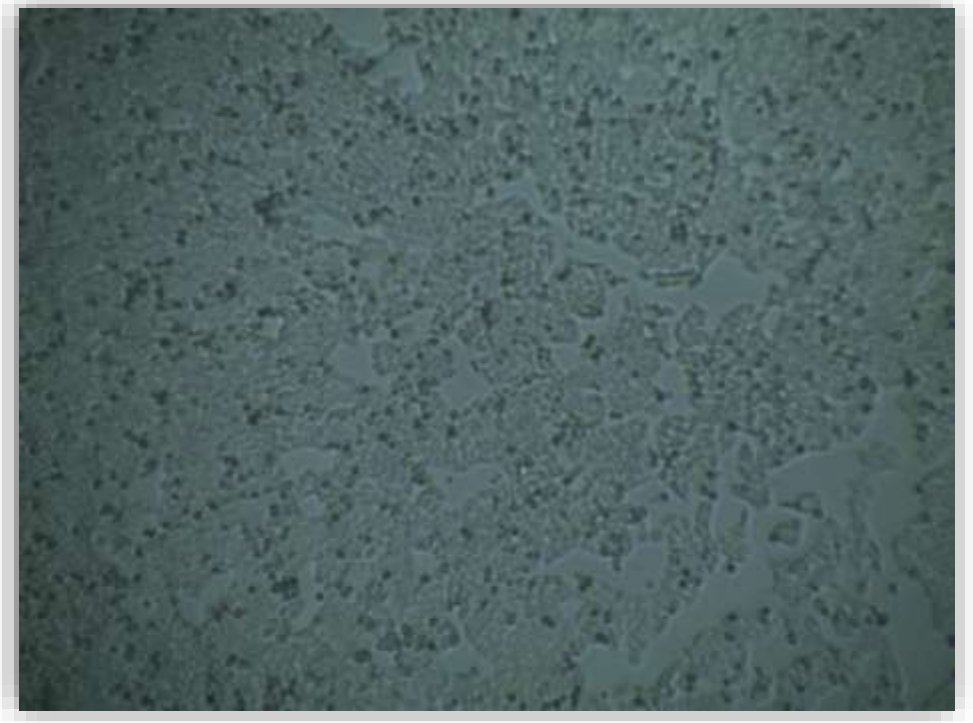


Fig 6: For 1.25 mg/ml

Fig 7: For 2.5 mg/ml

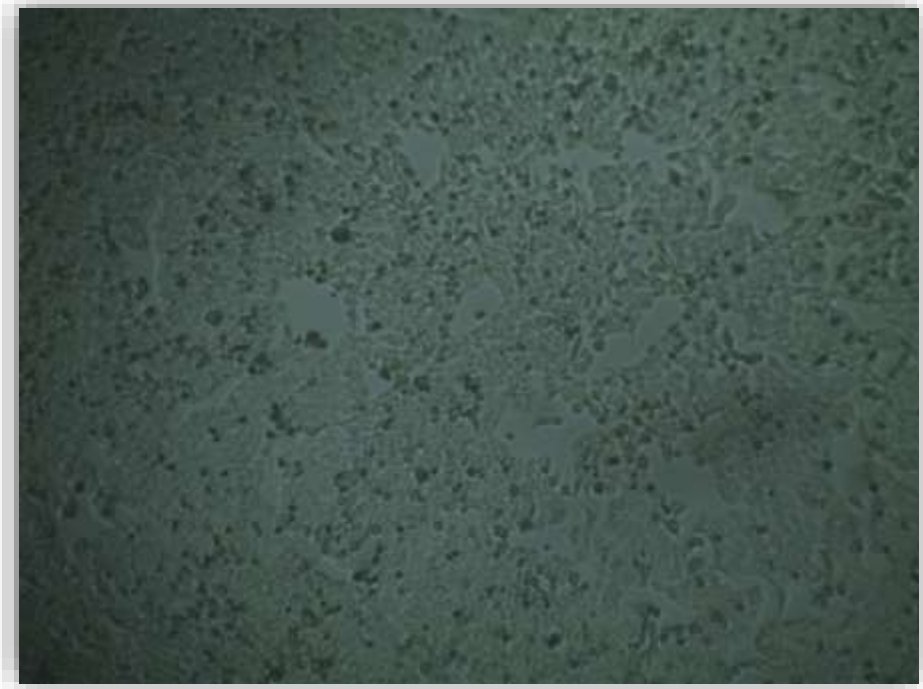


Fig 8: For 5 mg/ml

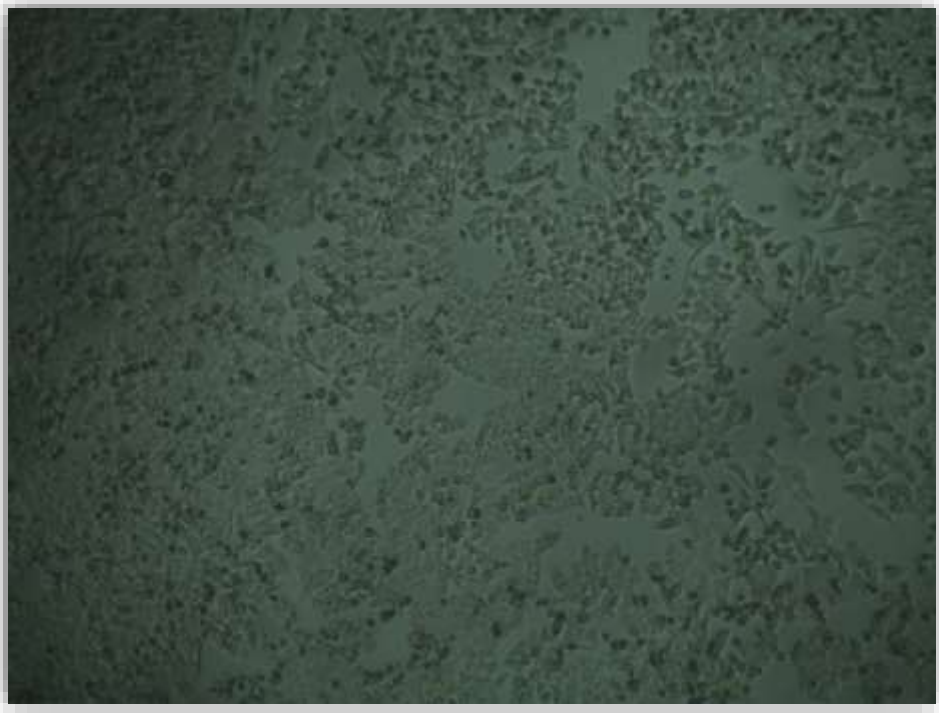


Fig 9: For 10 mg/ml

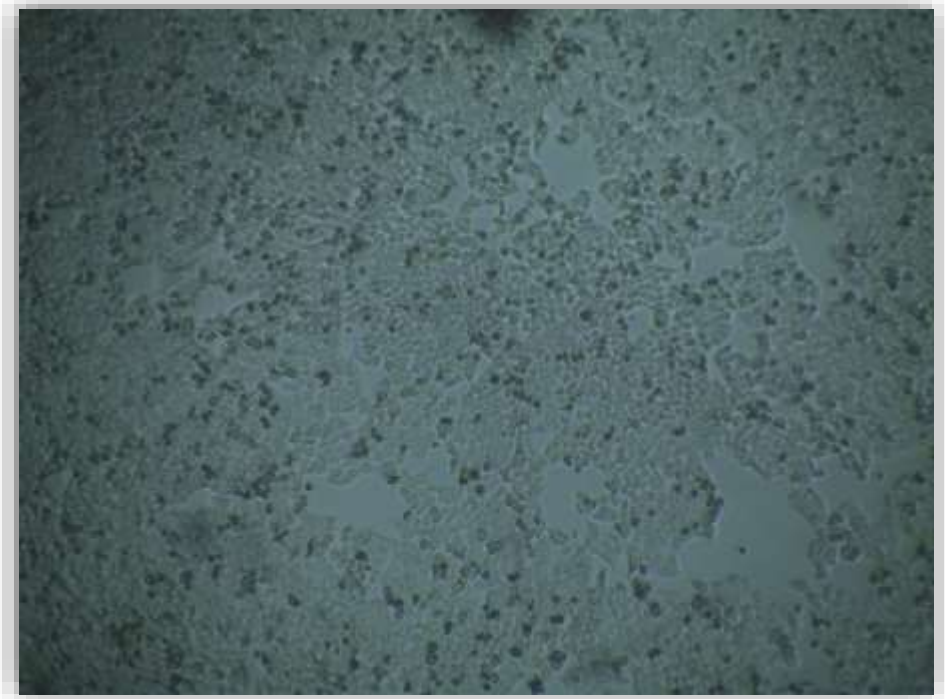


Fig 10: For Positive Control

At every tested concentration of the *Bougainvillea spectabilis* extract, the percentage survival of HeLa cells showed a considerable decrease; the most noticeable effect was shown at a concentration of 10 mg/mL, where cell viability dropped to 41.58%. Additionally, the extract's LD50 value was found to be approximately 8.7 mg/ml. These results strongly imply that the extract from *Bougainvillea spectabilis* has significant cytotoxic action, suggesting that it may be a source of anti-cancer medicines. To investigate its mode of action and therapeutic potential, more research is necessary.

7.1: Pesticidal Activity Test

7.1.1: Materials Required

1. **Rice Weevils** (*Sitophilus oryzae*)
2. **Rice** in a plastic container (for rice weevil growth)
3. **Methanolic extract of Bougainvillea spectabilis** (sample)
4. **Methanol** (co-solvent)
5. **Permethrin** (as standard)
6. **Micro Pipettes** (for measurements)
7. **Petri dish** (for medium and treatment)

7.1.2: Experimental Organism (*Sitophilus oryzae*)

Preparation of Rice Weevils

To cultivate rice weevils, 200–300 gm of dry, untreated rice were placed in a sterile, airtight container with ventilation covered with fine mesh to enable airflow but restrict escape. To replicate their natural habitat, 10–20 adult weevils were placed in the container, which was kept at 27–30°C, 60–70% relative humidity, and in a dark atmosphere. Within the grains, the weevils lay their eggs, and throughout their about 26–32 day life cycle, the larvae matured into adults. To make sure there was no mold or predators present, the culture was routinely checked.

Fig 11: Preparation of Rice

7.1.3: Experimental Design

A total of 3 groups were prepared in 6 petri dishes, where each petri dish containing 10 insects.

Group	Treatment	Concentration per disk	Number of insects per disk	Comments
Negative Control	MeOH	400µg	10	Ensures no mortality is caused by the solvent.
Positive Control	Permethrin	2mg	10	Verifies the reliability of the experimental setup.
Test Group	Methanolic extract of *Bougainvillea spectabilis*	2 mg 3 mg 4 mg 5 mg	10	Assesses the toxicity of the plant extract at different concentrations.

Table 6: Contains the experimental design for the Pesticidal Activity Test.

7.1.4: Experimental procedures

Various mg of plant extract was diluted in 400 µL of methanol (MeOH) and applied on rice wafer discs, each measuring one cm in diameter and weighing roughly thirty mg. A negative control was 400 µL of methanol, while a positive control was 2.0 mg of permethrin on the disc. After being weighed and allowed to dry under a fume hood, the discs were put in Petri dishes.

Fig 12: Experimental setup

Ten mature S. oryzae insects were placed in each Petri dish, which was thereafter sealed and maintained at 29 ± 1°C, 50–60% relative humidity, and an 8-hour dark and 16-hour light cycle.

After treatment, the number of dead insects was counted 24 and 48 hours later. If the appendages of an insect did not move when gently pushed with a fine brush, the insect was considered dead.

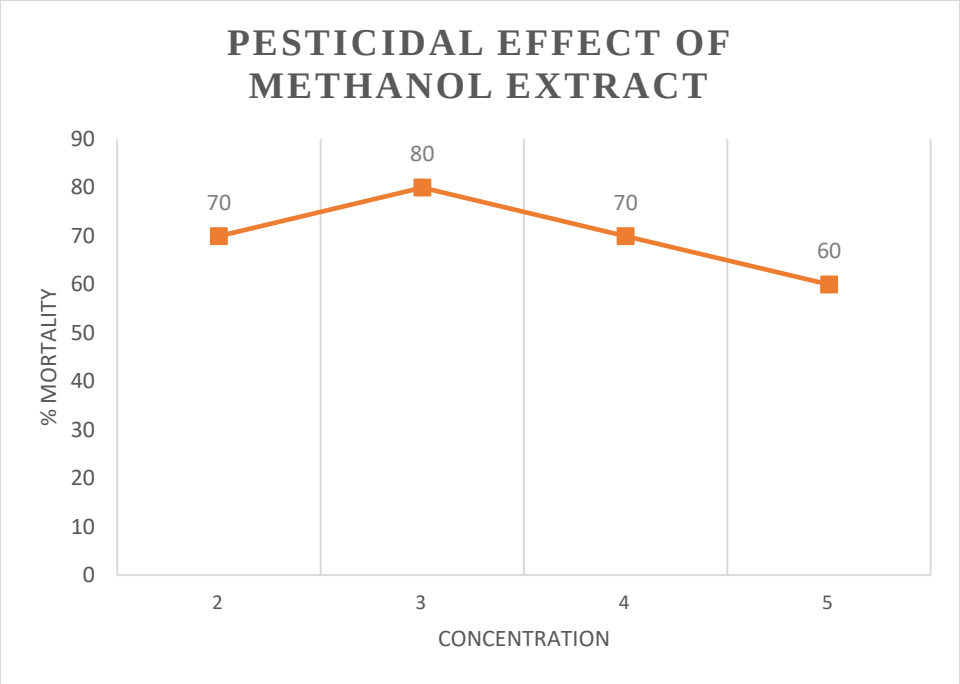
Fig 13: Rice Weevils inside the prepared disk

7.1.5: Results & Discussion

After exposing rice weevils to different concentrations of the methanolic plant extract, positive and negative control, the number of dead insects in each test tube was calculated.

Treatment	No. of insects	No. of dead *Sitophilus oryzae*							
Methanol extract	10	2mg		3mg		4mg		5mg	
		Hours after treatment							
		24h	48h	24h	48h	24h	48h	24h	48h
		7	3	8	2	7	3	6	3
Permethrin	10	24h				48h			
		10				0			
MeOH	10	1				1			

Table 7: Contains the data of dead insects.



Graph 2: Contains the data of Mortality for Pesticidal Activity Test.

According to the findings, the methanolic extracts of *Bougainvillea spectabilis* demonstrated insecticidal efficacy, resulting in 80–100% mortality at varying doses during the treatment period. Notably, at 3 mg, the extract resulted in over 80% mortality in 24 hours, rising to 100% in 48 hours. Permethrin, a common insecticide, reached 100% death in just 24 hours. MeOH, the negative control, only produced a 10% death rate, ensuring the solvent's inefficiency.

Chapter Six: Total Results & Discussions

6.1: Total Results & Discussions

The current study evaluated the bioactivity of the tested sample through the brine shrimp lethality test (BSLT), HeLa cell cytotoxicity assay, and a pesticidal activity test, with positive results observed across all assays.

Tests	Measurements	Results
1. **Lethality test**	% Mortality	100%
2. **Cytotoxicity test**	LD50	~8.7 mg/ml
3. **Pesticidal activity test**	% Mortality	100%

Table 8: Contains the Total Results.

The positive results across all three assays underline the multi-functional potential of the *Bougainvillea spectabilis* leaves extract. The cytotoxic effects in both brine shrimp and HeLa cells indicate that the sample contains compounds with therapeutic relevance, particularly in anti-cancer research. Additionally, the observed pesticidal activity suggests an opportunity for developing environmentally friendly biopesticides. To further explore the sample's potential, bioassay-guided fractionation and identification of the active compounds are essential. Advanced techniques such as chromatography and mass spectrometry could help elucidate the chemical structure of the bioactive constituents.

In conclusion, the observed results suggest that the sample holds significant promise for both pharmaceutical and agricultural applications. Its multi-faceted bioactivity warrants further investigation to unlock its full potential.

Chapter Seven: References

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