CHEMICAL AND BIOLOGICAL ANALYSIS OF LEAVES EXTRACTS OF *Bauhinia variegata* L. FROM SYANGJA DISTRICT OF NEPAL

A DISSERTATION SUBMITTED TO THE

DEPARTMENT OF CHEMISTRY

AMRIT CAMPUS

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

KATHMANDU, NEPAL

FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS OF MASTER OF SCIENCE IN CHEMISTRY

BY

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DECLARATION

The dissertation entitled **"CHEMICAL AND BIOLOGICAL ANALYSIS OF LEAVES EXTRACTS OF *Bauhinia variegata* L. FROM SYANGJA DISTRICT OF NEPAL"** is being submitted to the Department of Chemistry, Amrit Campus , Institute of Science and Technology (IOST), Tribhuvan University, Nepal, for the partial fulfillment of the Master Degree of Science in Chemistry is carried out by me under the supervision of **Assoc. Prof. Dr. Ram Lal (Swagat) Shrestha**, Department of Chemistry, Amrit Campus, Kathmandu.

I declare that this dissertation has been composed by myself and has not been published or submitted elsewhere for the requirement of a master's degree program.

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RECOMMENDATION

This is to recommend that Ms. **Soniya Bhusal** has carried out research entitled **"CHEMICAL AND BIOLOGICAL ANALYSIS OF LEAVES EXTRACTS** OF ***Bauhinia variegata*** **L. FROM SYANGJA DISTRICT OF NEPAL"** for the partial fulfillment of the requirements of a Master of Science Degree in Chemistry under our supervision. To best of our knowledge, this work has not been submitted for any other degree.

She has fulfilled all the requirements laid down by the Amrit Campus, Institute of Science and Technology (IOST), Tribhuvan University, Kathmandu, Nepal for the submission of the dissertation for the partial fulfillment of the requirements for the Master of Science Degree in Chemistry.

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This thesis entitled **"CHEMICAL AND BIOLOGICAL ANALYSIS OF LEAVES EXTRACTS OF *Bauhinia variegata* L. FROM SYANGJA DISTRICT OF NEPAL"** completed by **Ms. Soniya Bhusal**, under the supervision of **Assoc. Prof. Dr. Ram Lal (Swagat) Shrestha** and Co-supervision of **Assoc. Prof. Dr. Bhushan Shakya**, Department of Chemistry, Amrit Campus, Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal is hereby approved for the partial fulfillment of the Master of Science (M.Sc.) Degree in Chemistry.

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LETTER OF FORWARD

On the recommendation of **Assoc. Prof. Dr. Ram Lal (Swagat) Shrestha** and **Assoc. Prof. Dr. Bhushan Shakya**, this M.Sc. thesis submitted by Ms. Soniya Bhusal entitled **"CHEMICAL AND BIOLOGICAL ANALYSIS OF LEAVES EXTRACTS OF *Bauhinia variegate* L. FROM SYANGJA DISTRICT OF NEPAL"** is forwarded by the Department of Chemistry, Amrit Campus, Tribhuvan University to the Dean, IOST, T.U.

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ABSTRACT

Bauhinia variegata L. is the flowering plant from Fabaceae family. The aim of this study is to investigate phytochemicals and evaluate their biological functions. Hexane, chloroform, and methanol extract were extracted from the leaves of *B. variegata* using the ultrasonic extraction technique. The highest percentage yield was obtained from chloroform extract i.e. 1.46%. Flavonoids, alkaloids, steroid, tannins, resin and phenol were detected by phytochemical screening. The methanol extract was found to have the highest phenolic content (138.44±1.79 mg GAE/g), while the chloroform extract had the highest flavonoid content (116.97±1.76 mg QE/g). DPPH free radical scavenging assay was employed to assess the antioxidant activity. The IC_{50} value of methanol extract showed that it was more powerful natural antioxidant than hexane and chloroform extracts. The antimicrobial susceptibility test was performed using the agar well diffusion method, in which methanol extracts revealed the maximum Zone of Inhibition (ZOI) of 1.8 cm against *Escherichia coli* and also methanol extract exhibited good antifungal activity with a ZOI of 1.7 cm against *Candida albicans*. The α-amylase inhibition was performed using the 3,5- Dinitro Salicylic acid (DNSA) and among the three extracts, the hexane (IC_{50}=192.5µg/mL) and methanol (IC_{50}=772.011µg/mL) extract showed significant and moderate antidiabetic properties respectively. The toxicity assessment of the extracts was done using a Brine Shrimp Lethality Assay, in which all the extract demonstrated non-toxic behaviour. The TLC analysis of extracts revealed many spots on the TLC plate indicating the presence of multiple compounds and FTIR confirmed the large number of functional groups within the different plant extracts.

Keywords: *Bauhinia variegata*, Phytochemical, Antibacterial, Antioxidant, α-amylase inhibition, toxicity

शोध सार

कोइरालो (*Bauhinia variegata* L.) फेबेसिआइ (Fabaceae) परिवारको एक फुल फुल्ने विरुवा हो । यसको औषधीय गुणहरुको कारणले गर्दा यस अध्ययन विरुवाको फाइटोकेमिकल र वायोलोजीकल गतिवधिहरुको अनुसन्धान गर्न सञ्चालन गरिएको थियो । अल्ट्रासोनिक एक्स्ट्रयाक्टसन विधिबाट हेक्सेन, क्लोरोफर्म र मेथेनोल एक्स्ट्रयाक्ट निकालिएको थियो जसमा क्लोरोफर्मबाट उच्चतम एक्सट्रयाक्ट (१.४६%) निकालियो। फ्लेभोनोइड्स, अल्कालोइड्स, स्टेरोइड, ट्यानिन्स, रेजीन र फिनोल रहेका कुरा एक्स्ट्रयाक्टहरुको फाइटोकेमिकल स्क्रीनिङ द्वारा पत्ता लगाइयो। सबैभन्दा धेरै फेनोलिक कन्टेन्ट मेथेनोल एक्ट्रयाक्टमा पाईयो (१३८.४४±१.७९ mg GAE/g) भने सबैभन्दा धेरै फ्ल्याभोनोइड कन्टेन्ट क्लोरोफर्ममा (११६.९७±१.७६ mg QE/g) देखियो । एन्टिमाइक्रोवियल सवेंदनशीलता परिक्षण गर्न अगार वेल डिफ्युजन विधि प्रयोग गरिएको थियो, जसमा मेथेनोल एक्स्ट्रयाक्टले परिक्षण गरिएको ढुसि (*C. albicans*) विरुद्ध १.७ cm को अधिकतम निषेधित क्षेत्र देखाएको थियो र परिक्षण गरिएको व्याक्टेरियामध्यले *E.coli* (१.८ cm) को अधिकतम निषेधित क्षेत्र देखायो । DPPH फ्रि रेडिकल स्क्याभेन्जिङ विधिद्वारा एन्टिअक्सिडेन्ट गतिविधिको आँकलन गरिएको थियो । IC_{50} को मान अनुसार सबैभन्दा धेरै शक्तिशाली एन्टिअक्सिडेन्ट हेक्सेन र क्लोरोफर्म भन्दा मेथेनोलमा देखियो । DNSA विधिद्वारा अल्फा एमाइलेज इन्हिविसनको मापन गरिएको थियो र तीनओटा एक्स्ट्रयाक्टहरुमध्ये मेथेनोल (IC_{50}=७७२.०११ µg/mL) र हेक्सेनले (IC_{50}=१९२.५० µg/mL) महत्वपुर्ण एन्टिडायविटक गुण देखायो । एक्स्ट्रयाक्टहरुको विषादि मुल्याङकन गर्न ब्राईन श्रीम्प लिथालिटी ऐस्से प्रयोग गरिएको थियो जसमा हेक्सेन र क्लोरोफर्म एक्स्ट्रयाक्टहरुको साइटोटोक्सिसिटी कम थियो । एक्स्ट्रयाक्टहरुको TLC विश्लेषणले प्लेटमा धेरै स्पटहरुको सङख्या देखाउँदै धेरै यौगिकहरुको उपस्थितीलाई संकेत गर्यो। विभिन्न एक्स्ट्रयाक्टहरुको FTIR विश्लेषणले एक्स्ट्रयाक्टमा उपस्थित भएका कम्पाउण्डहरुमा धेरै फङसनल ग्रुप भएको पुष्टि गर्यो ।

किवर्ड : कोइरालो, फाइटोकेमिकल, एन्टिव्याक्टेरियल, एन्टिअक्सिडेण्ट, अल्फाएमाइलेज, टक्सीसीटी

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LIST OF ACRONYMS AND ABBREVIATIONS

B. subtilis	*Bacillus subtilis*
C. albicans	*Candida albicans*
cm	Centimeter
DCM	Dichloromethane
DM	Diabetes millitus
DMSO	Dimethyl Sulfoxide
DPPH	2,2-Diphenyl-1-1Picrylhydrazyl
E. coli	*Escherichia coli*
FCR	Folin Ciocalteu reagent
FDA	Food Drug Administration
FTIR	Fourier Transform Infrared
g	gram
g/mol	gram per mole
GAE	Gallic Acid Equivalent
IC50	Inhibitory Concentration, 50%
IR	Infrared
K. pneumonie	*Klebisella pneumoniae*
kg	Kilogram
L	Liter
LDL	Low density lipids
LD_{50}	Lethal dose 50%
LC_{50}	Lethality concentration,50%
m	meter
mm	Millimeter
mcg/mL	Microgram per milliliter

MHA	Mueller- Hinton Agar
MeOH	Methanol
QE	Quercetin Equivalent
s	Second
SARS-CoV-2	Severe Acute respiratory syndrome coronavirus -2
TFC	Total Flavonoid Content
TLC	Thin Layer Chromatography
TPC	Total Phenolic Content
VLDL	Very Low Density Lipids
WHO	World Health Organization
ZOI	Zone of Inhibition
µg	Microgram
µg/mL	Microgram per milliliter
µL	Microliter
^{o}C	Degree Celsius

TABLE OF CONTENT

CHAPTER 1: INTRODUCTION

1.1. General Introduction

Nepal's land area of 147,516 km^2 is less than 0.1% of the world's total, but it has a diverse range of plants and animals (Kumar *et al*., 2011). Nepal is one of the world's 34 biodiversity hotspots and one of the most species broad regions in the eastern Himalayas (Joshi & Joshi, 2022). Several articles estimated plant diversity for both flowering and non-flowering species. These estimates show that there are 2,467 species of fungi, 1078 species of lichens, 1,217 species of bryophytes, 550 species and 33 subspecies of pteridophytes, 23 species of gymnosperms, and 998 species of algae. In flowering plants, there are 5309 species under 1515 genera and 193 families, and 5,606 species and 214 infra-species under 1541 genera and 200 families (Rajbhandary, 2017). More than 5,800 species of flowering plants have been identified in Nepal as a result of several botanical excursions carried out by researchers, either on their own or as part of formal expeditions (*Sahetal,2003*). According to Lewis & Elvin-Lewis (1995) and Mahmood *et al*. (2011), plants are well-recognized for producing common compounds that support human health about 80% worldwide. Even in industrialized nations, a significant number of people utilize traditional remedies in one way or another way (Ihsan-ul-Haq *et al*., 2013). The examination of plant sources is essential for a better knowledge of their efficacy, safety, and pharmacological qualities as more than 30% of all pharmacological treatments are based on the active compounds of plants (Shinwari and Gilani, 2003). Flowers, leaves, bark, stems, and roots are the most often utilized plant elements (Ali *et al*., 2015). Research on medicinal plants continues to provide innovative and beneficial treatments (Patil, 2011).

1.2. Introduction of *Bauhinia variegata*

Bauhinia, a tropical plant, has approximately 250 species worldwide. Shrubs, trees, and vines are often planted for their colorful flowers and beautiful leaves (Modh *et al*., 2011). This plant thrives on rocky soil on hillsides, as well as sandy loam and loamy soil in valleys. It prefers acidic soil and cannot survive salty circumstances (Kumar, 2014).

1.2.1 Morphology of *B. variegata*

Bark: The bark is scaly, smooth to slightly fissured, and light brownish grey. The inner bark is bitter, fibrous, and pink (Khare *et al*., 2018).

Leaves: Its roughly ovate to circular lamina features minute stipules about 1-2 mm. Base cordate, lobe tips widely rounded.

Seeds: Pods are dehiscent, strap-shaped, horizontally striate, and measure 20–30 to 2–25 cm. Each pod is lengthy, rigid, and stretched out, containing 10–15 brown, nearly round seeds with coriaceus testa.

Flowering: April to May.

1.2.2. Classification of plant

Kingdom	Plantae
Sub Division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Bauhinia*
Species	*variegata*
Botanical Name	*Bauhinia variegata* L.
Local Name	Koiralo (Nepali), kachnar (Hindi), Ebony (English)

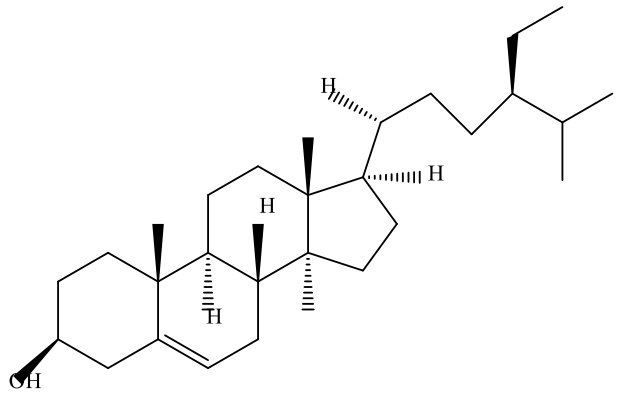
1.2.3. Traditional use of *B. variegata*

B. variegata L. has historically been used to treat bronchitis, leprosy, inflammation, bacterial infection, liver illnesses, diarrhea, dysentery, skin illness, leprosy, intestinal worms, wounds, ulcers, and fungal infections (Khare *et al.*, 2018). The bark has various uses, including fever treatment, tonic and astringent, antileprotic, skin problems, and wound healing (Mali *et al*., 2007). Nature is a brilliant indicator of the widespread occurrences of cohabitation. The foundation for treating is natural ingredients derived from plants, animals, and minerals for human illnesses (Jamshidi-Kia *et al*., 2018). The Indian tribal people employ *Bauhinia variegata* L. as a medicinal plant, and it is well-known in Ayurvedic, Unani, and homeopathic medicine systems. After the numerous conventional assertions on the usefulness of this plant, Researchers have worked hard to confirm the plant's potential for treating a variety of illnesses through pharmacological testing (Shah *et al*., 2010). The plant *B. variegata* is a well-known and

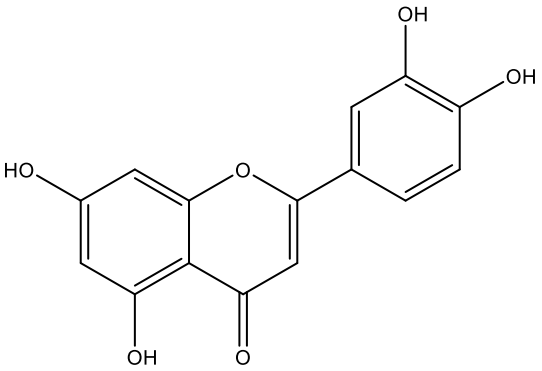
historically effective ethnomedicine. Studying *B. variegata's* ameliorative qualities to find novel secondary metabolites is therefore imperative (Kamal *et al*., 2022).

1.2.4. Chemical constituents of *B. variegata*

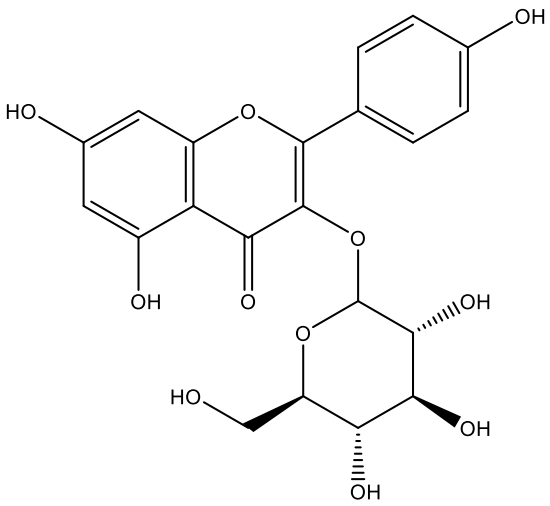
The genus *Bauhinia* comprises plants with a high percentage of alkaloids, terpenes, steroids, and flavonoids in their chemical composition. Luteolin, 5,7-dimethoxyflavonone-4–O–α–L–rhamnopyranosyl–β–D glucopyranoside, β-sitosterol, and kaempferol–3–glucoside were isolated from the stem of *B. variegata* (Sharma *et al*., 2019).



Beta-sitosterol



Luteolin



kaempferol–3–glucoside

1.3. Objectives

The objectives of this study are as follows:

General Objective

- The main objective of this research work is to carry out biological and chemical analysis of *B. variegata.*

Specific Objectives

- Performance of ultrasonic extraction using various solvents, such as hexane, chloroform, and methanol.
- To evaluate Phytochemicals and perform TLC on them and to evaluate FTIR of the extract.
- To calculate total phenolic content (TPC) and total flavonoid content (TFC) of the extract.
- To investigate the bioactivity of extracts such as antibacterial, antioxidant, anti-diabetic, and cytotoxicity.

CHAPTER 2: LITERATURE REVIEW

Shendge *et al.* in 2021 investigated *B. variegata's* antiproliferative potential extracts from a range of parts repeatedly macerating organic liquids in increasing sequence of polarity, (flowers, leaves, bark, stem, and roots) were created (n-hexane, ethyl acetate, methanol, and water). The most important details are that the disc diffusion assay was used to find antibacterial properties, the alpha-amylase assay was used to track antidiabetic potential, and the greatest phenolic and flavonoid concentrations were found in the methanolic extract of leaves and bark. Brine shrimp lethality assay showed greater than 90% death .Root and stem extracts in n-hexane and ethyl acetate also showed antiproliferative action against the human breast cancer MCF-7 cell line (Shendge *et al*., 2021).

A research was conducted by More-Adate et al. for seeking possible inhibitors of the primary protease of SARS-CoV-2. With regard to SARS-CoV-2 M^{pro}, it was shown that 3 main compounds: 2,5 dimethyl 1-H Pyrrole, 2,3 diphenyl cyclopropyl methyl phenyl sulphoxide, and Benzonitrile m phenethyl had the maximum binding affinity. These compounds could be potential principal contenders for SARS-CoV-2 therapy, however, more in vivo and in vitro research is required (More-Adate *et al*., 2022).

In 2009, Rajani and Ashok tested the ethanolic and aqueous extracts of *B. variegata* L. for in vitro antioxidant and antihyperlipidemic activities. The stem bark and root extracts were made and tested for in vitro antioxidant activity using a variety of methodologies, including total reducing power and scavenging of various free radicals. Next, the percentage of different free radicals scavenged was compared to antioxidants that are commonly used. The extracts showed a significant reduction in cholesterol, triglyceride, LDL, and VLDL. The extracts also showed a significant increase in HDL. The findings shows that *B. variegata* L. extracts, both alcoholic and aqueous, can successfully raise plasma HDL levels while lowering plasma levels of triglycerides, LDL, and VLDL (Rajani & Ashok, 2009).

A research was conducted by Mishra *et al*. in 2013 in which they found that Petroleum ether and chloroform fractions showed significant inhibition against Klebsiella pneumoniae in disc diffusion experiment. The extracts' power was reduced in a dose-

dependent manner. At lower concentrations, polar extracts demonstrated significant metal ion chelating activity. Several extracts had a high antioxidant response in the beta-carotene bleaching assay. *B. variegata* leaf extracts have been shown to have antibacterial, antioxidant, and anticancer properties. AQ fraction showed cytotoxicity against DU-145, HOP62, IGR-OV-1, MCF-7, and THP-1 human cancer cell lines, while ethyl acetate fraction produced cytotoxicity against MCF-7 and THP-1 cell lines (Mishra *et al*., 2013).

Flowering buds of *B. variegata* was used by Abbasi *et al.* in 2021 to performed phytochemical screening in both qualitative and quantitative terms of aqueous and methanol extracts according to accepted procedures, and a lethality test on brine shrimp was carried out to assess cytotoxicity.The methanol extract showed the highest concentrations of phenolics, flavonoids, and terpenoids. The research discovered that the presence of plant derived compounds in Both types of extracts are used by ancient cultures to treat a variety of illnesses and as food, along with methanol extract has cytotoxic activity, necessitating additional technical involvement to increase its value (Abbasi *et al*., 2021).

In order to overcome pharmacological limitations brought on by low bioavailability, insolubility in water, poor gastrointestinal absorption, and challenges in delivering the medication to the intended site of action, a research was conducted to employ nanotechnology in medicine. It was aimed to investigate the impact of gold nanoparticles (Au-NPs) on the antioxidant, antidiabetic, and hypolipidemic properties of *B. variegata* extract against streptozotocin-induced diabetic mascular degeneration (DM) in rats. Histopathological study revealed that In addition to its antioxidant and hypolipidemic effects, the B. variegata nano-extract showed a greater anti- diabetic impact by returning pancreatic cells to their normal architecture than the extract itself (Abdel-Halim *et al*., 2020).

In 2020, Kulkarni and Laddha investigated the impact of *B. variegata* L. leaves in the treatment of diabetic cardiomyopathy. A single intraperitoneal injection of streptozotocin was administered to induce diabetes in male Sprague Dawley ratsMice were administered AlcE directly for 28 days at dosages of 250, 500, and 1000 mg/kg following a 6-week period of diabetic induction. The raised amount of aspartate transaminase was dramatically decreased by therapy with AlcE at an amount of 1000

mg/kg, lactate dehydrogenase, and creatinine kinase-MB. AlcE therapy also dramatically avoided the loss of antioxidant enzymes such superoxide dismutase, catalase, and glutathione and decreased the development of lipid malons (Kulkarni & Laddha, 2020).

Because of its hypoglycemic qualities, *Morus nigra* and Bauhinia variegata are used in Egyptian medical practices by Hago *et al.* in 2021. In the model of streptozotocin-induced diabetic mice, standardized ethanolic extracts of both plants significantly reduced fasting blood glucose levels at two separate doses. In addition, tests for a-glucosidase inhibition and in vitro antioxidant activity were performed. Insulin levels and indicators for liver and kidney function were also measured. The beneficial effects of BMLE and BVLE in the treatment of diabetes include the prevention of diabetes-related liver and kidney tissue damage. Both extracts block the α-glucosidase enzyme, which may contribute to their anti-diabetic effects. More thorough research is required to explore the mechanism of action of both plants (Hago *et al*., 2021).

Many medications that are directly derived from plants have been found as a result of the isolation of active plant components. Shamran *et al*. in 2020 used *B. variegata*'s methanol leaf extract to separate and purify Glucokinin. Attempts were made to identify and describe the plant's functional groups using FTIR spectroscopy and HPLC. Using FTIR spectroscopy, several functional groups of Glucokinin were observed (Shamran *et al*.,2020).

To create a fingerprint of the medicinally and economically significant leaves of *B. variegata* L., the mobile phase of n-hexane, an ethanol extract of the leaves was created by Gunalan *et al*. in 2012. Using recognized procedures, ethyl acetate, formic acid, and acetic acid were scanned under visible light and UV light at 254 and 366 nm. The ethanol extract's HPTLC fingerprint revealed many peaks with various R_f values. Eleven spots were observed in 2.5µ liters of ethanol extract, compared to 13 spots in 5 and 10µ liters. In the solvent solution mentioned above, a 15µL concentration produced 14 spots. This species' authenticity and identification were aided by this fingerprint (Gunalan *et al*., 2012).

A research was conducted by Taia *et al*. in 2022 in which they examined thirteen species and two variants of *Bauhinia*. Morphological traits of pollen were examined and captured on camera with scanning electron and light microscopes. In addition to

identification keys, a pollen character-based clustering analysis of the taxa under study was conducted. There were variety of pollen morphological characteristics present in the pollen grains of *Bauhinia* species, including dispersal, polarity, symmetry, form, varying exine ornamentation, and diverse kinds and numbers of apertures. It has been suggested that the examined species have an evolutionary line. These differences support the genus's eurypalynous status (Taia *et al*., 2022).

CHAPTER 3: MATERIALS AND METHODS

3.1. Materials

3.1.1. Solvents required

Plant extract preparations used hexane, chloroform, and methanol as solvents.

3.1.2. Chemicals, Plants Materials

- TLC Aluminium sheets Silica gel 60 F_{254}
- Concentrated HCl
- Concentrated H_2SO_4
- Ammonia solution
- 2, 2-Diphenyl-1- picrylhydrazyl (DPPH)
- Mercuric chloride
- Potassium iodide
- NaOH
- Dimethyl Sulfoxide (DMSO)

Plant material: leaves of *B. variegata.*

3.1.3. Instrument and equipments

The following machinery was employed.

- Grinder
- Digital balance
- Refrigerator
- Rotary evaporator (RV10DS96)
- Herbal Medicine Disintegrator (FW177)
- Ultrasonic bath (FSF-040S)
- Pre-coated TLC plates
- UV-Vis Spectrophotometer (V1700)
- FT-IR (PerkinElmer Spectrum IR; Version 10.6.2)
- UV lamp (UV 2510TS)
- Water bath (2XT2)

- Capillary tube

3.2. Methods

3.2.1. Collection of plant

Leaves of *B. variegata* were collected from Galyang Municipality, Shyangja Nepal at about 913m altitude.

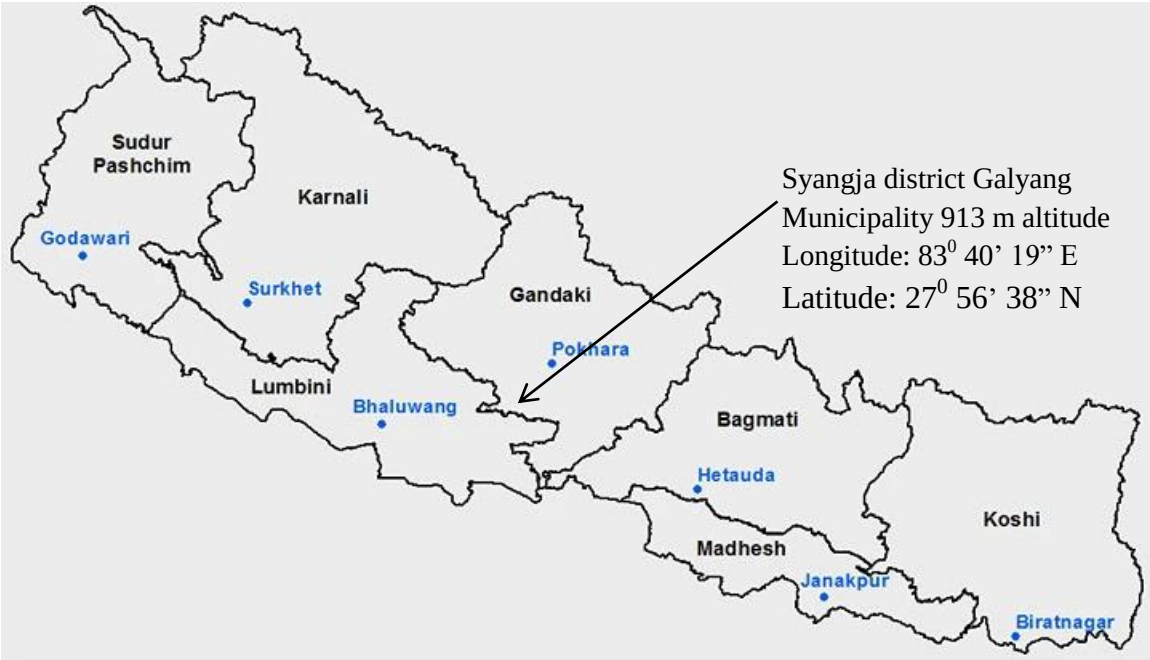
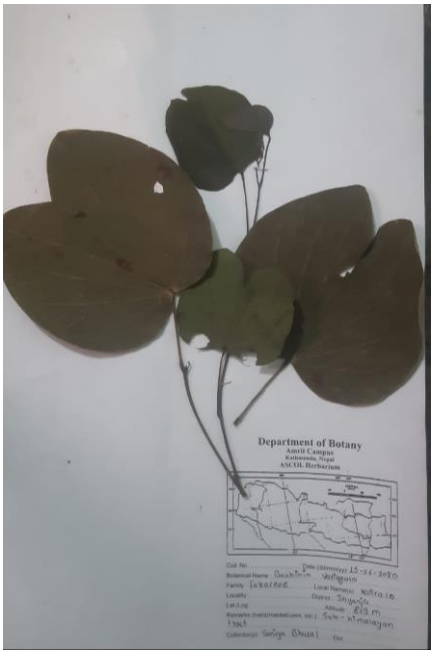


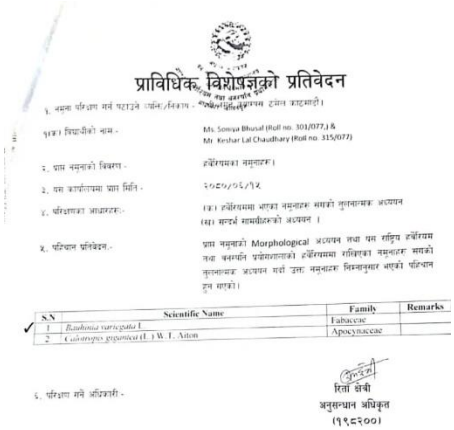
Figure 3.1: Map of Nepal showing sample collection site of *B. variegata*

Figure 3.2: Whole plant of *B. variegata*

3.2.2. Preparation of Herberium

The collected plant sample was squished between sheets of newspaper. Every day until the paper had completely dried, it was replaced. The dried sample was fixed and placed on a standard-sized herbarium sheet that had been precisely marked.




प्राविधिक विशेषज्ञको प्रतिवेदन

१. नमूना परिक्षण गर्न पठाउने व्यक्ति/निकाय :-

१(क) विद्यार्थीको नाम :- Ms. Soniya Bhusal (Roll no. 301/077.) &
Mr. Keshar Lal Chaudhary (Roll no. 315/077)

२. प्राप्त नमूनाको विवरण :- हर्बेरियमको नमूनाहरु ।

३. यस कार्यालयमा प्राप्त मिति :- २०८०/१२/१२

४. परिक्षणका आधारहरु :- (क) हर्बेरियममा भएका नमूनाहरु साथको तुलनात्मक अध्ययन
(ख) सन्दर्भ सामग्रीहरुको अध्ययन ।

५. परिक्षण प्रतिवेदन :- प्राप्त नमूनाको Morphological अध्ययन तथा यस राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशालाको हर्बेरियममा राखिएका नमूनाहरु साथको तुलनात्मक अध्ययन गर्दा उनिहरु निम्नानुसार भएको पहिचान हुन आएको ।

SN	Scientific Name	Family	Remarks
1	*Bauhinia variegata* L.	Fabaceae	
2	*Calotropis gigantea* (L.) W. T. Aiton	Apocynaceae	

६. परिक्षण गर्ने अधिकृतको :-

रिता शर्मी
अनुसन्धान अधिकृत
(१९८२००)

Figure 3.3: Herberium sheet of *B. variegata*

3.2.3. Identification of plant

The plant of *B. variegata* was identified from the National Herbarium and Plant Laboratories (KATH), Lalitpur, Nepal.

3.2.4. Drying and Grinding

The fresh leaves of *B. variegata* were plugged from the tree and it was washed with clean water to remove dust and soil particles. The leaves were dried completely at room temperature. It was grinded in Amrit Science Campus on a Herbal medicine Disintegrator with a speed of 24000 r.p.m.

3.2.5. Preparation of Extracts

Three extracts from different solvents, including hexane, chloroform, and methanol, were made.

3.2.6. Extraction procedure

The 800g dried powder of *B. variegata* was dipped in the 2L of hexane and it was sonicated for an hour then filtered. The filtrate was concentrated in the rotary evaporator to concentrate the sample and save the solvent. The process was repeated 2

times. Similarly, the process was further carried out in a similar way for the chloroform and methanol. The procedure was accomplished according to the polarity order.

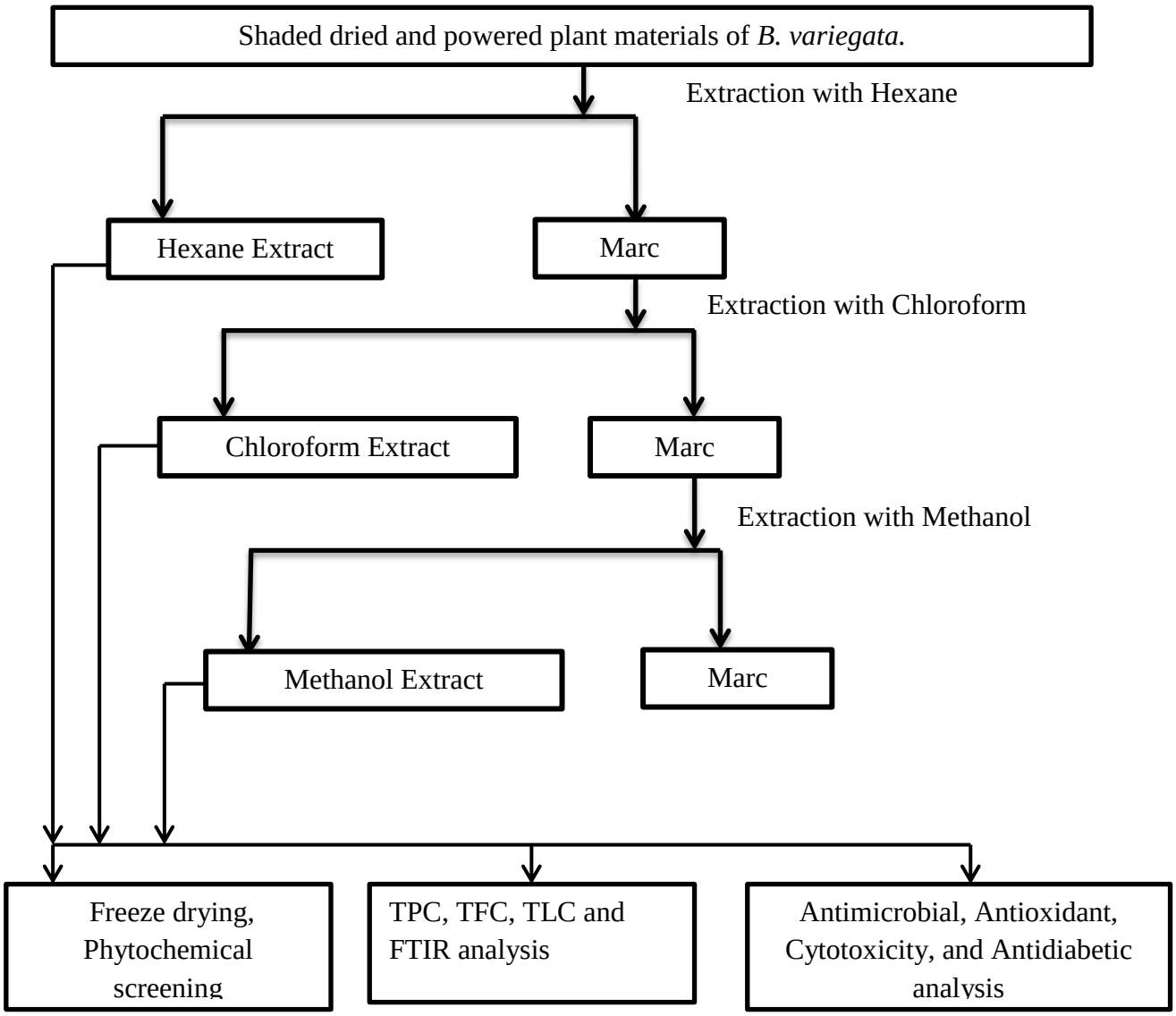


Figure 3.4:Research process for extraction, screening,chemical analysis and biological activities.

3.2.7. Determination of percentage yield

The resultant percent of the extract produced after the extraction procedure was estimated using the following formula (Duniya *et al*., 2018).

$$\% \text{ Yield} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant sample}} \times 100$$

3.2.8. Freeze drying of plant extracts

Freeze-drying imperfectly preserves essential kinds of therapeutic chemicals such as volatiles, phenolics, and carotenoids (Abascal *et al*., 2005).

The extract was freeze-dried to eliminate solvents and water. Initially, the plant extract was freeze-dried at -90°C. This stage converted the extract's moisture and solvent content into ice, which was then left for 5 hours. The vacuum was drawn into the chamber under increased pressure. As the pressure increased, the vacuum removed all solvents and moisture from the extract, leaving only plant extracts.

3.2.9. Phytochemical screening analysis

The process outlined in Appendices A and B of was followed in screening the extracts for the presence of different phytoconstituents (Banu & Cathrine, 2015).The unique coloring or precipitation of the extract solution indicated the presence of phytochemicals.

3.2.10. Total phenolic content

The FC assay examines additional chemicals that are easily oxidized and is typically used to determine total phenolic content (Ainsworth & Gillespie, 2007). The FC assay gauges an antioxidant's capacity for reductive breakdown via an electron-transfer-based reaction. It has been extensively utilized in nutritional and clinical research to determine the overall polyphenol content of food made from plants and biological materials (Lamuela-Raventós, 2017).

3.2.10.1. Total phenolic content assay

Many of the current methods in which phenolic compounds react with a colorimetric reagent to enable evaluation in the visible light spectrum provides the basis for calculating the overall phenolic content in food or biologic specimens.The Folin-Ciocalteu (F-C) test has been proposed as a standardized approach for frequent quality control and testing of the antioxidant capacity of food items and dietary supplements (Ainsworth & Gillespie, 2007). SPE is only suggested for extracts containing flavonoids rather than phenolic acids, as they are weakly retained in the matrix, resulting in exaggeration of TPC levels (Sánchez-Rangel *et al*., 2013).

3.2.10.2. Preparation of the Standard Gallic Acid Solution

A stock solution of 1000 µg/mL gallic acid was made by dissolving 50 mg in 50 mL of 30% DMSO. Different concentration of gallic acid such as; 500 µg/mL,250 µg/mL,

100 µg/mL, 50 µg/mL and 25 µg/mL were generated through serial dilution from the stock solution.

3.2.10.3 Construction of Calibration Curve

To create the calibration curve, 1 mL of each concentration of gallic acid solution was obtained in a beaker and 1 mL of DMSO as the blank. Each beaker received one mL of Folin-Ciocalteu reagent (FCR), which was allowed to stand for 5 minutes. Each mixture was thoroughly shaken before to adding 10 mL of a 7% Na_2CO_3 solution and 13 mL of filtered water. The reaction mixture was incubated for 90 minutes at 23°C. After incubation, a UV spectrophotometer at 750 nm was used to measure absorbance for each concentration and blank solution. The technique was conducted twice more to get triplicate data. The calibration curve was plotted using the average absorbance readings at different gallic acid concentrations.

3.2.10.4. Preparation of Sample Solution

To generate a stock solution of 5,000 µg/mL, a tiny amount of extract was dissolved in a calculated volume of 100% DMSO. Diluting 1mL of stock solution in 4 mL of 30% DMSO resulted in an extract concentration of 1000 µg/mL. The triplicate absorbance readings were acquired following the same approach as for gallic acid.

3.2.10.5. Calculation of Total phenolic content

Using equation 1, the total phenolic content was determined,

$$C = \frac{c \times V}{m} \quad \ldots\ldots\ldots\ldots (1)$$

where,

C = Total phenolic component content (mg/g) in gallic acid equivalent.

c = Gallic acid concentration as determined by the calibration curve (mg/mL).

V = Volume of extract (mL)

m = Weight of the plant extract (mg)

Data were recorded as a mean of three determination of absorbance for each concentration, from which the linear correlation coefficient (R^2) value was calculated.

The regression equation is given as,

$$y = mx + c \quad \ldots\ldots\ldots\ldots (2)$$

where,

y = Absorbance of the extract

m = Slope from the calibration curve

x = Concentration of the extract

c = Intercept

Using this regression equation, the concentration of the extract was calculated. Thus with the calculated value of the concentration of the extract, the total phenolic content was calculated from equation (2).

3.2.11. Total flavonoid content (TFC)

Following solvent extraction, the overall flavonoid content (TFC) of plants is often measured colorimetrically. Al(III) is used as a complexing agent in the aluminum chloride colorimetric assay, which is one of the commonly used techniques for measuring TFC in plant extracts (Shraim *et al*., 2021). The benchmark for quality was quercetin.

3.2.11.1. Preparation of the standard quercetin solution

To create the stock solution with a concentration of 1000 µg/mL (1 mg/mL), 10 mg of quercetin was weighed and dissolved in 10 mL of 100% DMSO. Subsequently, stock solution was serially diluted to create quercetin solutions with concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.125 µg/mL.

3.2.11.2. Construction of calibration curve

400 µL of the produced quercetin solution at each concentration was placed in a different test tube, and 400 µL of DMSO served as the blank. Each test tube received the following additions: 2.2 mL of distilled water, 1.2 mL of ethanol, 100 µL of 0.3M $AlCl_3$, and 100 µL of 10% sodium potassium tartarate solutions. After thoroughly mixing the entire mixture, it was allow to sit at room temperature until 30 minutes in the dark. Using the UV spectrophotometer set at 415 nm, absorbance was measured for each concentration and the blank solution following incubation. The procedure was repeated to collect triplicate data, and the calibration curve was plotted using the average absorbance values obtained at various quercetin concentrations.

3.2.11.3. Preparation of sample solution

The little amount of sample was dissolved in a determined volume of 100% DMSO to create a stock solution of 5,000 µg/mL of the extract. By dissolving 1 mL of stock

solution in 4 mL of 30% DMSO, the extract concentration was increased to 1000 µg/mL. The triplicate absorbance of the sample was then determined using the same method as described for the standard quercetin.

3.2.11.4. Calculation for total flavonoid content

The following formula was used to calculate the total flavonoid content of the extract,

Total Flavonoid Content (C) = $\frac{c \times V}{m}$

Where, C = Total Flavonoid Content (in mg/g) in Quercetin Equivalent (QE)

c = Concentration of quercetin established from calibration curve in mg/mL

V = Volume of the extract (in mL)

m = Weight of the plant extract (in mg)

3.2.11.5. Statistical Analysis

Data were recorded as a mean of three absorbance determinations absorbance for each concentration, from which the Linear Correlation Coefficient (R^2) value was calculated. The regression equation is given as,

$y = mx + c$

Where, y = Absorbance of the extract

m = Slope from the calibration curve

x = Concentration of the extract,

c = Intercept Using this regression equation,

the extract concentration was calculated. Thus with the calculated value of the concentration of extract, the flavonoid content was calculated.

3.2.12.Antimicrobial analaysis

3.2.12.1 Preparation of microbial culture media

The liquid broth (LB) media was made by mixing 13 g of LB powder (Sisco Research Laboratories Pvt. Ltd, India) in 1 liter of water. The combination has been sterilized at 15 psi pressure and 121 °C for 25 minutes. The sterilized media was cooled to 40-50 degrees Celsius before being transferred into sterile 15 mL falcon tubes (5 mL each). The produced mixture was used to coexistence bacterial seed cultures in separate tubes, which were incubated for 24 hours.

3.2.12.2 Collection of test organism

This study used microbial strains from the Himalaya Research Institute of Biotechnology in Bhaktapur, Nepal (Suryabinayak-5). The strains studied included two types of bacteria and one fungus.

Gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus*

Gram negative bacteria: *Escherichia coli* and *Klebsiella pneumonia*

Fungus: *Candida albicans*

3.2.12.3. Preparation of MH media plates and antimicrobial assay:

The Mueller-Hinton Agar plates were made by mixing 39 g of MH agar powder (Sisco Research Laboratories Pvt. Ltd, India) in 1 liter of water. The mixture was sterilized for 25 minutes at 15 psi and 121 °C. After freezing to 40-50 °C, the sterilized medium was transferred into 25 mL Petri dishes. The already assembled plates were kept in a fridge until needed. Label the medium plates with sample names and sprinkle 150 µL of liquid bacterial seed on the surface using a sterile cotton swab. The wells were created on the outermost layer of agar and every sample aliquot (100µL, 100mg/mL in DMSO) and standard kanamycin 5 mg/mL (10µL) were placed into the prepared well. The media plates were allowed to incubate for 24 hours at 37 degrees Celsius. Antimicrobial test results were obtained after 24 hours.

3.2.13. Antioxidant activity

Since antioxidants regulate the degree of oxidation, it is possible to quantify their activity indirectly rather than directly (Antolovich *et al*., 2002). Numerous assays using various processes, such as metal chelation, hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and others, can be used to track antioxidant activity (Shahidi & Zhong, 2015).

3.2.13.1. Preparation of 0.1 mM DPPH Solution

The preparation of 50 mL of 0.5 mM 2,2-Diphenyl-1-picrylhydrazyl-hydrate (DPPH) with a molecular weight of 394.32 g/mol involved carefully weighing 10 mg of DPPH, dissolving it in a small amount of methanol, adding methanol up to the mark, and shaking the mixture well. Subsequently, 0.1 mM DPPH was made by diluting 10 mL of 0.5 mM DPPH in a 50 mL volumetric flask, adding 40 mL of methanol, and filling the mark. All of these DPPH handling procedures were done in a dark environment and stored in a dark place until needed.

3.2.13.2. Preparation of sample solution

To make a stock solution, a tiny amount of sample was dissolved in a calculated volume of 100% DMSO, yielding 5,000 µg/mL of extract. By dissolving 1 mL of stock solution in 4 mL of 30% DMSO, the extract concentration was reached at 1000 µg/mL. Next, extract solutions containing 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.625 µg/mL, and 7.81 µg/mL were made by serial dilution.

3.2.13.3. Measurement of DPPH radical scavenging activity

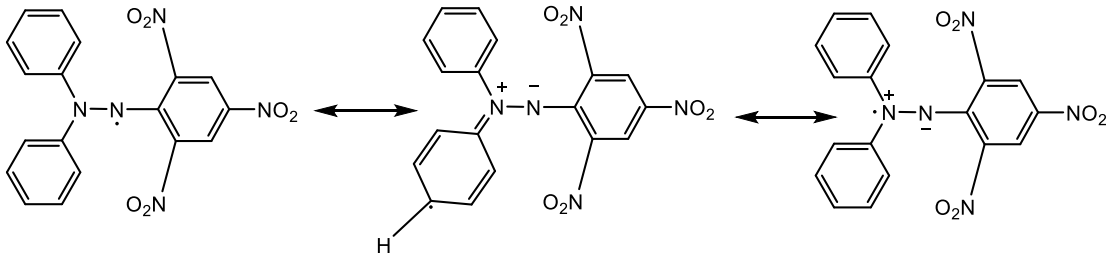
1 mL of each serially diluted concentration of the standard quercetin was placed in another test tube, and 1 mL of 0.1 mM DPPH solution was included to creating the dark environment. Similarly, 1mL of 30% DMSO was mixed with 1 mL DPPH and taken as the blank. The reaction mixture was left in the dark for thirty minutes at 37°C before being measured with a spectrophotometer at 517 nm. Each of the tests were carried out in a total of three and the percentage of DPPH free radical scavenging activity of each concentration was estimated using the following relation:

$$\% \text{ Scavenging} = \frac{Ac - As}{As} \times 100$$

Where,

A_C = Absorbance of the control (DMSO + DPPH)

A_S = Absorbance of the test sample



Stable DPPH Free Radical

Figure 3.5: DPPH free radical

The free radical scavenging of each plant extract of concentration 500µg/mL was determined following the same procedure. The only extracts with more than 50% scavenging value at 500µg/mL concentration were further serially diluted and respective percentage scavenging were determined. By plotting the % scavenging versus concentration, IC_{50} value of respective extract was calculated.

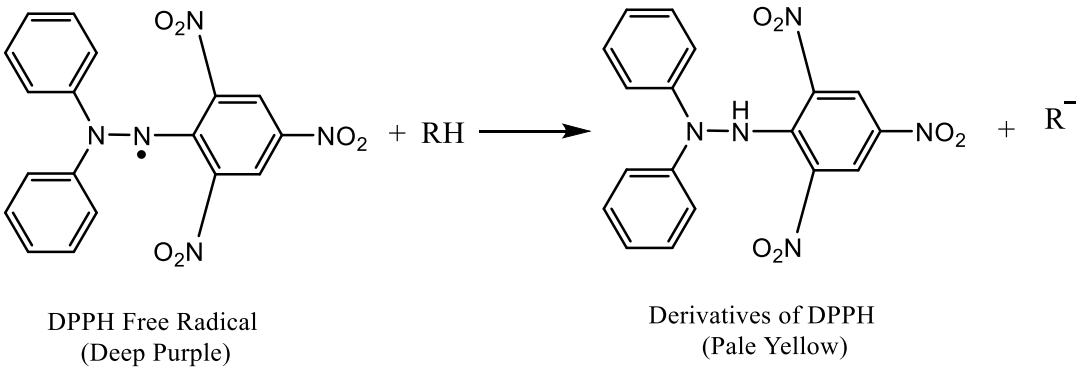


Figure 3.6: Mechanism of free radical

3.2.14. Brine Shrimp Lethality Bioassay

A quick and low-cost bioassay to evaluate the potency of phytochemicals in plant extracts is the Brine shrimp lethality assay (BSLA) (Waghulde *et al*., 2020). Brine shrimp growth condition: Artificial seawater was made using 38 g/L salt in water and brine shrimp eggs were hatched at 22-29° C under a continuous air pump supply. The naupulis hatched after 30-35 hours approximately were used for the cytotoxicity assay (Olowa & Nuñeza, 2013). 20 naupulis were used for each sample concentration in 96 well plates. The results tabulated here are obtained after 24 hours of assay.

3.2.14.1. Preparation of artificial seawater

Artemia salina leach were emerged in artificial saltwater made by mixing 38 g of sea salt in 1 L of distilled water (Olowa & Nuñeza, 2013).

3.2.14.2. Hatching of Brine shrimp eggs

Using brine shrimp eggs, Artemia salina were hatched for 48 hours at a temperature between 22 and 29 degrees Celsius, with continuous aeration and light. The beaker (1 liter) was filled with sterile artificial seawater (produced with 38 g/L sea salt). Following hatching, active nauplii from each concentration that were free of egg shells were chosen and used in the cytotoxicity test.

3.2.14.3. Preparation of samples

A clean e-tube was used to weigh 10 mg of the sample carefully, and 1 mL of DMSO was used to dilute it to create a stock solution with a concentration of 10,000 ppm (μg/mL). After that, 9 mL of pure water was added to dilute it until the concentration reached 1000 ppm. Using the serial dilution method, solution concentrations of 10 ppm, 50 ppm, 100 ppm, 500 ppm, and 800 ppm were created from 1000 ppm. After solution preparation, a 96-well plate with a volume capacity of 0.5 mL was employed for

cytotoxicity testing. Each well plate was inoculated with 20 nauplii, followed by a diluted sample (0.4 mL) in each of the wells. The experiment was carried out for 24 hours, with close observation of 8 hours, and each sample was analyzed in triplicate.

3.2.14.4. Calculation of lethality percentage

The percentage of brine shrimp nauplii that perished from the tested material is the lethality percentage in the brine shrimp assay calculation. The lethality percentage of each sample was determined by counting the number of nauplii that survived, and this was expressed as follows:

$$\% \text{ Mortality} = \frac{Number\ of\ dead\ shrimps}{Total\ Number\ Shrimps} \times 100$$

3.2.15. α-amylase inhibition assay

Chronic metabolic condition known as diabetes mellitus is typified by elevated blood glucose levels (Hu *et al*., 2016).

3.2.15.1. Protocol for anti-diabetic assay

α-amylase was inhibited using 3, 5-dinitro salicylic acid (DNSA) technique. B. *variegata* extracts were diluted with at least 10% DMSO. To produce various concentrations, the sample was combined with DMSO and then dissolved in buffer and NaCl at pH 6.9. Combine 200 µL of plant extract with 200 µL of α-amylase solution and agitate at 30°C for 10 minutes. Each of the tubes received 200 µL of 1% starch solution in water and was incubated for 3 minutes.The reaction was stopped by adding 200 µL of DNSA reagent. The end product was heated for 10 minutes in a water bath at 85-90 ° C. The liquid was cooled to room temperature and diluted with 5 mL of distilled water. The absorbance of the sample was measured at 540 nm with a UV spectrophotometer and compared to a blank solution. To generate a blank solution with 100% enzyme activity, 200 µL of buffer was used instead of the plant extract. The blank reaction was then set up using the sample plant extract at each concentration without the enzyme. Acarbose was used as a positive control in the anti-diabetic screening.

3.2.16. Thin layer chromatography

Thin-layer chromatography (TLC) is a widely used technique that can aid many laboratories in quality control, particularly those that deal with plant extracts and phytotherapeutics (Braz *et al*., 2012). Chromatograms generated by the TLC Analyser

are highly informative; nonetheless, it is crucial to precisely adhere to the band that is taken into consideration throughout the chromatogram generation process. (Sima *et al*., 2015). In addition to aiding in the identification and quality control of a specific species, TLC/HPTLC fingerprinting of plant species can provide foundational data that is useful in the separation, cleaning, characterisation, and identification of the species' indicate chemical compounds.(Gunalan *et al*., 2012).

$$\text{Retention factor (Rf)} = \frac{\text{Distance travelled by component}}{\text{Distance travelled by the solvent}}$$

3.2.17. FTIR analysis

Two popular analytical methods for determining the stability of compost are thermal analysis and Fourier transform infrared spectroscopy (FTIR) (Carballo *et al*., 2008). Plant composition and structure can be analyzed to a significant extent using Fourier Transform Infrared Spectroscopy (FTIR). Furthermore, FTIR spectroscopy is a validated, time-saving approach for detecting and characterising functional groups (Dhivya & Kalaichelvi, 2017). The most effective instrument for determining the kinds of chemical bonds (functional groups) that are present is probably the Fourier Transform Infrared Spectrophotometer (FTIR). The annotated spectrum illustrates how the chemical bond is characterized by the wavelength of light absorbed (Shamran *et al*.,2020).

CHAPTER 4: RESULT AND DISCUSSION

4.1. Extract yield

The extraction of *B. variegata* leaves was done using three different solvents of different polarity such as Hexane, Chloroform, and Methanol. Through the measurement of extract yields, their efficacy in isolating the active ingredients in *B. variegata* leaves that are responsible for their biological activity was assessed. It was sonicated using ultrasonification for 1 hour. It was crucified using a Rota-evaporator and dried in a water bath. The amount of extracts recovered in grams after drying them in vails is displayed in Table 4.1.

Table 4.1: Number of extract collected from each experiment

Plant Name	Parts Used	Method of Extraction	Solvents	Colour and consistency	Weight of extract (g)	% yield
B. variegata	Leaves	Ultrasonic extraction	Hexane	Light green	2.2 g	0.275
			Chloroform	Light green	11.7 g	1.46
			Methanol	Brownish green	7.6 g	0.95

4.2. Phytochemical screening analysis

To determine whether the secondary metabolites in the gathered *B. variegata* plant extract were present,a phytochemical screening study was conducted. The presence of the secondary metabolites and their phytoconstituents was discovered after a number of experiments. The result of phytochemical screening has been encapsulated in table 2.

Table 4.2: phytochemical analysis of extracts of *B. variegata*

S.N	Class of phytochemicals	Hexane extract	Chloroform extract	Methanol extract
1.	Alkaloids	-	+	-
2.	Flavonoids	+	+	+
3.	Steroids	-	-	+
4.	Quinones	-	-	+
5.	Tannins	-	-	+
6.	Phenolic Compounds	-	-	+
7.	Carbohydrates	-	+	-
8.	Proteins	-	-	-
9.	Cardiac Glycosides	+	-	-
10.	Resins	+	-	+
11.	Saponins	-	-	+

"+" denotes presence, whereas "-" signifies absence.

4.3. Total Phenolic Content Analysis

4.3.1. Construction of calibration curve

The total phenolic content in plant extracts was determined using the oxidation-reduction method based on the calorimetric Folin-Ciocalteu reagent (FCR). A combination of phosphotungstic acid and phosphomolybdic acid is known as the Folin-Ciocalteu reagent. Gallic acid was utilized as the reference chemical to create a calibration curve, and its absorbance was evaluated at different levels (500 µg/mL, 250 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, and 25 µg/mL). The resulting graphical curve, with the X- and Y-axes denoting concentration and absorbance, respectively, shows the relationship between absorbance and the standard gallic acid concentration. Given figure displays the conventional gallic acid calibration curve.

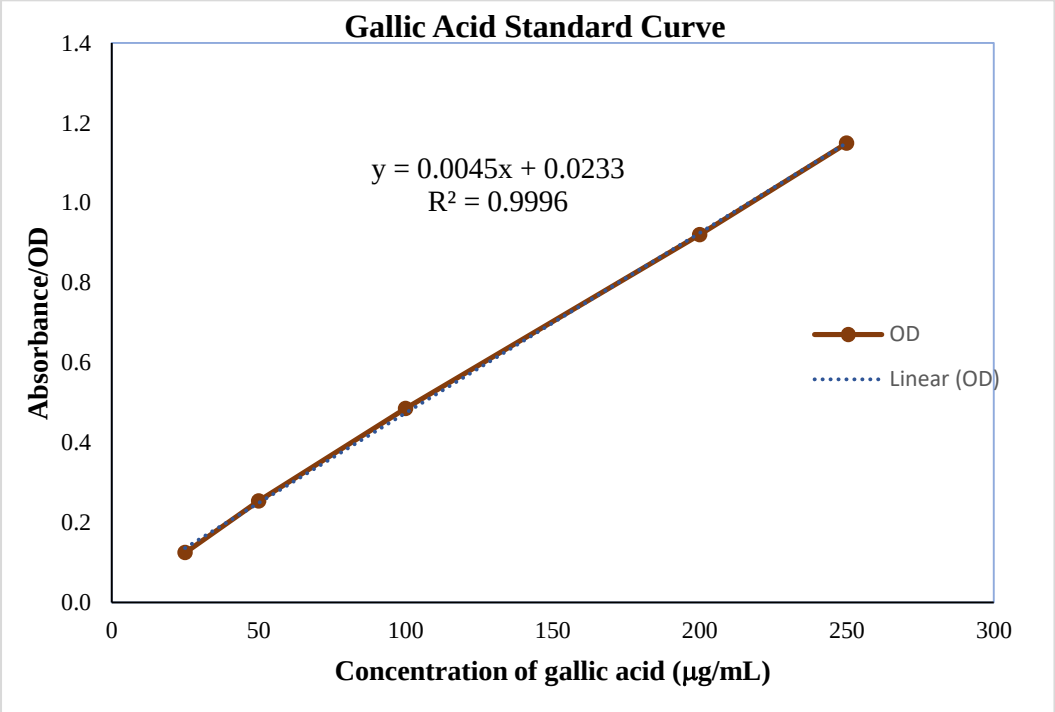


Figure 4.1: Calibration curve for standard gallic acid

4.3.2. Calculation of total phenolic content in different extracts

The total phenolic content of the various extracts was determined using triplicate absorbance readings of 1000 µg/mL and a calibration curve. The results are displayed in Table 4.3.

Table 4.3: Total phenolic content of different extract

Extracts	OD of Samples			OD of Control	TPC (mg GAE/g)			TPC Mean (mg GAE/ g)	Std Dev.
	I	II	III		I	II	III		
Hexane	0.18	0.22	0.16	0.07	15.93	29.04	13.39	19.45	8.20
Chloroform	0.35	0.38	0.31	0.07	56.82	61.26	48.60	55.56	6.42
Methanol	0.69	0.70	0.70	0.07	147.04	134.15	134.15	138.44	1.79

The total phenolic contents of hexane, chloroform, and methanol extracts were found to be 19.45±8.20 mg GAE/g, 55.56±6.42 mg GAE/g, and 138.44±1.79 mg GAE/g, respectively. It demonstrates that the methanol extract had the highest phenolic content, whereas the hexane extract had the fewest of the three.

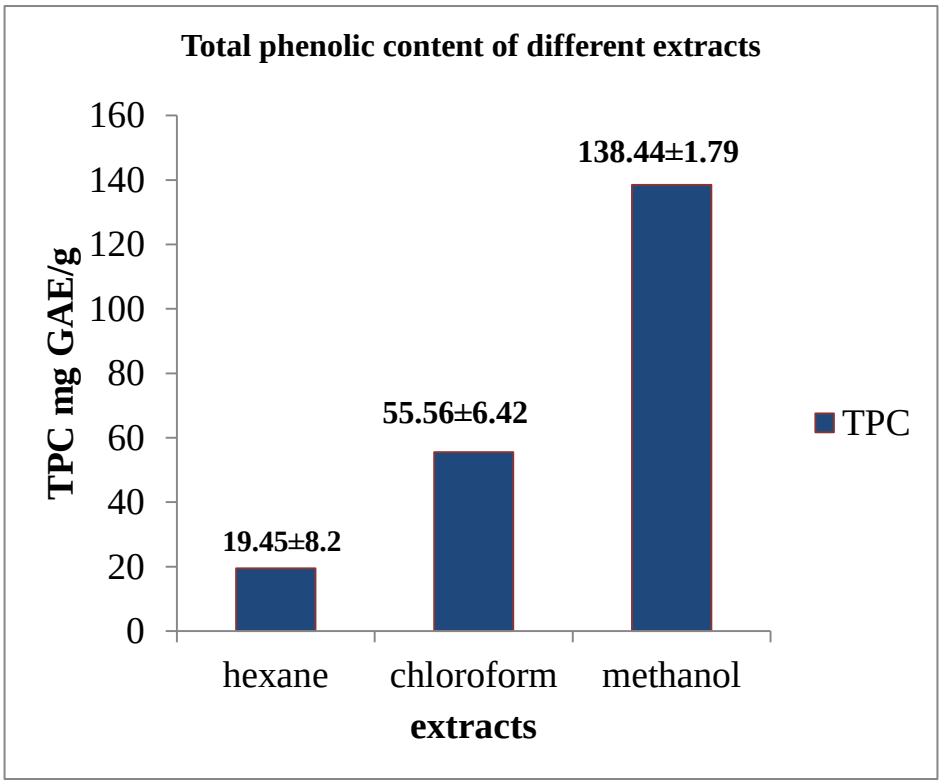


Figure 4.2:Total phenolic content in different extracts

4.4. Estimation of total flavonoid content

4.4.1 Construction of calibration curve

The total flavonoid concentration in plant extracts was determined using the aluminum chloride colorimetric technique. To create a calibration curve, quercetin was employed

as the standard compound. The absorbance of different concentrations (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.125 µg/mL) was measured at 415 nm. The graphical curve was created by graphing absorbance against various concentrations of standard quercetin. The X-axis represents concentration, while the Y-axis represents absorbance. Figure 8 shows the calibration curve for standard quercetin.

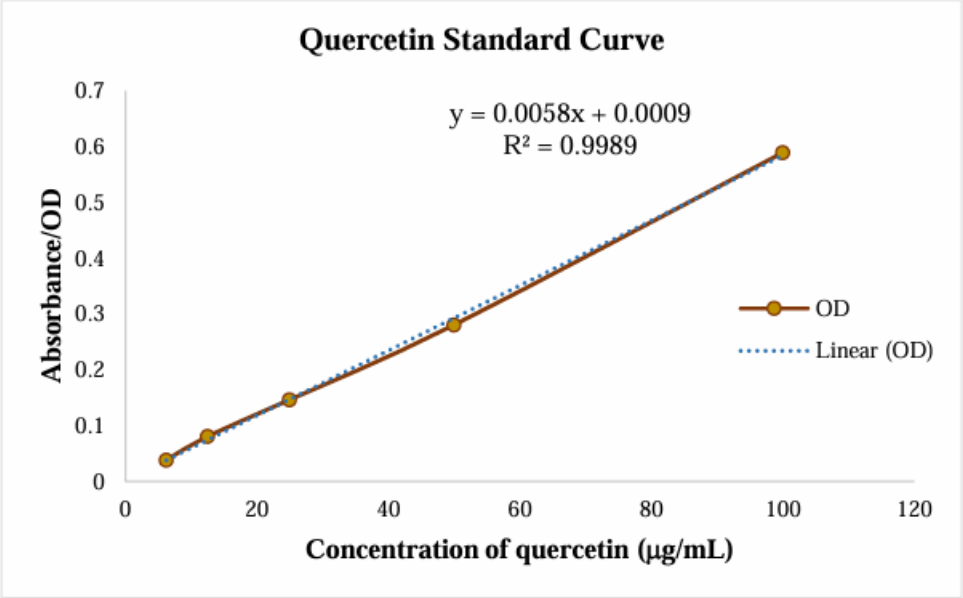


Figure 4.3: Calibration curve for standard quercetin

4.4.2. Calculation of total flavonoid content in different extracts

The total flavonoid content in each extract was determined using the calibration curve and absorbance values (triplicate of 1000 µg/mL), as indicated in the table 4.4.

Table 4.4: Total flavonoid content of different extract

Extracts	OD of Samples			OD of Control	TFC (mg GAE/g)			TFC Mean (mg QE/g)	Std Dev
	I	II	III		I	II	III		
Hexane	0.60	0.50	0.63	0.06	37.52	30.42	39.31	35.75	4.70
Chloroform	1.11	1.24	1.13	0.058	72.69	203.81	74.42	116.97	1.76
Methanol	0.42	0.41	0.42	0.061	24.69	24.34	25.24	24.75	0.45

The total falvonoid contents of hexane, chloroform, and methanol extracts were found to be 35.75±4.70 mg QE/g, 116.97±1.76 mg QE/g, and 24.75±0.45 mg QE/g, respectively. The total flavonoid content of the several *B. variegata* extracts is shown in the bar graph. Among the three extracts, the flavonoid concentration of the chloroform

extract was the highest, while the flavonoid content of the Methanol extract was the lowest.

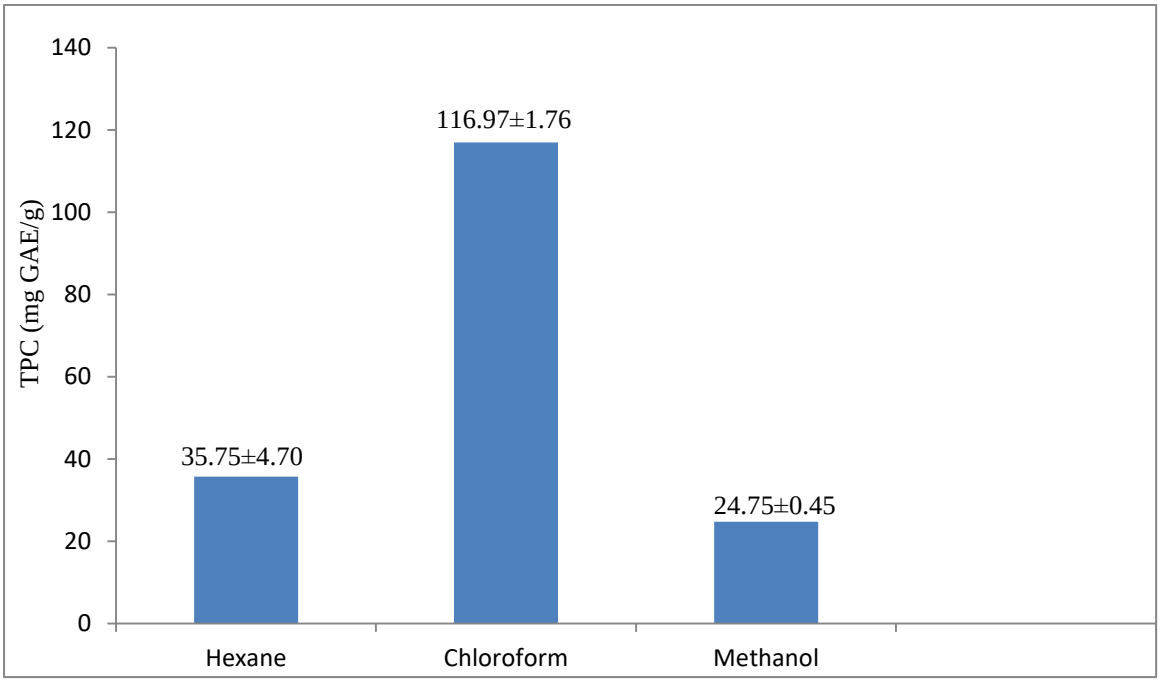


Figure 4.4: Total flavonoid content in different extracts

4.5. Antimicrobial Analysis

Numerous earlier investigations have demonstrated that plants are abundant providers of vital phytoconstituents. The antimicrobial activity of extract of different solvent (50mg/mL) are shown in table 4.5.

Table 4.5: Antimicrobial test of different solvent extract

Bacterial strain	Reference culture	Type	Positive control (c+) cm	Hexane	Chloroform	Methanol
E. coli	ATCC 8739	Gram -ve	2.7	1.3	1.7	1.8
B. subtilis	ATCC 6051	Gram +ve	2.5	1.2	1.5	1.6
S. aureus	ATCC 700603	Gram +ve	2.1	1.2	1.6	1.7
K. pnuemoniae	ATCC 700603	Gram -ve	2.1	1.2	1.6	1.2
C. albicans	ATCC 2091	Fungi	2.5	1.3	1.6	1.7

*****zone of inhibition (ZOI) have been tabulated in centimeters (cm).

*No activity was found in negative control (c^-, DMSO).

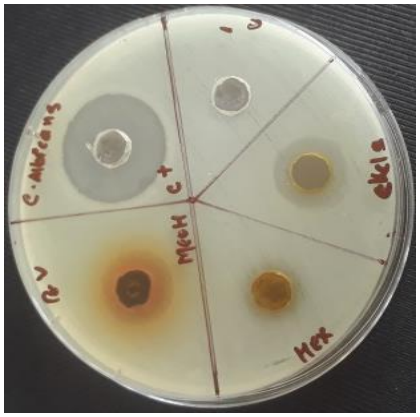


Figure 4.5: Antifungal activity shown by fungi *C. albicans*

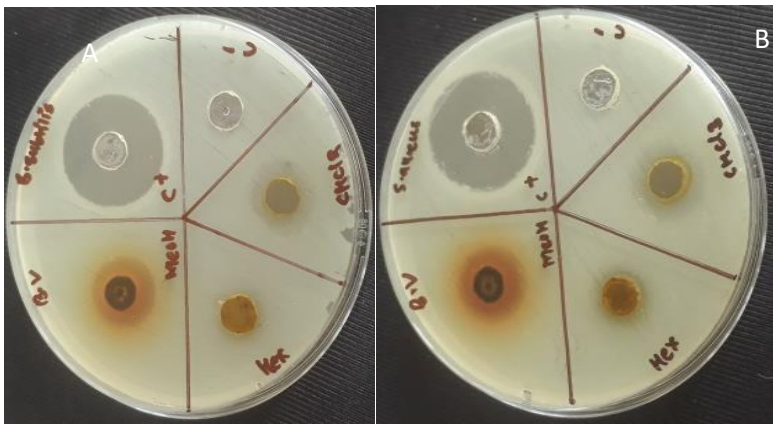


Figure 4.6: Antibacterial activity shown by Gram +ve bacteria (A) *B. subtilis* and (B) *S. aureus*

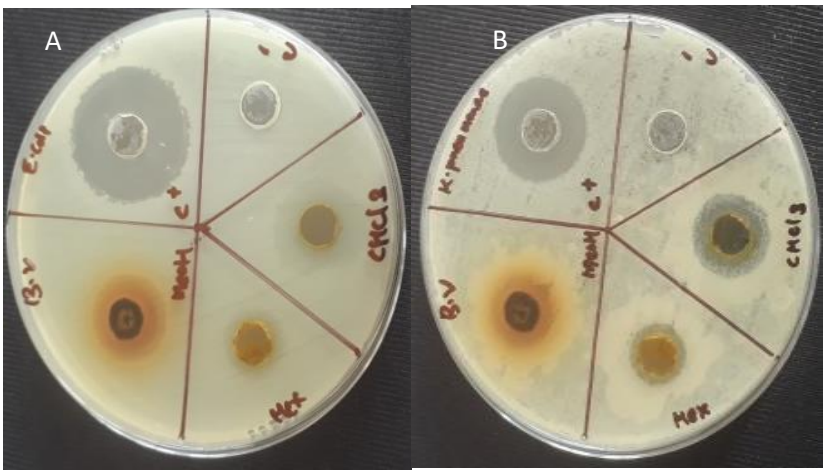


Figure 4.7: Antibacterial activity shown by Gram -ve bacteria (A) *E. coli* and (B) *K. pnuemoniae*

4.6. Antioxidant Activity

Examining the absorbance shift brought about by lower DPPH allowed researchers to gauge how well the test compounds scavenged free radicals. There is an inverse association between antioxidant activity and the IC_{50} value, which was obtained using

linear regression analysis of percentage inhibition versus antioxidant activity. As the IC_{50} values decrease, the antioxidant activity rises. The level of antioxidant activity is higher when the IC_{50} value is lower. The antioxidant capacity of several *B. variegata* extracts was assessed through the plotting of the percentage of free radical scavenging versus concentration. Using the absorbance values acquired at 517 nm, the IC_{50} value of the respective extracts was calculated by computing the percentage inhibition of DPPH radicals against the sample displayed in the table below.

Table 4.6: Antioxidant activity of different extract in 500 µg/mL concentraction

Extracts	**Concentration (µg/mL)**	**OD of samples**	**OD of control**	**% Scavenging**
Hexane	500	0.81	0.88	6.61
Chloroform	500	0.72	0.88	17.55
Methanol	500	0.20	0.88	76.51

4.6.1. Antioxidant activity of standard quercetin

By serially diluting the concentration and calculating the percentage scavenging value, the antioxidant activity of the quercetin was ascertained. The percentage of DPPH radical inhibition for each concentration was calculated, and the outcomes are displayed in the table 4.7.

Table 4.7: Antioxidant activity of standard Quercetin

Sample	**Concentration (µg/mL)**	**OD of Samples**			**OD of Control**	**% Scavenging**			**Avg. % Scavenging**
		I	**II**	**III**		**I**	**II**	**III**	
Quercetin	25	0.176	0.242	0.248	0.957	81.60	74.71	74.08	76.79
	12.5	0.218	0.218	0.23	0.957	77.22	77.22	75.96	76.8
	6.25	0.37	0.372	0.25	0.957	61.33	61.12	73.87	65.44
	3.125	0.621	0.631	0.501	0.957	35.10	34.06	47.64	38.93
	1.56	0.779	0.791	0.711	0.957	18.59	17.34	25.70	20.54
	0.78	0.858	0.86	0.815	0.957	10.34	10.13	14.83	11.76

The figure displays the IC_{50} value of standard quercetin, which was determined to be 4.76 µg/mL.

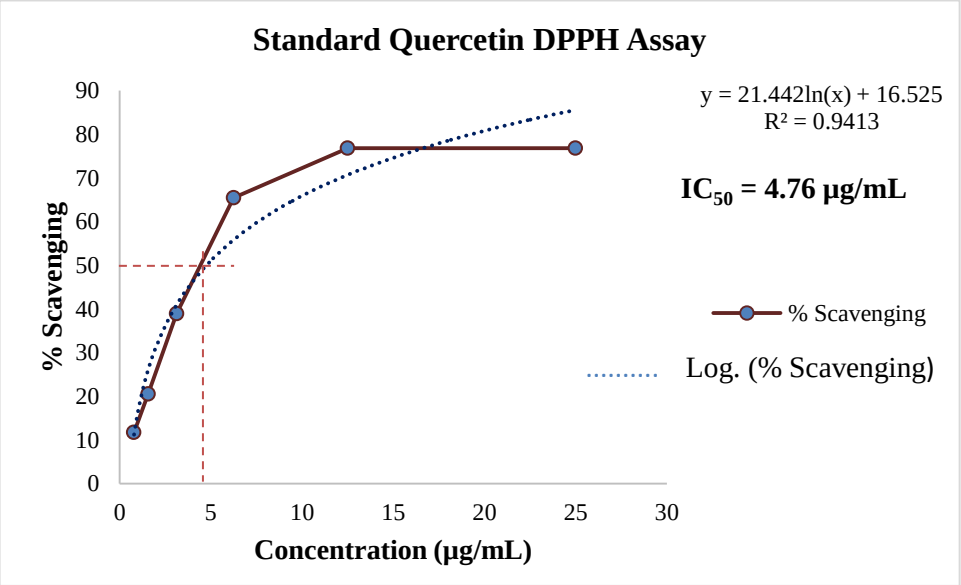


Figure 4.8: Antioxidant activity of standard quercetin

4.6.2. Antioxidant activity of hexane extract

At 500 µg/mL, the hexane extract exhibited a comparatively modest free radical scavenging activity of only 6.61%. This result suggested that *B. variegata*'s hexane extract had a limited capacity for antioxidant activity. These results pointed that hexane extract's poor ability to neutralize free radicals.

4.6.3. Antioxidant activity of chloroform extract

At 500 µg/mL, the *B. variegata* chloroform extract showed only 17.55 % free radical scavenging activity. This indicates that 17.55% of the DPPH radicals in the test solution can be neutralized by it. This finding indicates that the chloroform extract has a comparatively low antioxidant activity.

4.6.4. Antioxidant activity of methanol extract

The % scavenging activity of the methanol extract of *B. variegata* at 500 µg/mL was found to be 76.51%. Due to the high % scavenging activity, the serial dilution was performed and the percentage inhibition of DPPH radicals for each concentrated was calculated which is shown in Table 4.8.

Table 4.8: DPPH radical scanvenging of methanol extract

Sample	Concentration (µg/mL)	OD of Samples			OD of Control	% Scavenging			Avg. % Scavenging
		I	II	III		I	II	III	
Methanol extract of *B. variegata*	250	0.260	0.227	0.195	0.877	70.35	74.11	77.30	77.76
	125	0.265	0.301	0.199	0.877	69.78	65.67	77.30	70.91
	62.5	0.277	0.480	0.281	0.877	68.41	45.26	67.95	60.54
	31.25	0.508	0.575	0.483	0.877	42.07	34.43	44.92	40.47
	15.625	0.719	0.641	0.633	0.877	18.01	26.90	27.82	24.24
	7.81	0.744	0.76	0.747	0.877	14.82	13.34	14.82	14.32

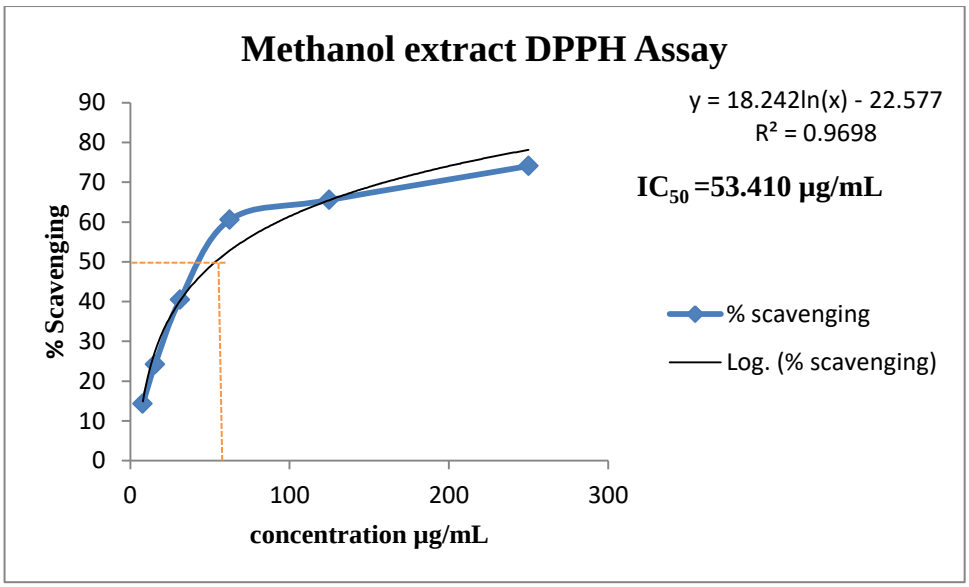


Figure 4.9: Antioxidant activity of Methanol extract

The figure display the IC_{50} value of Methanol extract is 53.410 µg/mL.

4.7. α-Amylase Assay

Utilizing the 3, 5-dinitro salicylic acid (DNSA) technique, the α-amylase inhibition assay was assessed. This approach evaluates the antidiabetic potential using the logarithmic regression equation, which has an inverse relationship with the IC_{50} value.

Every extract's absorbance was calculated at 540 nm. The following table and picture present the percentage of α-amylase inhibition for several *B. variegata* extracts.

4.7.1. α-amylase inhibition of standard acarbose

Table 9 displays the percentage of normal α-amylase function that is inhibited by standard acarbose drug.

Table 4.9: α-Amylase inhibition of standard acarbose

Sample	Concentration (µg/mL)	% Inhibition
Acarbose	62.5	52.48
	31.25	45.7
	15.625	35.02
	7.81	29.08
	IC_{50}	**49.57 µg/mL**

The IC_{50} value of the standard acarbose drug was found to be 49.57 µg/mL as exhibited in Figure 4.10.

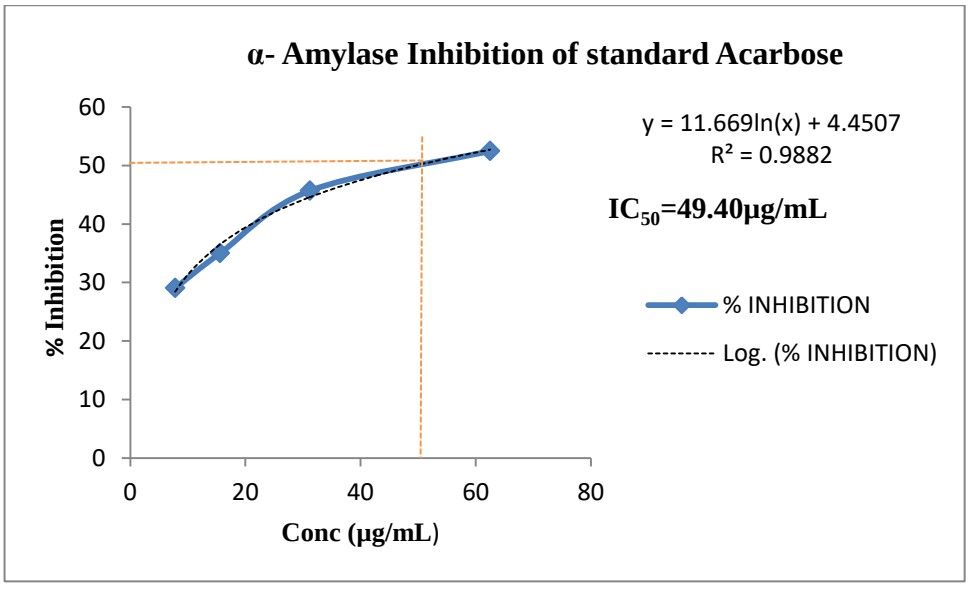


Figure 4.10: α- Amylase Inhibition os standard Acarbose

4.7.2. α-amylase inhibition of hexane extract

Table 4.10 shows the proportion of α-amylase inhibition caused by hexane extract.

Table 4.10: α-Amylase inhibition of Hexane extract

Sample	Concentration	% Inhibition
B. variegata	2000	78.71
	1000	58.67
	500	54.54
	125	49.79
	IC_{50}	**192.5µg/mL**

IC_{50} value of Hexane extract was found to be **192.5µg/mL** as shown in figure 4.11. This indicate that it was more effective against α-amylase Inhibition activity.

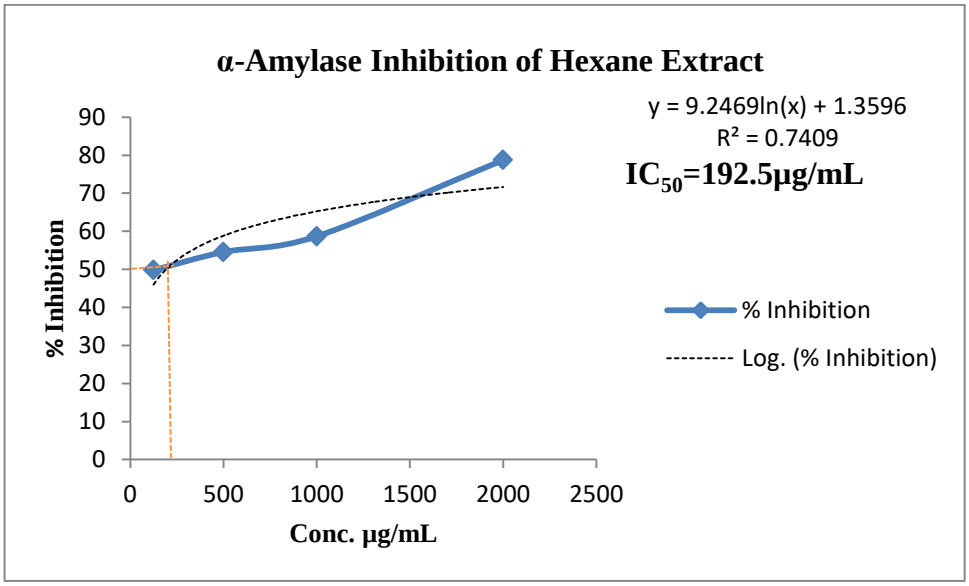


Figure 4.11: α-amylase inhibition of hexane extract

4.7.3. α-amylase inhibition of chloroform extract

Table 4.11 displays the percentage of *B. variegata* chloroform extract-induced inhibition of α-amylase functioning.

Table 4.11: α- amylase inhibition of chloroform extract

Sample	Concentration	% Inhibition
	4000	54.56
	2000	43.20

Chloroform extract of *B. variegata*	1000	32.51
	500	27.17
	IC_{50}	**3127.53µg/mL**

The IC_{50} value of Chloroform extract of *B. variegata* is found to be 3127.53. This suggested that the chloroform extract has limited efficacy to inhibit α-amylase.The IC_{50} value is shown in figure below.

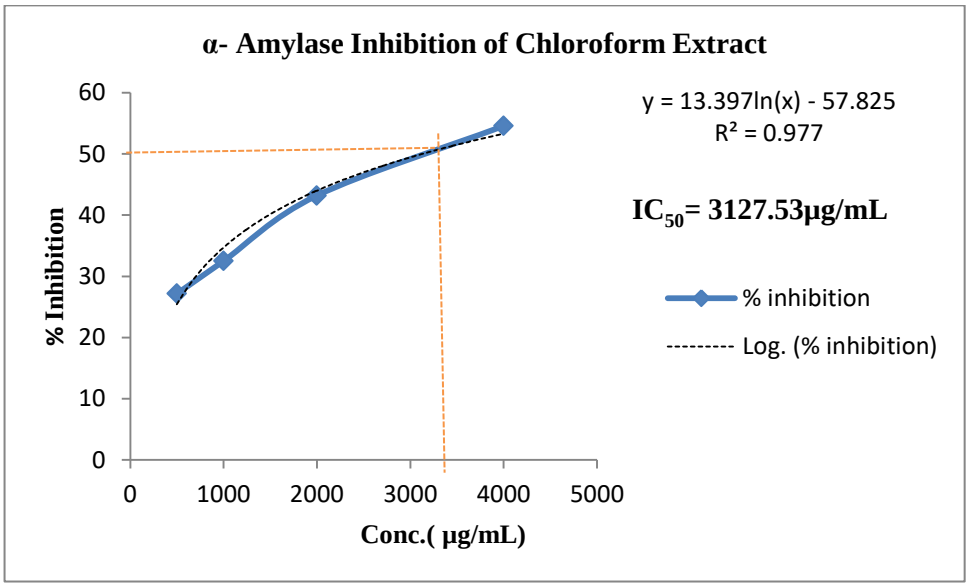


Figure 4.12: α- amylase inhibition of chloroform extract

4.7.4. α-amylase inhibition of methanol extract

The percentage inhibition of different concentration of methanol extract is shown in below table 4.12.

Table 4.12: α-amylase inhibition of methanol extract

Sample	Concentration (µg/mL)	% Inhibition
Methanol extract of *B. variegata*	4000	87.22
	2000	68.96
	1000	56.79
	500	40.36
	IC_{50}	**772.011µg/mL**

The methanol extract's IC_{50} value was determined to be 772.011 µg/mL. This finding implies that, methanol extract can also be useful in inhibiting α-amylase.

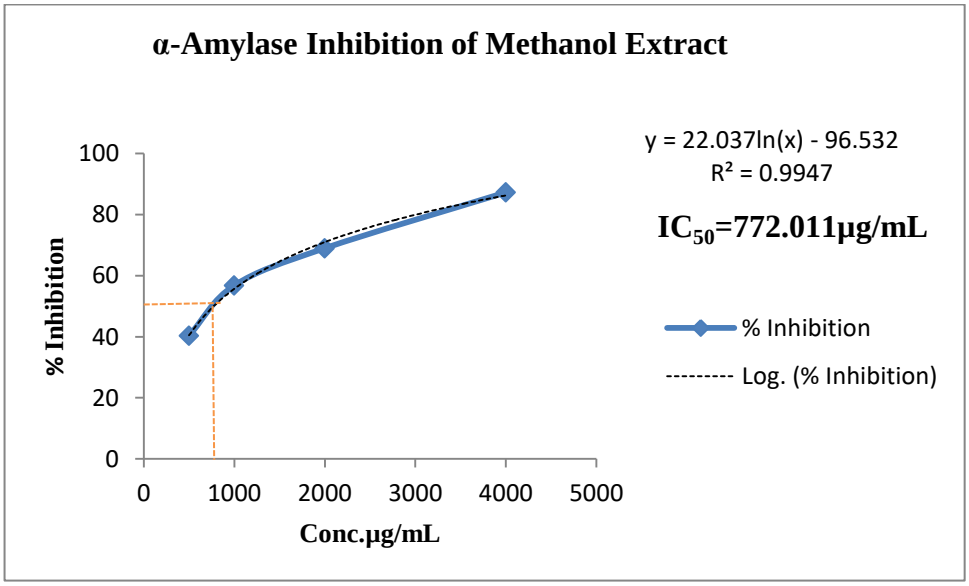


Figure 4.13: α-amylase inhibition of methanol extract

Hexane and methanol extracts were shown to be the most efficient in managing diabetes because they were potent α-amylase inhibitors, while chloroform extract was found to have less potent α-amylase inhibitory qualities.

4.8. Cytotoxicity Screening Analysis

4.8.1. Sample preparation and concentration

10 mg of sample was carefully weighed into a clean e-tube and was dissolved in 1 mL of DMSO. It was then further diluted with 9 mL of distilled water to make the concentration of 1000 ppm. Serial dilution was made for lower concentration needed for analysis. Lower concentrations of 10, 50, 100, 500, and 800 ppm were made from 1000 ppm. 96 well plates were used for cytotoxicity analysis with a volume capacity of 0.5 mL of each well. Each well was inoculated with 20 naupulis, after which each diluted sample (0.4 mL) was loaded in each well. Each samples were analysed in triplicate. The experiment was continued for a complete 24 hours with close observation for 8 hours. All the naupulis died after 24 hours of analysis. The provided samples were analyzed as per the above-mentioned procedure, and the results obtained are tabulated below.

Table 4.13: Brine shrimp lethality assay of hexane extract in different concentration

Sample	Concentration ppm	No. of alive naupuli after 24hrs			Mortality rate %
		T1	T2	T3	0
Hexane extract	0	20	20	20	0
	10	20	20	20	0
	50	20	20	20	0
	100	20	20	20	0
	500	20	20	20	0
	800	20	20	20	0
	1000	19	18	17	10
	LC_{50} >1000 µg/mL				

Since, only 10% mortality was found in 1000 ppm, after hours of sample analysis. LC_{50} was above **1000 ppm**. Hence the hexane extract was considered to be non toxic.

Table 4.14: Brine shrimp lethality assay of chloroform extract in different concentration

Sample	Concen. ppm	No. of alive naupuli after 24hrs			Mortality rate %
		T1	T2	T3	
Chloroform extract	0	20	20	20	0
	10	20	20	20	0
	50	20	20	20	0
	100	20	20	20	0
	500	18	19	17	10
	800	10	8	11	51
	1000	9	7	10	56
LC_{50}= 920 µg/mL					

Naupulis started dying at concentration of 500 ppm. 50% of the tested Naupulis died at the higest concentration of 800 ppm. Hence, LC_{50} of chloroform extract was **920 µg/mL.**

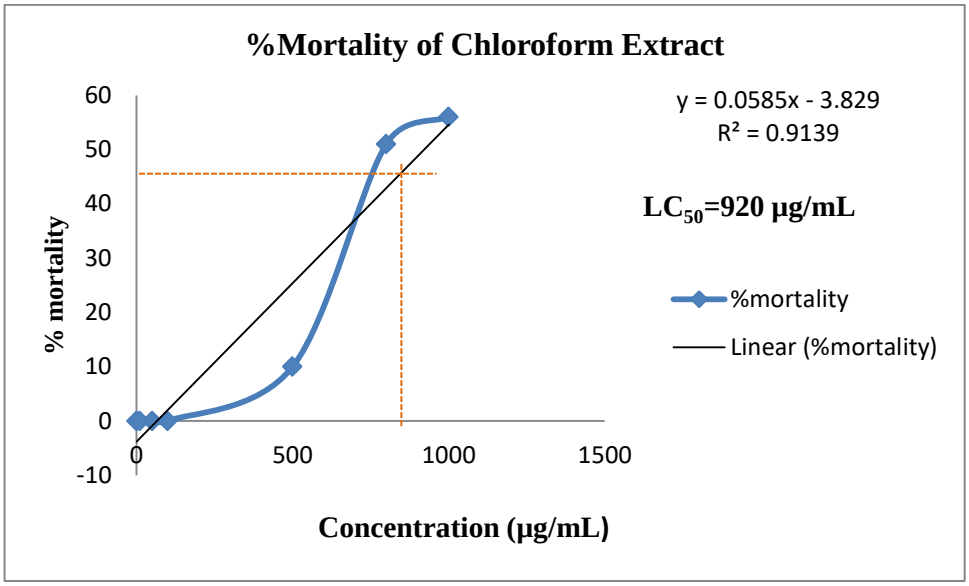


Figure 4.15: LC_{50} value of chloroform extract

Table 4.15: Brine shrimp lethality assay of Methanol extract in different concentrations

sample	Concentration ppm	No. of alive naupulis after 24 hrs			Mortality rate %
		T1	T2	T3	
Methanol extract	0	20	20	20	0
	10	20	20	20	0
	50	20	20	20	0
	100	20	20	20	0
	500	10	10	9	51
	800	9	8	8	58
	1000	2	2	3	88
LC_{50}= 601.965 µg/mL					

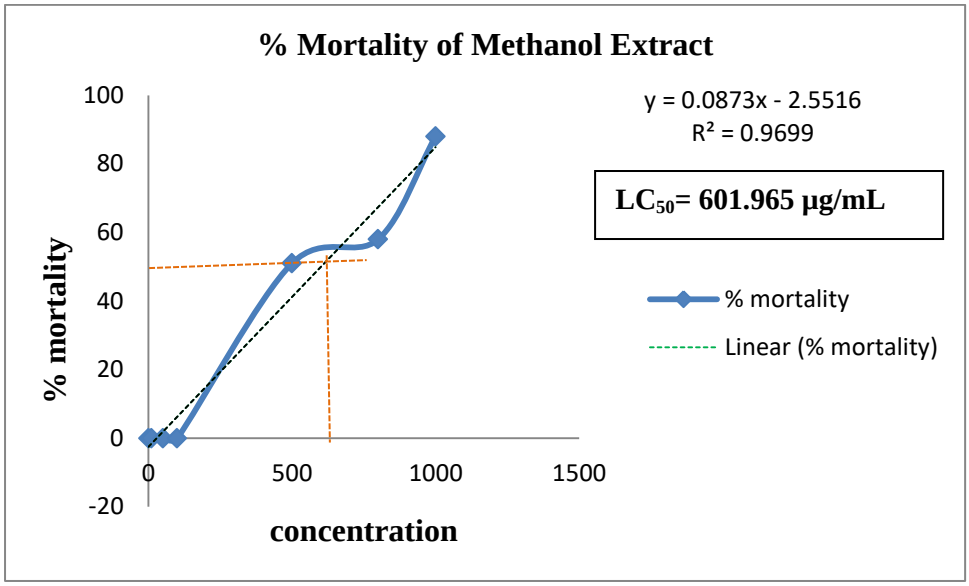


Figure 4.16: LC_{50} value of methanol extract

Naupulis started dying at a concentration of 500 ppm. 50% of the tested Naupulis died at the highest concentration of 500 ppm. Hence, LC_{50} of Methanol extract is **601.965 µg/mL.**

4.9. TLC Analysis

TLC analysis was performed on several extracts of *B. variegata* in hexane, chloroform, and methanol. There were seen more number of spots in different extract which further indicate the presence of large number of compound which can be separated by column chromatography.

Table 4.16: Rf value of different solvent systems such as hexane, chloroform, methanol leaf crude extract of *B. variegata*

Different extracts	Solvent system ratio(%)	Number of spots	Rf value
Hexane	Ethylacetate : hexane (20:80)	5	0.08, 0.2, 0.28, 0.44, 0.91
Chloroform	Acetone: hexane (30:70)	7	0.05, 0.14, 0.35, 0.39, 0.45, 0.48, 0.54
Chloroform	Acetone: hexane (35:65)	6	0.22, 0.46, 0.5, 0.53, 0.56, 0.59
Methanol	Acetone: hexane (35:65)	7	0.24, 0.44, 0.46, 0.5, 0.54, 0.57, 0.61
chloroform	Ethyl acetate: hexane	5	0.24, 0.28, 0.38, 0.48,

	(25:75)		0.52

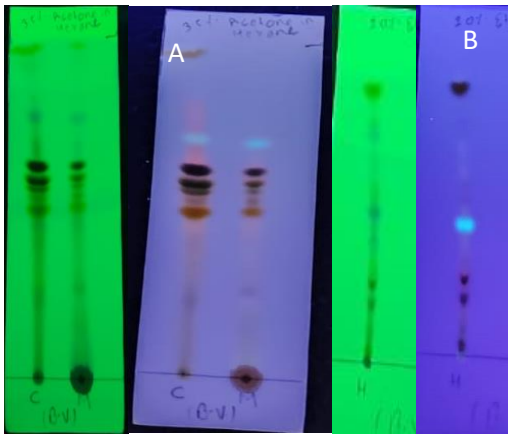


Figure 4.17: TLC plate of (A) hexane and methanol extract in 35% acetone in hexane and (B) hexane extract in 20% ethylacetate in hexane

4.10. FTIR analysis of *B. variegata*

Fourier transform infrared spectroscopy, was employed to determine the extract's distinctive functional groups. It offers structural information about molecules, which is often gleaned from their absorption spectra (Reddy *et al*., 2003).

4.10.1. FTIR analysis of hexane extract

The band at 3377 cm^{-1} is OH groups while at 2958 cm^{-1} might be of aliphatic CH_2, 2916 cm^{-1} and 2848 cm^{-1} (possibly connected to ethanol), 1735 cm^{-1} (primarily due to C=C and C=O vibrations), 1462 cm^{-1} (CH and aromatic vibrations), 1245 cm^{-1} (presumably related to polyol C-O), 1108 cm^{-1} (secondary alcohols and C-O-vibrations), and 955 cm^{-1}. Additional bands were observed in addition to those previously mentioned. A band at 1710 cm^{-1} could be related to the stretching vibration of carboxyl groups (Jain P *et al*., 2016).

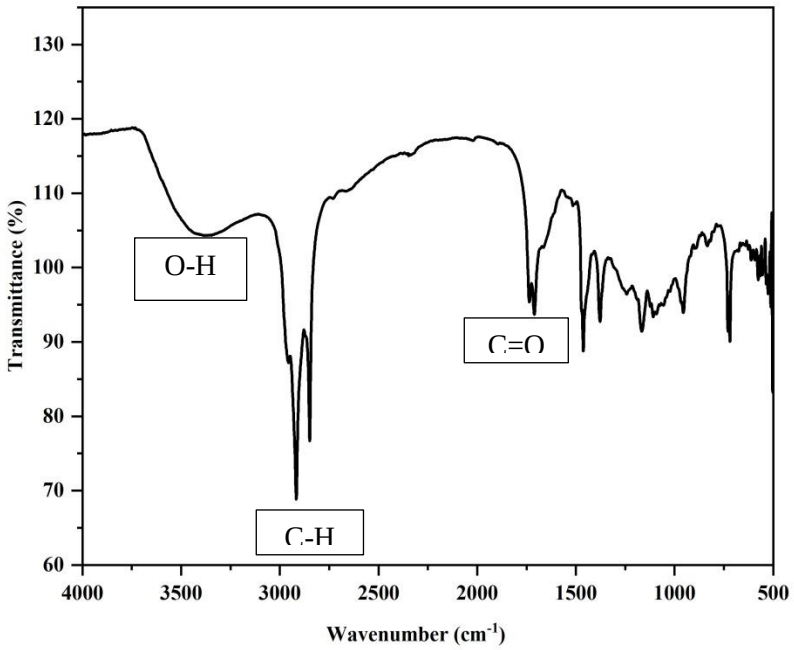


Figure 4.18: FTIR Peak of Hexane extract

Table 4.17: FTIR peak assignments of Hexane extract

wavelength(cm^{-1})	Functional group
3377.37	O-H stretch
2958	C-H stretch
2848.84	C-H stretch
1377.57	C-H bending
1245.7	C-O stretching
1108.08	C-O stretching

The sharp point on 3377.37cm^{-1} indicate the presence of OH functional group. The two medium absorption band at 2958 cm^{-1} and 2848.84 cm^{-1} specify the existance of C-H bond in aliphatic group. The broad peak at 1377.57 cm^{-1} indicated the appearance of C-H bond. The peak at 1108.08 cm^{-1} indicated the appearance of –C=O group.

4.10.2. FTIR analysis of chloroform extract

The amide II band and amide III band values were 540 cm^{-1} (N-H bends/C-N stretch) and 1240 cm^{-1} (C-N stretch/N-H bent), accordingly. Additionally, it was believed that protein side-chain COO- caused the peak near 1400 cm^{-1} and that N-H bending caused

the peak near 3300 cm^{-1}. The peak value at 2858cm^{-1} was supposed to be methyl group $–CH_3$. The band at 1724cm^{-1} is C=O carbonyl group (Shamran *et al*.,2020).

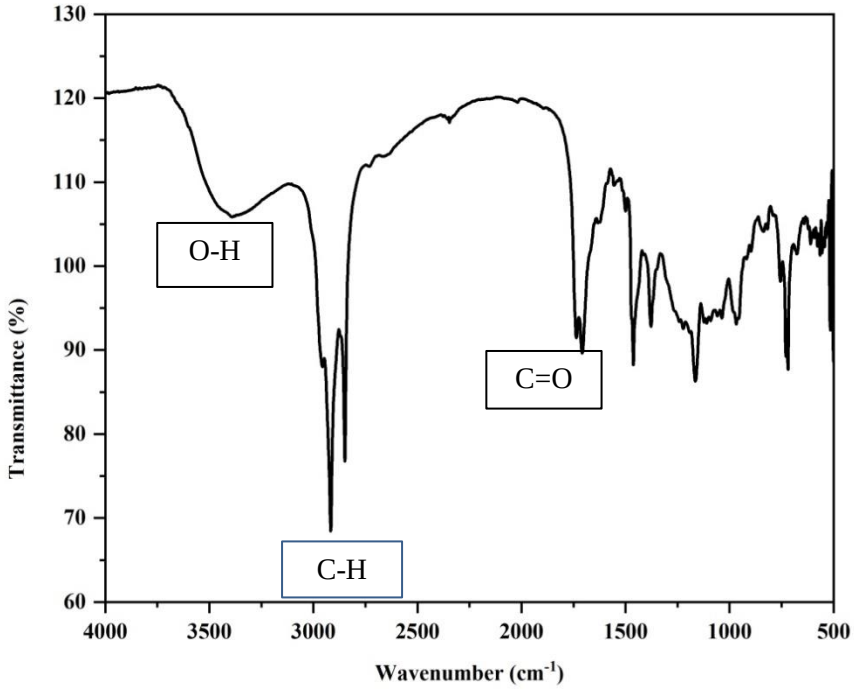


Figure 4.19: FTIR peak of chloroform extract

Table 4.18: FTIR peak assignments of chloroform extract

wavelength(cm^{-1})	Functional group
3391.44	O-H group
2960.4	C-H stretching
2916.65	C-H stretching
2848.86	C-H stretching
1708.13	C-O stretch
1462.53	C-H bending
1377.75	C-H bending
1038	C-O stretching

The presence of the OH functional group was indicated by the sharp point on 3391.44 cm^{-1}. The two medium absorption bands at 2960.4 cm^{-1} and 2916.65 cm^{-1} showed that an aliphatic group had a C-H bond. The presence of the C-H bond was indicated by the large peak at 1377.75 cm^{-1}. The existence of the –C=O group was indicated by the peak at 1038 cm^{-1}.

4.10.3. FTIR analysis of methanol extract

N-H stretching was detected by the FT-IR, which produced a broad peak at 3175.82 cm-1. It produced a prominent peak at 2864.8, 2963.65, and 2.730.26 cm-1, indicating the existence of C-H stretching. O-H bending was evident in the peaks measured at 1249.88, 1299.07, and 1461.57 cm -1. The presence of C-H Bend out of plane was suggested by the peak measured at 811.56 cm-1. The presence of C-C stretching was indicated by the peak seen at 890.16 cm^{-1}. The presence of silicates and nitrates was indicated by the peaks measured at 1377.19 and 984.19 cm^{-1}(Kalaichelvi & Dhivya, 2017).

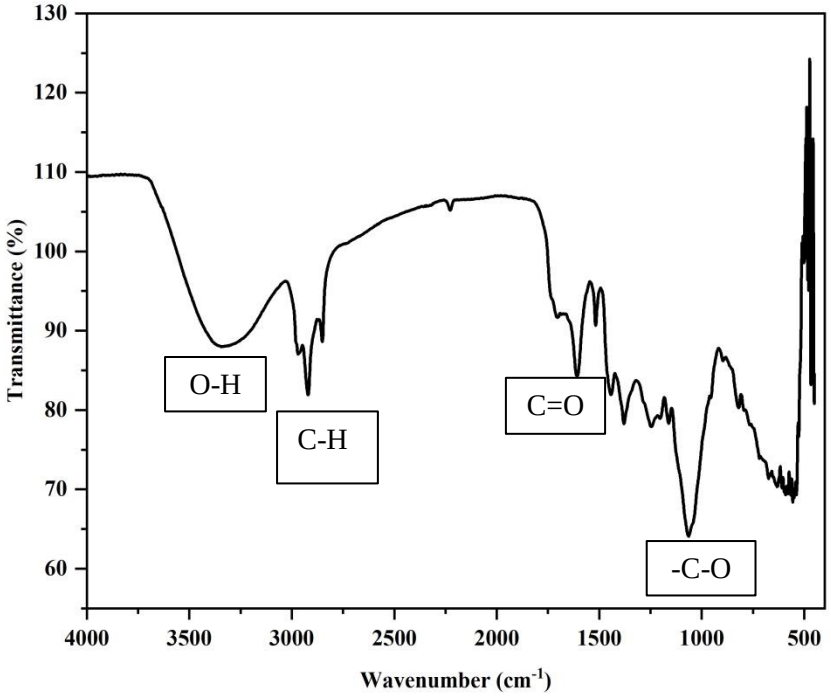


Figure 4.20: FTIR peak of methanol extract

Table 4.19: FTIR peak assignments of methanol extract

wavelength(cm^{-1})	Functional group
3344	OH stretching
2920	C-H stretching
2851	C-H stretching
1442.53	C-H bending
1379.19	C-H bending

818.82	C-H bending in aromatic ring
1063.80	C-O stretching

CHAPTER 5: CONCLUSIONS AND RECOMMENDATION

5.1. Conclusions

Plant extracts include a high concentration of secondary metabolites, according to phytochemistry research. The extraction process was carried out by ultrasonic extraction. Among different solvent extracts, the highest percentage yield was found in chloroform extract i.e. 1.46 %.The phytochemical screening analysis showed the different phytochemicals such as: flavonoids, alkaloids, saponins, phenolic compounds, resin, steroids, and tennins. Chloroform extract was initiate to contain high flavonoid compounds i.e. 116.97±1.76 mg GAE/g but the Methanol extract was found to contain high phenolic compounds i.e. 138.44±1.79 mg QE/g. When assessing the antibacterial activity of *B. variegata* leaf extracts , methanol extracts had great bacterial activity against *B. subtilis*, they demonstrated significant inhibitory activity against the growth of a Gram negative bacteria, *E. coli*. Compared to chloroform and hexane extract, the methanol extract of *B. variegata* exhibited antifungal efficacy against *C. albicans*. The antibacterial activity of the Gram-positive bacteria *Staphylococcus aureus* was more pronounced on methanol extract. The DPPH radical scavenging activities and IC_{50} of the plant extracts were found to exhibit different levels of antioxidant capability. The *B. variegata* methanol extract was shown to be the most effective natural antioxidant compared to the chloroform extract, as indicated by the IC_{50} values of the various extracts. The 3,5-dinitro salicylic acid (DNSA) technique was used to assess the extracts' antidiabetic properties. Strong antidiabetic effects were shown by the hexane and methanol extracts, while the methanol IC_{50} value of 3127.53 µg/mL indicated substantially low antidiabetic effects of the extract. Brine shrimp lethality assay revealed that the methanol extract was toxic then the chloroform and hexane extract. Hexane extract was found to be non toxic as the LC_{50} geater than 1000 µg/mL and methanol essance was found to be virulent as compared with hexane with LC_{50} value of 601.965 µg/mL. In hexane, chloroform, and methanol extract, TLC revealed 5, 7, and 7 spots respectively in 20% ethyl acetate and 30% acetone in hexane.The FTIR analysis revealed the existence of a considerable number of functional groups present in the plant extracts such as: C=O, C-H, OH etc.

5.2.Recommendation

The plants' phytochemical study revealed the existence of numerous physiologically active substances. These different secondary metabolites are which provide medicinal

plants their therapeutic qualities. To extract a greater diversity of phytochemicals in bigger levels, It is recommended to synthesize extracts from plants in other primary solvents using different extraction procedures than those described there. Due to the plant's great extraction capacity, a wide range of active ingredients for various biological activities can be produced; these elements could be utilized to create a potent medication. To extract unique active components from this plant, more investigation is required. This could result in a fresh approach to treating a range of illnesses. Furthermore, more investigation is required to separate and identify the bioactive substances found in *B. variegata.* LC-MS analysis can be used to identify the phytoconstituents included in the plant extracts, and column chromatography can be used to ease their pure extraction. Such pure chemicals' biological examination may result in the creation of innovative medications with potential uses in a variety of industries, such as biotechnology, healthcare, and medicine.

REFERENCES

Abascal, K., Ganora, L., & Yarnell, E. (2005). The Effect of Freeze-drying and its Implications for Botanical Medicine *: A Review*. *660*(December 2004), 655–660.

Abbasi, A. S., Najam-Us-Saqib, Q., Ur-Rehman, A., & Ur-Rahman, N. (2021). Phytochemical analysis and cytotoxicity evaluation of flowering buds of *Bauhinia variegata* L. *Herba Polonica*, *67*(3), 27–36. https://doi.org/10.2478/hepo-2021-0013

Abdel-Halim, A. H., Fyiad, A. A. A., Aboulthana, W. M., El-Sammad, N. M., Youssef, A. M., & Ali, M. M. (2020). Assessment of the anti-diabetic effect of *Bauhinia variegata* gold nano-extract against streptozotocin induced diabetes mellitus in rats. *Journal of Applied Pharmaceutical Science*, *10*(5), 77–91. https://doi.org/10.7324/JAPS.2020.10511

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols*, *2*(4), 875–877. https://doi.org/10.1038/nprot.2007.102

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *Analyst*, *127*(1), 183–198. https://doi.org/10.1039/b009171p

Banu, K. S., & Cathrine, L. (2015). *General Techniques Involved in Phytochemical Analysis*. *2*(4), 25–32.

Braz, R., Wolf, L. G., Lopes, G. C., & de Mello, J. C. P. (2012). Quality control and TLC profile data on selected plant species commonly found in the Brazilian market. *Revista Brasileira de Farmacognosia*, *22*(5), 1111–1118. https://doi.org/10.1590/S0102-695X2011005000204

Carballo, T., Gil, M. V., Gómez, X., González-Andrés, F., & Morán, A. (2008). Characterization of different compost extracts using Fourier-transform infrared spectroscopy (FTIR) and thermal analysis. *Biodegradation*, *19*(6), 815–830. https://doi.org/10.1007/s10532-008-9184-4

Dhivya, S. M., & Kalaichelvi, K. (2017). Uv-Vis Spectroscopic and Ftir Analysis of Sarcostemma. *International Journal of Current Pharmaceutical Research*, *9*(3),

46–49. https://doi.org/10.22159/ijcpr.2017v9i3.18890

Duniya, S. V., Ojonugwa, M. C., Adamu, A. D., John, O., Eleojo, S. I., & Salifu, U. O. (2018). *Phytochemical constituent , percentage yield and phenolic content estimation of different solvent system of Caricapapaya leave A R T I C L E I N F O International Journal of Chemistry and Phytochemical constituent , percentage yield and phenolic content e*. *January*.

Gunalan, G., Saraswathy, A., & Vijayalakshmi, K. (2012). HPTLC fingerprint profile of *Bauhinia variegata* Linn. leaves. *Asian Pacific Journal of Tropical Disease*, *2*(SUPPL.1). https://doi.org/10.1016/S2222-1808(12)60117-0

Hago, S., Mahrous, E. A., Moawad, M., Abdel-Wahab, S., & Abdel-Sattar, E. (2021). Evaluation of antidiabetic activity of *Morus nigra* L. and *Bauhinia variegata* L. leaves as Egyptian remedies used for the treatment of diabetes. *Natural Product Research*, *35*(5), 829–835. https://doi.org/10.1080/14786419.2019.1601094

Hu, X., Cheng, D., & Zhang, Z. (2016). Antidiabetic activity of Helicteres angustifolia root. *Pharmaceutical Biology*, *54*(6), 938–944. https://doi.org/10.3109/13880209.2015.1077871

Jain P, Soni A, Jain P, & Bhawsar J. (2016). Phytochemical analysis of mentha spicata plant extract using uv-vis, ftir and gc/ms technique. *Journal of Chemical and Pharmaceutical Research*, *March*, 1–6.

Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. *Journal of HerbMed Pharmacology*, *7*(1), 1–7. https://doi.org/10.15171/jhp.2018.01

Joshi, M. D., & Joshi, C. (2022). Areas of species diversity and endemicity of Nepal. *Ecosphere*, *13*(3), 1–13. https://doi.org/10.1002/ecs2.3969

Kalaichelvi, K., & Dhivya, S. M. (2017). Screening of phytoconstituents, UV-VIS Spectrum and FTIR analysis of Micrococca mercurialis (L.) Benth. *40 ~ International Journal of Herbal Medicine*, *5*(6), 40–44.

Kamal, Y., Khan, T., Haq, I., Zahra, S. S., Asim, M. H., Shahzadi, I., Mannan, A., & Fatima, N. (2022). Phytochemical and biological attributes of Bauhinia variegata L. (Caesalpiniaceae). *Brazilian Journal of Biology*, *82*, 1–10.

https://doi.org/10.1590/1519-6984.257990

Khare, P., Kishore, K., & Sharma, D. K. (2018). Historical aspects, Medicinal uses, Phytochemistry and Pharmacological review of Bauhinia variegata. *Asian Journal of Pharmacy and Pharmacology*, *4*(5), 546–562. https://doi.org/10.31024/ajpp.2018.4.5.3

Kulkarni, Y. A., & Laddha, A. P. (2020). *Bauhinia variegata* leaf extract: An effective management option for diabetic cardiomyopathy. *South African Journal of Botany*, *132*, 50–58. https://doi.org/10.1016/j.sajb.2020.04.021

Kumar, A. (2014). *1 * 1 ,. 1*(5), 279–287. https://doi.org/10.13040/IJPSR.0975-8232.IJP.1(5).279-87

Kumar, P., Bishnu, P., Academy, B., Republic, C., Kindlmann, P., & Globe, C. (2011). *An Overview of the Biodiversity in Nepal Metadata of the chapter that will be visualized online* (Issue February 2016). https://doi.org/10.1007/978-94-007-1802-9

Lamuela-Raventós, R. M. (2017). Folin-Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. *Measurement of Antioxidant Activity and Capacity: Recent Trends and Applications*, 107–115. https://doi.org/10.1002/9781119135388.ch6

Mali, R. G., Mahajan, S. G., & Mehta, A. A. (n.d.). Phcog Rev. : Plant Review Rakta Kanchan (*Bauhinia variegata*): Chemistry, Traditional and Medicinal uses-a review. In *Pharmacognosy Reviews* (Vol. 1). http://www.phcogrev.com

Mishra, A., Sharma, A. K., Kumar, S., Saxena, A. K., & Pandey, A. K. (2013). *Bauhinia variegata* leaf extracts exhibit considerable antibacterial, antioxidant, and anticancer activities. *BioMed Research International*, *2013*. https://doi.org/10.1155/2013/915436

Modh, K. M., Parmar, P. T., Panigrahi, B., Anand, I. S., & Patel, C. N. (2011). Pharmacognostical and phytochemical evaluation of leaves of *Bauhinia variegata* Linn. *Pharmacognosy Journal*, *3*(24), 45–49. https://doi.org/10.5530/pj.2011.24.9

More-Adate, P., Lokhande, K. B., Swamy, K. V., Nagar, S., & Baheti, A. (2022). GC-MS profiling of Bauhinia variegata major phytoconstituents with computational

identification of potential lead inhibitors of SARS-CoV-2 Mpro. *Computers in Biology and Medicine*, *147*(March), 105679. https://doi.org/10.1016/j.compbiomed.2022.105679

Olowa, L. F., & Nuñeza, O. M. (2013). *Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City , Philippines*. *2*(11), 74–77.

Rajani, G., & Ashok, P. (2009). In vitro antioxidant and antihyperlipidemic activities of Bauhinia variegata Linn. *Indian Journal of Pharmacology*, *41*(5), 227–232. https://doi.org/10.4103/0253-7613.58513

Rajbhandary, S. (2017). Proceedings of International Conference on ASIC. In *Proceedings of International Conference on ASIC* (Vols. 2017-Octob, Issue June).

Reddy, M. V. B., Reddy, M. K., Gunasekar, D., Caux, C., & Bodo, B. (2003). A flavanone and a dihydrodibenzoxepin from Bauhinia variegata. *Phytochemistry*, *64*(4), 879–882. https://doi.org/10.1016/S0031-9422(03)00416-3

Sahetal2003-GhodaghodiFloralDiversity.pdf.crdownload. (n.d.).

Sánchez-Rangel, J. C., Benavides, J., Heredia, J. B., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2013). The Folin-Ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination. *Analytical Methods*, *5*(21), 5990–5999. https://doi.org/10.1039/c3ay41125g

Shah, B. N., Shah, M. R., Modi, D. C., Sahu, D., Students, P. G., Correspondence, F., & Shah, M. R. (2010). *Newsletter PHYTO-PHARMACOLOGICAL PROFILE OF Newsletter Shah et al . Figure : Bauhinia variegata*. *837*, 829–837.

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods*, *18*, 757–781. https://doi.org/10.1016/j.jff.2015.01.047

Shamran, D. J., Fadel, E., Al-Jumaily, A., & Al-Jumaili, F. (n.d.). *Phytochemical Screening by HPLC and FTIR Spectroscopy of Glucokinin Isolated from Methanol Extract of Bauhinia Variegata* (Vol. 20, Issue 2). https://www.researchgate.net/publication/342625051

Sharma, N., Sharma, A., Bhatia, G., Landi, M., Brestic, M., Singh, B., Singh, J., Kaur,

S., & Bhardwaj, R. (2019). Isolation of phytochemicals from *Bauhinia variegata* L. Bark and their in vitro antioxidant and cytotoxic potential. *Antioxidants*, *8*(10), 1–19. https://doi.org/10.3390/antiox8100492

Shendge, A., Basu, T., & Mandal, N. (2021). Evaluation of anticancer activity of Clerodendrum viscosum leaves against breast carcinoma. *Indian Journal of Pharmacology*, *53*(5). https://doi.org/10.4103/ijp.IJP_565_19

Shraim, A. M., Ahmed, T. A., Rahman, M. M., & Hijji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *Lwt*, *150*(June), 111932. https://doi.org/10.1016/j.lwt.2021.111932

Sima, I. A., Sârbu, C., & Naşcu-Briciu, R. D. (2015). Use of TLC and UV–visible spectrometry for fingerprinting of dietary supplements. *Chromatographia*, *78*(13), 929–935. https://doi.org/10.1007/s10337-015-2884-0

Taia, W., Mahdy, R., Taia, W. K., & Bassiouni, E. M. (2022). *Pollen morphological study in some Bauhinia L. species and their phylogenetic indications*. www.botanyjournals.com

Waghulde, S., Kale, M. K., & Patil, V. (2020). *Brine Shrimp Lethality Assay of the Aqueous and Ethanolic Extracts of the Selected Species of Medicinal Plants*. *2*, 47. https://doi.org/10.3390/ecsoc-23-06703

APPENDICES

1. Test for Alkaloids

Three different experiment were carried out for alkaloid test.

Mayer's test: 1 ml of the filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Yellow color formation indicates the presence of alkaloids.

Dragendroff's test: 1ml of extract and 0.2ml dilute HCl was taken in testube. 1 ml of Dragendroff 's reagent was added and left for a few minutes. Orange-brown ppt. indicate the positive result.

Wagner's test: 3 drops of freshly prepared Wagner reagent and 1ml of extract were added to the test tube. Reddish brown ppt indicates the presence of alkaloids.

2. Test for flavonoids

Alkali reagent test: Extract was treated with a few drops of sodium hydroxide solution. The intense yellow color formation which on the addition of dil. acid becomes colorless indicates the presence of flavonoids.

Lead acetate test: Extracts were treated with a few drops of lead acetate solution. The formation of yellow color indicates the presence of flavonoids.

Shinoda test: A few pieces of magnesium were mixed with 3 ml extract followed by dropwise addition of concentrated HCl and boiled for 5 min. Megneta color indicate the presence of flavonoids.

3. Test for Steroid Compound

Salkowaski's test: A test tube containing 1 milliliter of plant extract was filled with 10 milliliters of chloroform, and then the test tube was topped off with an equivalent volume of concentrated sulfuric acid by sides. The test tube's top layer needs to be made red, and the presence of steroids should be shown by a yellow color with green fluorescence in the sulphuric acid layer.

4. Test for Quinones

Add a few drops of concentrated H_2SO_4 or aqueous NaOH solution to 2 ml of extract. When the quinoid component is present, color creation occurs.

5. Test for Saponins

Foam test: After diluting the extracts with 20 mL of distilled water, they were agitated for 15 minutes in a graduated cylinder. Saponins are present when a layer of foam forms, about 1 cm in thickness.

6. Test for tannins

$Fecl_3$ test: 1 ml of 5% ferric chloride solution was added to five ml of extract solution. The presence of tannins was suggested by the greenish-black coloring.

Potassium dichromate test: 1 ml of a 10% aqueous potassium dichromate solution was added to 5 ml of the extract. The development of a precipitate with a yellowish-brown color indicates the presence of tannins.

Lead acetate test: Five ml of extract were combined with a few drops of freshly produced 1% lead acetate. The yellow precipitate indicates a successful outcome.

7. Detection of carbohydrates

Benedict's test: Benedict's reagent was applied to the filtrates, and they were then gently heated. Orange-red precipitate suggests that reducing sugars are present.

Fehlings test: Filtrates were heated using Fehling's A & B solutions, neutralized with alkali, and hydrolyzed with diluted HCl. Red precipitate development is a sign that reducing sugars are present.

8. Detection of resins

Separately, 3–4 ml of $CuSO_4$ solution were added to 0.5 mL of extract, and the tubes were rapidly shaken for one to two minutes. It came down to let the resultant solution separate. The presence of resins was revealed by the production of a green precipitate.

9. Test for proteins

Xanthoproteic test: A few drops of concentrated nitric acid were applied to the extracts. The development of a yellow color signifies the existence of proteins.

10. Detection of amino acids

Ninhydrin test: Ninhydrin solution was added to the test solution, brought to boil, and the production of a violet color was monitored.

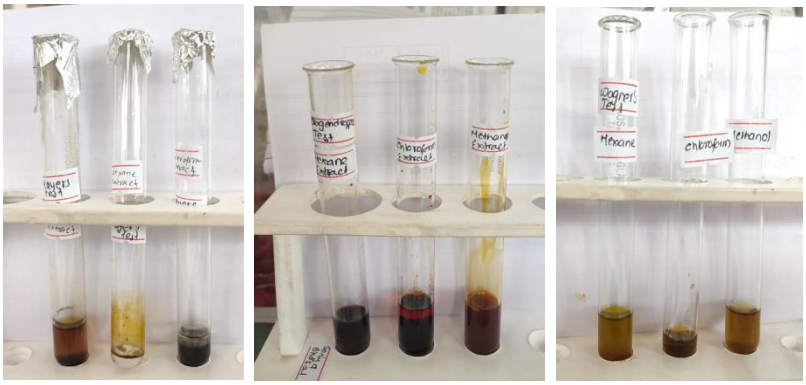
11. Test for phenols

Ferric chloride test: One percent ferric chloride solution and one percent potassium ferrocyanide were combined in equal parts. Three drops of this recently made mixture were added to two ml of extract. The development of a dark green or bluish-green coloration was interpreted favorably.

12. Test for cardiac Glycoside

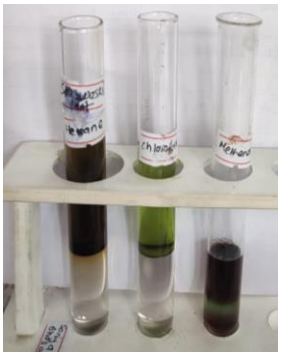
Killer-Killani test: After dissolving 1 mL of the extracts in 1 mL of glacial acetic acid and leaving it cool, add 2-3 drops of ferric chloride. Pour 2 mL of concentrated H2SO4 around the edges of the test tube into this solution. The presence of glycosides is indicated by the appearance of a reddish-brown ring at the intersection of two layers.

1. **Test for alkaloids**



2. **Test for flavonoids**

3. Test for steroids



4. Test for quinones

5.Test for quinones

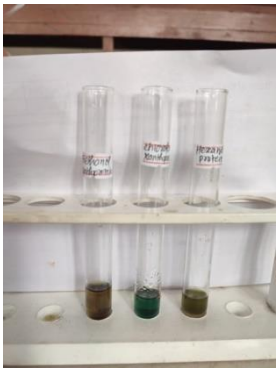
6. Test for saponins

7. Test for tannins

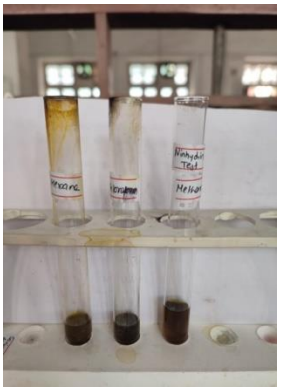
8. Detection of carbohydrates

9. Detection of resins

10.Test for proteins

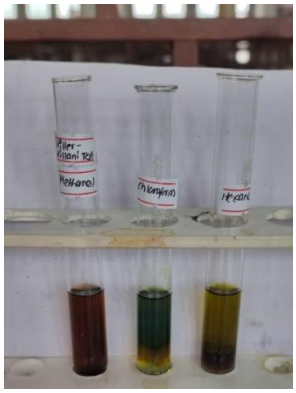


11. Detection of amino acids



1. 12. Test for phenol

13. Test for cardiac glycoside



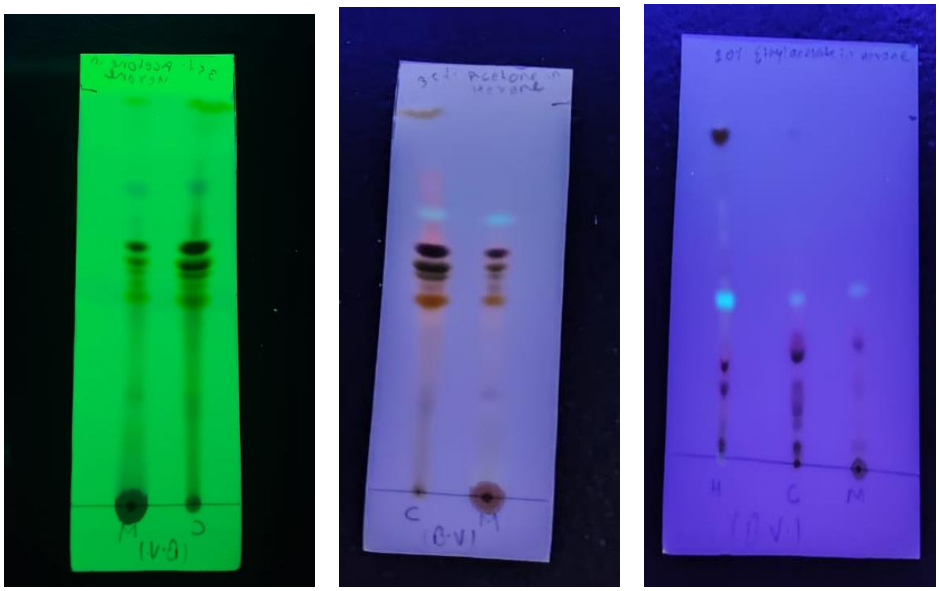


Fig: TLC of different extract


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Fig: Different activities during the process

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