ANTIOXIDANT AND BIOLOGICAL EVALUATION OF METHANOLIC, ETHYL ACETATE AND DICHLOROMETHANE EXTRACT OF *Zingiber officinale* COLLECTED FROM TWO DIFFERENT DISTRICTS OF NEPAL

A THESIS SUBMITTED TO THE

DEPARTMENT OF CHEMISTRY

BIRENDRA MULTIPLE CAMPUS

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

NEPAL

FOR THE PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE

IN CHEMISTRY

BY

PURNIMA BANJADE

EXAMS ROLL NO: CHE 1923/076

TU REGISTRATION NO: 5-2-49-199-2013

FEBRUARY 2025

DECLARATION

I declare that this dissertation entitled "**Antioxidant and Biological Evaluation of Methanolic, Ethyl Acetate and Dichloromethane Extract of *Zingiber officinale* Collected from Two Different Districts of Nepal**", are my own research work. This work has not been published or accepted and submitted for any degree award. Plagiarism checked at Birendra Multiple Campus Library also confirmed that the work is original and genuine.

Purnima Banjade

RECOMMENDATION

The dissertation entitles **"Antioxidant and Biological Evaluation of Methanolic, Ethyl Acetate and Dichloromethane Extract of *Zingiber officinale* Collected from Two Different Districts of Nepal"**, is submitted by **Mrs. Purnima Banjade** for the partial fulfilment of M.Sc. degree in Chemistry at Birendra Multiple Campus. The entire work is completed under our supervision. All the reports presented here are his finding. We confidently recommend this thesis for final evaluation.

.....

Ganga Raj Pokhrel, Ph.D.

Supervisor

(Associate Professor)

Birendra Multiple Campus

Tribhuvan University

Bharatpur, Chitwan,

Nepal

.....

Bodh Babu Bhattarai, Ph.D.

(Assistant Professor)

Co-supervisor

Birendra Multiple Campus

Tribhuvan University

Bharatpur, Chitwan,

Nepal

Feb 2025

FOREWORD

The thesis work "**Antioxidant and Biological Evaluation of Methanolic, Ethyl Acetate and Dichloromethane Extract of *Zingiber officinale* Collected from Two Different Districts of Nepal**", submitted by **Purnima Banjade** as a part of M.Sc. Coursework in Chemistry at Birendra Multiple Campus is carried out under my supervision. Any part of this thesis work has not been submitted for any other degree award.

.....

Ganga Raj Pokhrel, Ph.D.

Supervisor

(Associate Professor)

Birendra Multiple Campus

Tribhuvan University

Bharatpur, Chitwan,

Nepal

LETTER OF APPROVAL

Date: Feb, 2025

Tribhuvan University
BIRENDRA MULTIPLE CAMPUS
Bharatpur, Chitwan

On the recommendation of **Ganga Raj Pokhrel, Ph.D. (Assoc. Prof.)** and **Bodh Babu Bhattarai, Ph.D. (Asst. Prof.)**, this M.Sc. thesis submitted by **Purnima Banjade** entitled "**Antioxidant and Biological Evaluation of Methanolic, Ethyl Acetate and Dichloromethane Extract of *Zingiber officinale* Collected from Two Different Districts of Nepal**", is forwarded by Department of Chemistry, Birendra Multiple Campus (BMC) to the office of Dean, IOST, T.U.

.....

Head of the Department

Dept. of Chemistry, Birendra Multiple Campus, TU

Bharatpur, Chitwan, Nepal

BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL

Date: February 06, 2025

This dissertation entitled "**Antioxidant and Biological Evaluation of Methanolic, Ethyl Acetate and Dichloromethane Extract of *Zingiber officinale* Collected from Two Different Districts of Nepal**", by **Purnima Banajde**, under the supervision of ***Ganga Raj Pokhrel, Ph.D. (Assoc. Prof.)*** and ***Bodh Babu Bhattarai, Ph.D. (Asst. Prof.)***, Department of Chemistry, Tribhuvan University is submitted for the partial fulfillment of the Master of Science (M.Sc.) degree in Chemistry. It is importantly note that this work has not been previously presented to any other institution for pursue any degree.

.....
Head of Department
Ganga Raj Pokhrel, Ph.D

Department of Chemistry
Birendra Multiple Campus, Bharatpur, Nepal

.....
Supervisor
Ganga Raj Pokharel, Ph.D.
(Associate Professor)
Department of Chemistry
Birendra Multiple Campus, Bharatpur, Nepal

.....
Co-supervisor
Bodh Babu Bhattarai, Ph.D.
(Assistant Professor)
Department of Chemistry
Birendra Multiple Campus, Bharatpur, Nepal

.....
Internal Examiner
Dipendra Adhikari
(Assistant Professor)
Department of Chemistry
Birendra Multiple Campus, Bharatpur, Nepal

.....
External Examiner
Kedar Nath Ghimire, Ph.D.
(Professor)
Central Department of Chemistry
Tribhuvan University (T.U.), Kirtipur, Nepal

AKNOWLEDGMENTS

I would like to express my deepest gratitude to my supervisor, **Ganga Raj Pokhrel, Ph.D. (Assoc. Prof.)**, for his extraordinary support, guidance, and encouragement throughout the course of my dissertation. His insightful feedback, patience, and unwavering dedication were instrumental in shaping this work. Without his expertise and constant encouragement, this dissertation would not have been possible. I am truly grateful for the opportunity to learn under his mentorship and for the invaluable experience this journey has provided.

I wish to extend my heartfelt thanks to my co-supervisor **Bodh Babu Bhattarai, Ph.D. (Asst. Prof.)** for his extraordinary contribution to my thesis. His deep insights, constructive feedback, and constant encouragement have been invaluable to the development and completion of this work. His commitment to my research, attention to detail, and willingness to go the extra mile have greatly enriched my academic journey. I am immensely grateful for the time and effort he invested in guiding me, and for his unwavering support throughout this process.

My deepest appreciation goes out to the Campus Chief **Assoc. Prof. Dr. Prem Sagar Bhandari**, Central Department of Chemistry, Department of Chemistry, Birendra Multiple Campus and our Head of Department, **Ganga Raj Pokhrel, Ph.D. (Assoc. Prof.),** Department of Microbiology and Head of Department, Mrs. Pavitra Shrestha Madam, Lab in charge **Mr. Santa Prasad Dhungana**, my seniors **Mr. Ajaya Mahato**, **Miss Ranjana Khanal, Mr. Bipin Khanal, Mr. Rabi Bhatta** Chemicals and equipment suppliers, those who provided me plant samples for their invaluable assistance with my dissertation. My academic career has been greatly aided by the department's resources, advice, and encouragement. I would like to express my deepest gratitude to Zest Laboratory, the center for natural and applied sciences.

First and foremost, I would like to express my heartfelt gratitude to my parents, for encouraging me throughout this journey.

Purnima Banjade

Feb, 2025

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

$^{\circ}C$	Degree Celsius
µg/mL	Microgram per milliliter
µL	Microliter
ABTS	2,2-azino-bis (3-ethylbenzothiazoline-6-sulonic acid)
ATCC	American Type Culture Collection
BHT	Butylated Hydroxy Toluene
BSLT	Brine Shrimp Lethality Test
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
DPPH	2, 2-diphenyl-1-picryl-1-hydrazyl
F-C	Folin-Ciocalteu
g	Gram
GAE	Gallic Acid Equivalent
GC-FTD	Gas Chromatography Flame Ionization Detector
GC-MS	Gas Chromatography/Mass Spectroscopy
IC	Inhibition Concentration
IC_{50}	Inhibitory Concentration 50
LC	Lethal Concentration
MeOH	Methanol
mg	Milligram
N/M	Normality/Molarity
nm	Nanometer
ppm	part per million
QE	Quercetin Equivalent
RNS	Reactive Nitrogen Sulphate

ROS	Reactive Oxygen Species
TBARS	Thiobarbituric Acid Reactive Substances
TFC	Total Flavonoids Content
TPC	Total Phenolic Content
UV	Ultraviolet
ZOI	Zone of Inhibition

ABSTRACT

Antioxidant and biological evaluation of methanolic, ethyl acetate, and dichloromethane extract of *Zingiber officinale* collected from two different districts of Nepal is not clearly understood yet. This study investigates these properties in ginger extracts from Arghakhanchi and Dang districts. The ginger samples were gathered, dried, and extracted using the cold percolation method with three solvents: methanol (MeOH), dichloromethane (DCM), and ethyl acetate (EtOAc). Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity were measured via DPPH assay, antimicrobial activity, and toxicity using a brine shrimp bioassay. The methanolic extract from Dang district (S2(MeOH)) showed the highest TPC (123.02 ± 2.84 mg GAE/g) and TFC (25.42 ± 1.69 mg QE/g), with strong antioxidant activity (IC50 = 162.73 ± 3.22 µg/mL). The dichloromethane extract from Arghakhanchi (S1(DCM)) had the lowest TPC (36.23 ± 3.45 mg GAE/g) and TFC (14.32 ± 1.74 mg QE/g), with weaker antioxidant activity (IC50 = 279.63 ± 14.67 µg/mL). The ethyl acetate extract from Dang (S2(EtOAc)) showed the best antimicrobial activity, especially against *S. aureus* and *P. aeruginosa*. The methanolic extract demonstrated strong antifungal properties. Brine shrimp bioassay results indicated higher toxicity in the methanolic extract (LC50 = 3.0×10^3 µg/mL). Ginger from Dang district showed the most promising antioxidant and antimicrobial properties. Further research on active compounds is recommended for potential medicinal use.

Keywords: *Antimicrobial, Antioxidant, Ginger (Zingiber officinale), Total Flavonoid Content, Total Phenolic Content.*

सारांश

नेपालका अर्घाखाँची र दाङ जिल्लाबाट सङ्कलित *Zingiber officinale* का मेथानोल (MeOH), डाइक्लोरोमिथेन (DCM), र इथाइल एसेटेट (EtOAc) एक्स्ट्र्याक्टहरूको एन्टिअक्सिडेन्ट र जैविक मूल्याङ्कन गरिएको छ। अदुवाका नमूनाहरू सङ्कलन गरी सुकाइएपछि चिसो परकोलेसन विधिबाट एक्स्ट्र्याक्ट गरियो। कुल फिनोलिक कन्टेन्ट (Total Phenolic Content, TPC), कुल फ्लेभोनोइड कन्टेन्ट (Total Flavonoid Content, TFC), एन्टिअक्सिडेन्ट (DPPH विधि), एन्टिमाइक्रोबियल गतिविधि, र ब्राइन श्रिम्प बायोएस्से परीक्षण गरिएको थियो। दाङ जिल्लाको मेथानोलिक एक्स्ट्र्याक्ट (S2(MeOH)) मा उच्च TPC (१२३.०२ ± २.८४ mg GAE/g) र TFC (२५.४२ ± १.६९ mg QE/g) पाइएर उत्कृष्ट एन्टिअक्सिडेन्ट गतिविधि (IC_{50} = १६२.७३ ± ३.२२ μg/mL) देखियो, जबकि अर्घाखाँचीको DCM एक्स्ट्र्याक्ट (S1(DCM)) ले न्यूनतम TPC (३६.२३ ± ३.४५ mg GAE/g) र TFC (१४.३२ ± १.७४ mg QE/g) सहित कमजोर एन्टिअक्सिडेन्ट गतिविधि (IC_{50} = २७९.६३ ± १४.६७ μg/mL) देखायो। दाङ जिल्लाको इथाइल एसेटेट एक्स्ट्र्याक्ट (S2(EtOAc)) ले *Staphylococcus aureus* र *Pseudomonas aeruginosa* बिरुद्ध सबभन्दा राम्रो एन्टिमाइक्रोबियल गतिविधि देखायो, भने मेथानोलिक एक्स्ट्र्याक्टले उत्कृष्ट एन्टिफङ्गल गुण देखायो। विषाक्तता परीक्षणमा, मेथानोलिक एक्स्ट्र्याक्ट (LC_{50} = ३.० × $१०^{३}$ μg/mL) सबभन्दा उच्च विषाक्त देखियो। यी नतिजाहरूले दाङ जिल्लाको अदुवामा उच्च औषधीय सम्भावना रहेको संकेत गर्छन्, जसलाई थप अनुसन्धान गरी औषधीय उपयोगताका लागि विकास गर्न सकिन्छ।

कीवर्डहरू: एन्टिअक्सिडेन्ट, एन्टिमाइक्रोबियल, कुल फिनोलिक कन्टेन्ट, कुल फ्लेभोनोइड कन्टेन्ट, अदुवा (*Zingiber officinale*)।

CHAPTER 1: INTRODUCTION

1.1 Background

Fossil evidence shows that humans have used plants and other natural resources for medicinal purposes for at least 60,000 years, (QingWen et al., 2010).

Interest in natural products and their chemistry has recently surged, driven by their impact across scientific, technological, and economic fields. Previously, interest declined due to factors such as low prioritization, limited access to advanced tools and techniques, lack of modern analytical methods, insufficient knowledge in academic and industrial sectors, and inadequate funding for research and development, all of which stifled innovation, (Khan, 2018).

Natural products, shaped by extensive evolutionary processes, offer remarkable structural diversity and distinct biological activities, making them essential in medicine as the foundation for many FDA-approved drugs and widely used in food additives, textiles, plastics, health products, and energy sources, and they can be classified based on their biological function, biosynthetic pathway, or origin, (Abozenadah et al., 2017).

Natural products (NPs), sourced from various organisms like microbes, marine life, animals, fungi, and plants, offer exceptional chemical diversity beyond that of synthetic compounds, making them crucial for discovering new drugs and lead compounds due to their unique biological activities and drug-like characteristics, (Atanasov et al., 2021).

Following the discovery of streptomycin by Selman Waksman in the 1940s, significant natural product discovery efforts surged in pharmaceutical companies across the U.S., Europe, and Japan, with notable academic contributions from labs like those of Satoshi Ōmura, Hamao Umezawa, Hans Zähner, and Hans Reichenbach, who together identified over 1,000 novel natural products (Katz & Baltz, 2016). Mesopotamian clay tablets from 2600 BC list 1,000 plant-based remedies, including oils from Cypressus sempervirens and Commiphora species, still used for colds, coughs, and inflammation. (Bernardini et al., 2017).

Egyptian medicine from around 2900 BC is well-documented in a notable text from 1500 BC, which details over 700 remedies, primarily plants but also including some animal parts and minerals, used in various forms such as poultices, gargles, and pills,

often combined with beer, milk, wine, and honey, (Newman et al., 2000). In the Western world, the knowledge about plant-based therapeutics is primarily influenced by Greek and Roman cultures (Mukherjee et al., 2010).

In 1929, Fleming published his research findings on penicillin, (Fleming, 1929) a broad-spectrum antibiotic that is widely used to treat infections caused by gram-positive cocci and rods, most anaerobes, and gram-negative cocci, (Wood et al., 1994). From this discovery, scientific research was mainly focused on the isolation of natural products from microbial sources, (J & D, 2010). Following World War II, scientists directed their research towards discovering new antibiotic molecules, which not only gave rise to the modern pharmaceutical industry but also led to the identification of key antibiotics such as streptomycin, tetracycline, and gentamicin, prompting pharmaceutical companies to significantly enhance and expand their research programs with a strong emphasis on the discovery of natural products and the advancement of microbial fermentation technologies, (Baker et al., 2007).

The ability to synthesize the natural compounds and their structural analysis compounds and enabled chemists to alter them to enhance, (Ji et al., 2009).

In the present, many chemists and biologists believe that the biological effects of natural compounds result from long-term co-evolution, where interacting organisms developed compounds to influence each other's biology. These compounds were retained and improved through natural selection, and due to similarities in physiology, they also affect humans, (Newman, 2008).

In developing countries like Nepal, traditional medicine is essential for healthcare, particularly in rural areas where government facilities are limited. With 80% of Nepal's 28 million people residing in such areas, access to medical care is challenging. The country has only 2 physicians per 10,000 people, in contrast to Europe’s average of 33 per 10,000, ranging from 19 in Romania to 54 in Greece. The 2015 earthquake exacerbated these challenges by further disrupting medical access and increasing the prevalence of epidemics, particularly among children and the elderly, leading rural populations to rely heavily on the use of medicinal plants to meet basic health needs., (Ji et al., 2009).

Traditional knowledge of medicinal plants is passed down orally within families or small healer groups, encompassing folk, shamanistic, and Ayurvedic medicine. Nepal,

with its diverse altitude, topography, and climate, hosts 6,500 plant species, 2,000 of which are used in traditional healing. The country's 125 ethnic groups each have their own unique cultural practices and knowledge of medicinal plants, (Ambu et al., 2020).

Zingiber officinale, commonly referred to as ginger is a tropical perennial plant from the Zingiberaceae family, often cultivated annually. While the entire plant has a pleasant aroma, it is the underground rhizome, whether raw or processed, that is primarily valued as a spice (*Handbook of Herbs and Spices | ScienceDirect*, 2012) These Zingiberene plants possess potent aromatic and curative qualities and are distinguished by their tuberous or non-tuberous rhizomes, (Chen et al., 2007). The crop thrives in temperatures between 19°C and 28°C and requires humidity levels of 70% to 90%, (Adhikari & Bhandari, 2022).

In Nepal, ginger is cultivated across 22,132 hectares of land, yielding a total production of 284,427,000 kg (*Agriculture Information and Training Center*, 2020) with the involvement of 1.2 million people, which represents approximately 4.3% of the Nepali population, (Joshi & Khanal, 2021).

The Agriculture Development Strategy (2015–2035), which outlines the long-term vision for Nepal's agriculture sector, has emphasized the importance of developing the ginger sub-sector's value chain, (Agriculture Development Strategy (ADS) | Ministry of Agriculture and Livestock Development, 2072).

Nepal is the world's second-largest producer of ginger and ranks fourth globally in ginger production. Approximately 400,000 farmers are directly or indirectly involved in its cultivation. Additionally, Nepal is the fourth-largest exporter of ginger worldwide, (Aryal et al., 2016).

Grown-up ginger roots are tough, fibrous, and almost dry and can be used in a variety of dishes. They can be boiled to make ginger tea, often sweetened with honey and sometimes enhanced with orange or lemon slices. The juice from ginger roots is highly effective as a spice for flavoring seafood, mutton, snacks, and stews. Ground dried ginger, or ginger powder, is often used to bring a warm, spicy flavor to gingerbread and various dishes. It's also commonly found in sweets and used to flavor cookies, crackers, and cakes, and carbonated non-alcoholic beverages. Ginger (*Zingiber officinale*) is rich in essential compounds, including phenolic substances like gingerols, shogaols, and paradols, (Bhattarai et al., 2018).

Ginger is recognized for its anti-aging properties, helping to guard against oxidative stress and inflammation associated with degenerative diseases and aging. Although primarily used as a spice or flavoring in cooking, Z. officinale is also valued as a herbal medicine for treating various health issues, including diabetes, nausea, and migraines. Additionally, ginger acts as a mild chemical irritant with sialagogue effects, promoting saliva production, (Mohd Sahardi & Makpol, 2019).

Free radicals generated during oxidation play a key role in triggering various long-term illnesses, including cardiovascular diseases, diabetes, inflammatory conditions, Alzheimer's disease, rheumatoid arthritis, and cancer, (Aher & Wahi, 2011). Antioxidants are necessary for the prevention of oxidative damage cells, preservation of food from being oxidation. Secondary metabolites obtained from plants like phenolic compounds, alkaloids, flavonoids can delay or inhibit the initiation step of lipid peroxidation, (Al-Fatimi et al., 2007).

The DPPH (2,2-diphenyl-1-picrylhydrazine-hydrate) free radical scavenging assay is a widely used and reliable technique for assessing the antioxidant properties of plant extracts and secondary metabolites. This approach relies on the capability of antioxidants to donate electrons or hydrogen atoms, effectively neutralizing the free radical nature of DPPH. The antioxidant activity is conveniently quantified using a spectrophotometer by measuring changes in absorbance at a specific wavelength, (Najafian et al., 2016).

DPPH solutions typically exhibit a sharp absorbance at 517 nm, producing a deep violet color due to presence of an unpaired electron. When an antioxidant donates a hydrogen atom to the DPPH molecule, it transforms into DPPH-H, resulting in the loss of both the violet color and the paramagnetic properties. Standard antioxidants, such as cysteine and ascorbic acid, are commonly employed to assess the antioxidant activity, (Halliwell, 2007).

Quercetin was used as standard DPPH free radical scavenger. Quercetin is a flavonoid class of compounds with excellent free radical scavenging properties.

Phenolic acids, typically present as esters, glycosides, or amides, are produced by plants in response to stresses such as pests, UV radiation, and physical damage, influencing their aroma, with differences in number and placement of hydroxyl groups on the aromatic ring. (Quideau et al., 2011). This are classified into hydroxycinnamic acids

and hydroxybenzoic acids, are vital for plant cell walls as they play a key role in shielding plants from stressors like infections, physical damage, and UV radiation (Bhat & Jacobs, 1995).

The phenolic hydroxyl group acts as an effective hydrogen donor, enabling H-donating antioxidants to interact with ROS and reactive nitrogen species RNS in termination reactions that interrupt cycle of new radical formation, and following this interaction, the antioxidants produce a radical form that is significantly more chemically stable than the original radical; this stability arises from the connection of the phenolic hydroxyl groups with the π-electrons of the benzene ring, which imparts the ability to generate free radicals that are stabilized by delocalization, thereby modifying the radical-mediated oxidation process (Parr & Bolwell, 2000).

Phenolic compounds act as antioxidants by chelating transition metals like iron and copper, but they can also act as pro-oxidants by enhancing metal catalytic activity or reducing metals, increasing free radical production (Parr & Bolwell, 2000).

Flavonoids in food enhance color and flavor, prevent fat oxidation, and protect vitamins and enzymes; their strong antioxidant properties, free radical-scavenging abilities, and potential health benefits, including reducing inflammation, protecting the liver, and combating diseases like heart disease and cancer, have led to significant interest in their role in managing oxidative stress and disease prevention (Kumar & Pandey, 2013).

An antimicrobial substance, whether of synthetic, natural, or semi-synthetic origin, that kills the growth of microorganisms while causing minimal harm to the host. Antibiotics are crucial in combating microbial infections and have greatly benefited human health. However, overuse of antibiotics can reduce their effectiveness, leading to the development of resistance and potentially causing toxic reactions (Bhalodia & Shukla, 2011). Between 1981 and 2002, approximately 61% of new drugs developed were derived from natural products, demonstrating significant success in treating infectious diseases and cancer. This highlights the importance of natural compounds in pharmaceutical development and their potential therapeutic benefits, (Newman, D. J. 2008).

A significant number of researchers across various regions of the world are exploring natural products as a new source of antimicrobial agents, particularly those that may possess novel mechanisms of action, (Reddy et al., 2001). Plants contain a diverse range

of secondary metabolites, including tannins, glucosides, alkaloids, and terpenoids, which have been shown to exhibit antimicrobial activity in in-vitro studies (Gupta et al., 2010), (Cowan, 1999).

The initial stages of biofilm formation are driven by bacterial attachment, (Yasuyuki et al., 2010).

The food industry and hospitals have become key areas for the application of antibacterial materials helps prevent the damage and degradation of substances (Kumar & Anand, 1998), (Mangram et al., 1999). Ginger oil, extracted from the root using CO2 supercritical extraction, has demonstrated antibacterial activity against the gram-positive bacterium *Pseudomonas aeruginosa* and the gram-negative bacteria *Salmonella typhimurium* and *Shigella flexneri* (Hadi & Hameed, 2017). Both in vitro and in vivo toxicity tests are crucial for the preliminary evaluation of plant toxicity, with the brine shrimp lethality test (BSLT) being commonly used to assess the cytotoxic effects of various plant-derived products, (Mayorga et al., 2010).

This method employs brine shrimp as a straightforward bioassay in natural product research. It measures LC50 values (in µg/mL) of active compounds and extracts within a brine solution. The activity of various known active compounds is indicated by their toxicity to the shrimp, which has been extensively studied among Artemia species. (Campbell et al., 1994).

Numerous studies have demonstrated a strong correlation between the LC50 values obtained in the BSLT using A. salina and the results of oral acute toxicity tests in mice. BSLT is a simple, cost-effective, and fast method. Although observing can be extended up to 60 hours, results can typically be calculated after 24 hours of exposure, requiring only a small quantity of test material (Omeke et al., 2018).

1.2 Justification of the study

Many medicinal plants remain scientifically unexplored despite their rich content of bioactive compounds like terpenes, polyphenols, flavonoids, glycosides, and alkaloids, which play crucial roles in neutralizing free radicals and inhibiting hydrolytic and oxidative enzymes. With a global shift from synthetic drugs to herbal remedies as a natural approach to disease prevention, Nepal's diverse climate, geology, and biodiversity offer a vast reservoir of secondary metabolites with therapeutic potential. While synthetic drugs are effective in addressing antimicrobial and antioxidant

challenges, their harmful side effects highlight the need for safer, cost-effective alternatives. Natural products derived from Nepal's biodiversity present a promising avenue for discovering novel compounds and developing new therapeutic agents.

1.3 Scope of the study

This study highlights the significance of ginger and emphasizes the need for its conservation.

This study could further highlight the medicinal value of ginger and potentially assist local communities in enhancing their economic growth.

This study may provide scientific validation of ginger, contributing to the development of new therapeutic agents.

1.4 Objectives

1.4.1 General Objectives:

- To evaluate the biological activities of methanolic, ethyl acetate, and dichloromethane (DCM) extracts of Zingiber officinale from two different districts of Nepal.

1.4.2 Specific Objectives:

- To perform the phytochemical analysis of the secondary metabolites in ginger extract sourced from the Arghakhanchi and Dang districts.
- To estimate the TPC of ginger extracts.
- To analyze the TFC of ginger extracts.
- To determine the antioxidant property (DPPH assay) of ginger extracts.
- To examine the antibacterial and antifungal activity of ginger extracts and
- To evaluate the toxicity tests of ginger extracts (Brine Shrimp Toxicity).

CHAPTER 2: LITERATURE REVIEW

The research conducted on various Zinger species globally is based on information gathered from journal articles, books, and reputable websites such as Sci-Hub, Google, Google Scholar, and JSTOR, and is thoroughly discussed.

Traditional medicine remains the most affordable and accessible form of treatment within primary healthcare systems, with medicinal plants serving as its foundation. These plants have consistently proven to be a valuable resource for treating various diseases, whether through traditional practices or as pure active compounds derived from their crude extracts. In developing nations, synthetic drugs are often inaccessible and prohibitively expensive for disadvantaged populations. Consequently, drugs derived from natural products present a promising alternative for future disease treatment. Moreover, some synthetic inhibitory drugs have been associated with adverse effects and a range of undesirable side effects (Fatemeh Jamshidi-Kia et al., 2018), (Bhattacharjee et al., 2017), (Yuan et al., 2016).

Nepal's diverse plant species can be attributed to its remarkable altitudinal range, spanning from 60 meters to 8,848 meters, along with its varied topography and distinct climatic zones, making it a recognized biodiversity hotspot. The geo-climatic diversity of the Nepalese Himalayas has established the region as a significant repository of traditional medicinal plants, boasting a rich array of ethnomedicinal properties (Bhattacharjee et al., 2017).

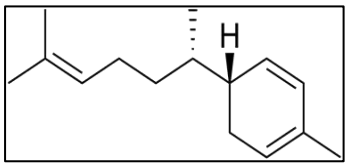
Bhattarai et al. (2018) studied the chemical components of essential oils and conducted biological assays on crude ethanolic extracts of ginger collected from the Sindhupalchowk, Tanahu, and Gorkha regions of Nepal. Using hydrodistillation, they identified monoterpenes and sesquiterpene derivatives as the primary constituents.

Malu et al. (2009) evaluated the antibacterial and bacterial growth inhibition activities of ginger extracts and found that all extracts, except the water extract, exhibited antibacterial properties. The inhibition of bacterial growth was found to be dose dependent.

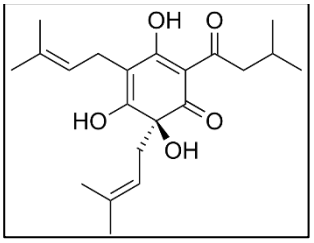
Freire et al. (2017) the antifungal activity of ginger was studied by testing different concentrations—1 g, 2 g, and 4 g of shuntichurna dissolved in 99.9% ethanol. The results showed that ginger paste at room temperature had a larger inhibition zone than

ethanol alone, while the cold ethanolic ginger extract exhibited the strongest inhibition zone after 24 hours.

Kamaliroosta et al. (2013) extracted ginger essential oil using a Clevenger apparatus and identified zingiberene, a sesquiterpene, as the dominant compound in the oily fraction.



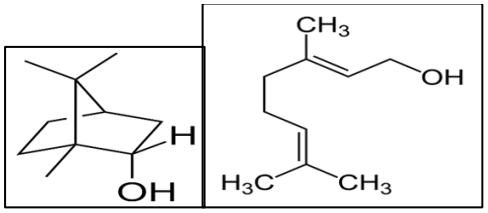
Zingiberene



Sesquiterpene

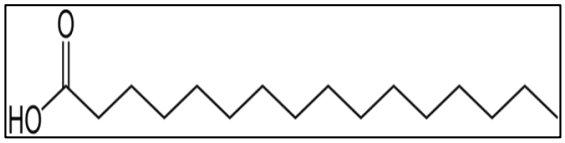
Shukla et al. (2018) analyzes the physical, nutritional, and antioxidant properties of nine northeast Indian ginger varieties, finding that the Sungro-sung variety from Nagaland has the highest levels of nutrients and antioxidants, with smaller rhizomes exhibiting greater antioxidant content, offering valuable insights for commercial production and future development of improved ginger varieties.

Fitriady et al. (2017) performed steam distillation to extract ginger essential oil and investigated the impact of steam flow rate and processing time. They found that linalool, borneol, and geraniol were extracted early in the process, while xanthorrhizol and n-hexadecanoic acid were extracted later, near the end of the process.



Borneol

Geraniol



n-hexadecanoic acid

Stoilova et al. (2007) studied ginger extract's antioxidant effects and phenol content. The DPPH radical scavenging activity was 90.1%, surpassing BHT with an IC50 of 0.64 g/mL.

Kanade et al. (2016) compared different ginger extraction methods, including ultrasound-assisted extraction, Soxhlet extraction, and autoclave agitator, at an optimal temperature of 80°C for 60 minutes. Their findings suggested that Soxhlet extraction

was the most effective technique due to its high recovery, simplicity, thermal stability, low energy requirements, and environmental friendliness.

Tohma (2016) demonstrated that ginger extracts possess strong antioxidant properties, which can help prevent or control the development on diseases triggered by oxidative stress resulting from inadequate antioxidant consumption.

Masuda et al. (2004The antioxidant activity of *Zingiber officinale* extract, which contained 181.41 mg GAE/g of polyphenols and 7.8% flavonoids (14.15 mg quercetin/g), was evaluated, with NMR analysis revealing phenolic ketones as the main components, and the extract showed stronger DPPH radical scavenging activity than ABTS cation radicals using both spectrophotometric methods.

Zancan et al. (2002) studied the impact of pressure, temperature, and co-solvents (ethanol and isopropyl alcohol) on the kinetics of ginger oleoresin extraction using GC-MS and GC-FID. They found that temperature, pressure, and solvent significantly influenced the levels of zingiberene, gingerols, and shogaols in the extracts.

Kamaliroosta et al. (2013) extracted ginger essential oil using a Clevenger apparatus and identified the TPC responsible for flavor, preservation, and antioxidant activities. Their findings revealed that zingiberene, a sesquiterpene, was the predominant compound in the oily fraction.

Nadeen Waleed Al-Areer et al. (2023) employed HPLC to examine phenolic and flavonoid compounds in fresh and powdered ginger extracts, revealing notable differences, with crude powdered extracts demonstrating stronger anticancer effects on HT-29, highlighting the need for further research to establish safe dosages for CRC therapy.

Great Iruoghene Edo et al. (2024) Zingiber officinale showed significant antibacterial activity, with the chloroform, ethanol, and n-hexane extracts, particularly the ethanol extract at 200 mg/mL, effectively inhibiting various pathogens, highlighting its dose-dependent antibacterial effects.

Mian et al. (2019) investigated the toxicity activity of bioactive compounds produced by endophytic fungi isolated from red ginger. The fungi were identified through macroscopic and microscopic examination, and bioactive compounds were extracted using 96% ethanol. The LC50 values of the bioactive compounds from the isolates were

determined to be 2.300 and 1.747 µg/mL, respectively.

A thorough literature review reveals that considerable research has been conducted on ginger, focusing on its chemical constituents and biological activities. While studies have been carried out in various parts of the world, only a limited number of studies have been done in Nepal. However, a comprehensive profiling of the biological activities of ginger from selected regions has yet to be undertaken. The proposed research aims to bridge this gap by examining the antioxidant, antimicrobial, and antifungal activities of plant extracts and essential oils.

CHAPTER 3: MATERIALS AND METHODOLOGY

3.1 Collection of plants materials

Ginger rhizomes were collected from Arghakhanchi and Dang districts of Nepal in June 2024.

The ginger samples were collected based on the ethnobotanical knowledge of local communities and a review of existing literature. Collected plant samples were identified in (CDB) Central Department of Botany, Tribhuvan University.

Table 1 : Sample of ginger from two districts of Nepal

S. N	Ginger collected Districts	Ginger sample
1	Arghakhanchi	S1
2	Dang	S2

3.2 Work plan

The research was done in the laboratory consists of a skeletal framework shown in the following flow chart of figure 2.

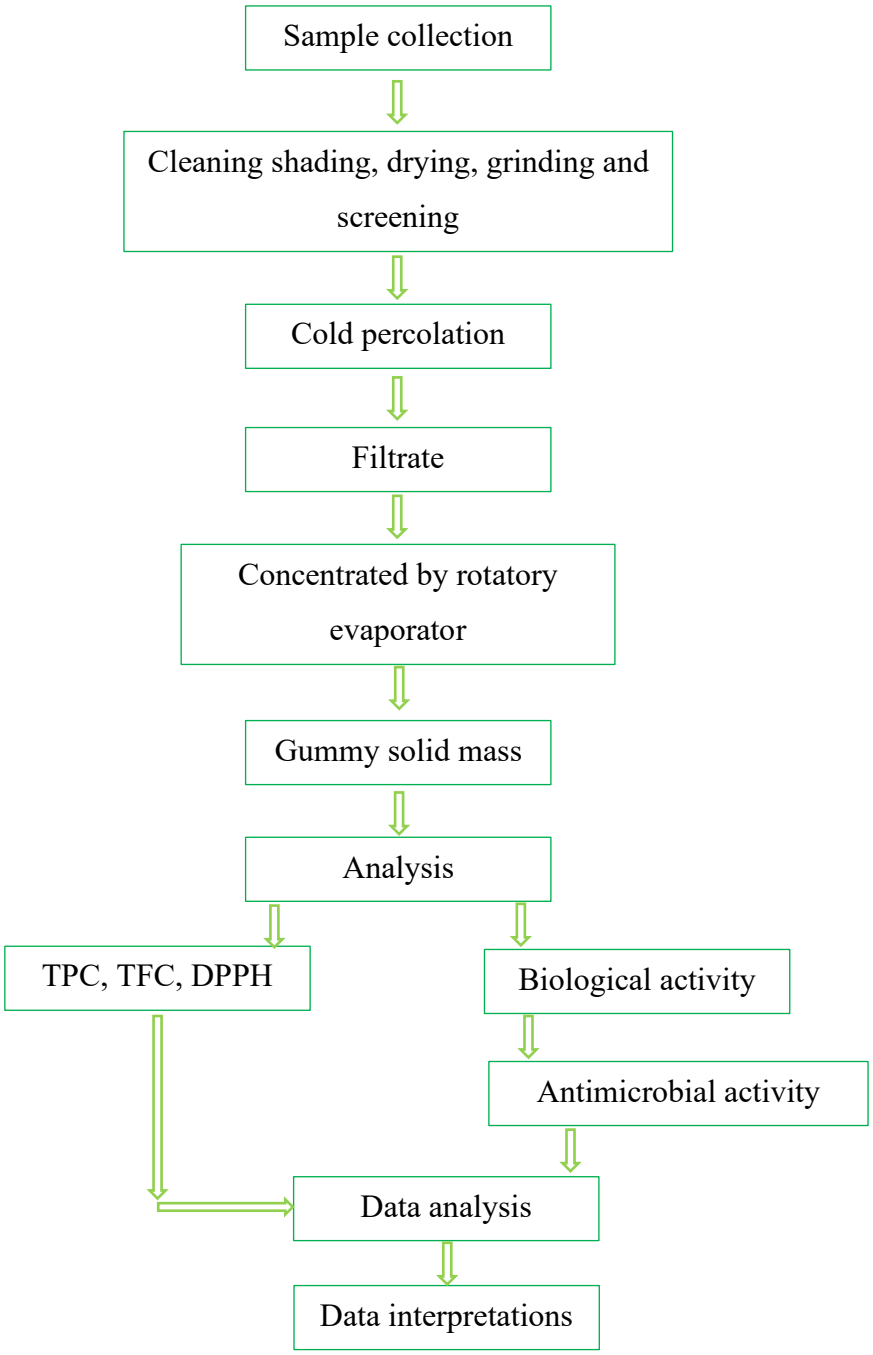


Figure 1 Flow chart of the work plan

3.3 Preparation of Herbarium

Herbarium specimen is a preserved plant sample that has been pressed and dried, often consisting of entire plants, and is typically mounted on a sheet of paper for future reference.

3.4 Preparation of extracts

3.4.1 Drying and pulverizing:

The ginger rhizome was chopped into pieces and dried in the shade at room temperature by avoiding direct sunlight according to the method describe by Ali et al.

3.4.2 Preparation of methanolic, dichloromethane, and ethyl acetate extract

Phytochemicals from the powdered rhizomes were extracted using the cold percolation method, with methanol as the solvent. A 100 g sample of the powder was weighed and placed in a conical flask, then 250 mL of methanol was added. The mixture was left undisturbed for 7 days, shaken periodically to facilitate thorough extraction. After the maceration period, the mixture was poured off and then strained through a cotton plug to separate the extract. The filtrate was concentrated by removing the solvent using a rotary evaporator under reduced pressure, ensuring that the temperature remained below 40°C. The concentrated extract was transferred to a pre-weighed 100 mL beaker, covered with perforated aluminum foil to facilitate further evaporation, and left to dry. The resulting semi-solid methanolic extract of ginger was analyzed % yield, and a portion of it was divided into vials for phytochemical and biological screening, while the remainder was stored for future use (Shrestha et al., 2015).

The methanolic ginger extract was further fractionated using ethyl acetate and dichloromethane (DCM) as solvents at room temperature. For the ethyl acetate fractionation, the methanol extract was dissolved in 200 mL of water in a separating funnel. Ethyl acetate was added of an equal volume, and the mixture was thoroughly shaken to ensure effective separation. The ethyl acetate layer was collected in a beaker and dried using a rotary evaporator under reduced pressure, followed by further drying in a water bath at 40 °C to remove residual solvent. Similarly, the methanolic ginger extract was fractionated with an equal volume of dichloromethane (DCM) at room temperature. The mixture was thoroughly shaken, and the dichloromethane layer was separated, collected in a beaker, and dried using a rotary evaporator. The dried DCM fraction was then subjected to further drying in a water bath at room temperature. Both the ethyl acetate and dichloromethane fractions were stored and later analyzed for biological activities.

$$\text{Percentage yield} = \frac{Dry\ weight\ of\ extracts}{Dry\ weight\ of\ plant\ material}\ 100\%$$

3.5 Phytochemical analysis

Process entails the targeted and step by step extraction of phytochemicals from ginger rhizome, adhering to established protocols. The presence of important plant extracts was composed of natural compounds assessed through color reactions with reagents. Detailed procedures for the phytochemical analysis and reagent preparation are outlined in Appendix I and II, (Naik et al., 2015).

3.6 Determination of Total Phenol Content

3.6.1 Preparation of reagents

A 1 M sodium carbonate solution was prepared by dissolving 5.29 g of sodium carbonate in 50 mL of distilled water. The Folin-Ciocalteu reagent was diluted 1:10 v/v by mixing 1 mL of the reagent with 10 mL of distilled water, (Ainsworth & Gillespie, 2007).

3.6.2 Preparation standard Gallic acid solution

A 500 µg/mL stock solution of gallic acid was prepared by dissolving 5 mg in 10 mL of ethanol. This was then diluted to create final concentrations of 10 to 80 µg/mL. The test solution should be made fresh before use, (Ainsworth & Gillespie, 2007).

3.6.3 Preparation of plant extract

Plant extracts were prepared at 500 µg/mL by diluting a 50 mg/mL stock in 50% DMSO in water. Total phenolic content was measured using the Folin-Ciocalteu reagent and gallic acid standards. The reagent reacts with tungsten and molybdenum oxides to produce a blue compound that absorbs light at 765 nm, with intensity correlating to phenol concentration. Total phenolic content was determined using methods by Ainsworth et al. and Lu et al., with slight modifications. (Baba & Malik, 2015).

3.6.4 Evaluation of total phenolic content

The total phenol content in the extracts was determined using the Folin-Ciocalteu reagent and a modified 96-well plate method. Gallic acid standards (10-80 µg/mL) and plant samples (500 µg/mL) were added in triplicate to the wells. Each well received 100 µL of Folin-Ciocalteu reagent and 80 µL of Na_2CO_3, followed by a 15-minute dark incubation. Absorbance at 765 nm was measured using a microplate reader, and the total phenolic content (TPC) was calculated from a gallic acid standard curve and expressed as mg GAE/g dry weight, (Ainsworth & Gillespie, 2007).

3.6.5 Calculation of total phenolic content (TPC)

The TPC content of each extract was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) and was calculated individually for each extract using the following formula.

$C = \frac{cV}{m}$ ……. (1)

Where,

C = Total phenolic content compounds in mg/g, in gallic acid equivalent (GAE)

c = Concentration of gallic acid established from the calibration curve in mg/mL

V = Volume of extract in mL

M = Weight of plant extract

3.6.6 Statistical analysis

Data were recorded as the average of three absorbance measurements for each concentration, and a linear correlation coefficient (R^2) was determined using Origin software. The regression equation is given as:

Y = mx + c……. (2)

Where,

Y = Absorbance of extract

m = Slope from the calibration curve

x = Concentration of extract

c = Intercept

The concentration of the extracts was determined using the regression equation. Then, based on the calculated concentration of each extract, the total polyphenolic content was calculated. by equation (2).

3.7 Determination of Total Flavonoid Content

3.7.1 Preparation of reagents

A 10% AlCl3 solution was made by dissolving 1 g of AlCl3 in 10 mL of water. A 1 M potassium acetate solution was prepared by dissolving 0.98 g of potassium acetate in 10 mL of water, (Chang et al., 2020).

3.7.2 Preparation standard quercetin solution

A 0.1 mg/mL quercetin stock solution was made by dissolving 1 mg in 10 mL of methanol. The standard solutions were prepared by diluting the stock to final concentrations of 10-80 µg/mL, (Chang et al., 2020).

3.7.3 Preparation of plant extract

The plant extracts were prepared at a concentration of 500 µg/mL by diluting a 50 mg/mL stock solution in 50% DMSO in water, (Najafian et al., 2016).

3.7.4 Procedure

Total flavonoid content was measured using a modified 96-well plate method. Standard quercetin solutions (10-80 µg/mL) and plant samples were added to the wells. Each well received ethanol, AlCl3, and potassium acetate, followed by a 30-minute dark incubation. Absorbance at 415 nm was measured, and total flavonoid content (TFC) was calculated as milligrams of quercetin equivalent per gram of dry weight (mg QE/g), (Chang et al., 2020).

3.7.5 Calculation of total flavonoid content

The total flavonoid concentration in the extracts was expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g). It was calculated for each extract individually using the following formula:

$$C = \frac{cV}{m} \quad \text{..... (3)}$$

Where,

C = Total flavonoid content compounds in mg/g, in Quercetin equivalent (QE)

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

V = Volume of extract in mL

M = Weight of plant extract

3.7.6 Statistical analysis

The procedure is mentioned in above chapter 3.6.6.

3.8 Determination of antioxidant activity

3.8.1 Preparation of DPPH solution

A 0.1 M DPPH solution was prepared by dissolving 3.9 mg of DPPH in 100 mL methanol and shielding it from light with aluminum foil, (Sabudak et al., 2013).

3.8.2 Preparation of quercetin solution

A quercetin stock solution was made by dissolving 5 mg in 5 mL of methanol. Serial dilutions were performed to obtain final concentrations of 50 to 0.390625 µg/mL, (Sabudak et al., 2013).

3.8.3 Preparation of plant extract

A 50 mg/mL plant extract stock solution was prepared by dissolving 50 mg in 1 mL of 50% DMSO in water. The final concentration of 500 µg/mL was achieved using the same DMSO solution, (Sabudak et al., 2013).

3.8.4 Procedure

Antioxidant activity was assessed using a modified 96-well plate method, where plant extracts reduced the purple DPPH radical to yellow diphenyl picrylhydrazine. Quercetin was the positive control, and 50% DMSO was the negative control. Each well contained 100 µL of extract or control, plus 100 µL of DPPH reagent. After 30 minutes of dark incubation, absorbance was measured at 517 nm, and DPPH scavenging ability was calculated using the formula:

$$\% \text{ Inhibition} = \frac{Acontrol - Asample}{Acontrol} \times 100\ \%$$

Where,

A is the absorbance of the sample and control, (Sabudak et al., 2013).

3.9 Biological activities

Different plant extracts are screened for their biological activities based on the compounds present. The biological screening involves studying the effect of crude plant extracts on organisms at different dosage levels to predict their potential effects.

3.9.1 Determination of Antimicrobial Activities

Antibacterial activity of ginger extract and antifungal activity of ginger extract were tested for antimicrobial activity.

3.9.1.1 Antibacterial activities

Ginger extract's antibacterial activity was tested using the agar well diffusion method, with the zone of inhibition (ZOI) measured in millimeters to assess effectiveness, (Tagg & McGiven, 1971).

3.9.1.1.1 Preparation of stock solution

A 50 mg/mL stock solution was made by dissolving 100 mg of crude extract in 2 mL of DMSO and stored in the refrigerator (2-8°C) for later use, (Tagg & McGiven, 1971)

3.9.1.1.2 Preparation of Standard Culture Inoculums

Bacteria from a primary culture plate were transferred to a test tube with sterile nutrient broth and incubated overnight at 37°C, (Tagg & McGiven, 1971)

3.9.1.1.3 Preparation of media

The media were prepared according to the manufacturer's instructions. The detailed procedure is given below.

3.9.1.1.3.1 Nutrient agar

Nutrient agar was prepared by dissolving 28 g in 1000 mL of distilled water, boiling, and autoclaving at 121 ºC for 15 minutes. After cooling to 50 ºC, it was poured into sterilized Petri dishes. Plates were incubated for 24 hours, and bacterial growth inhibition was observed by a clear zone around the wells. The zone of inhibition (ZOI) was measured in millimeters, with no zone indicating no activity. Each plate received 25 mL of media and was aseptically labeled to solidify, (Tagg & McGiven, 1971)

3.9.1.1.3.2 Nutrient broth solution (HiMEDIA)

To prepare nutrient broth, 0.65 g of powder was dissolved in 50 mL distilled water (13 g/L), then cooled at room temperature. After transferring 10 mL into a capped bottle, it was autoclaved at 121 ºC for 15 minutes.

3.9.1.1.3.3 Muller Hinton agar (MHA) plates (HiMEDIA)

9.5 g of media was dissolved in 250 mL of distilled water, boiled to dissolve, and autoclaved at 121 ºC for 15 minutes. After cooling to about 50 ºC, 20 mL of the sterilized medium was poured into each Petri dish and left to solidify.

3.9.1.1.4 Qualitative screening and evaluation of antibacterial activity

Agar (MHA) plates were dried to remove excess moisture and labeled with the bacterial species and disc code. The bacterial inoculum was spread evenly across the agar using a sterile cotton swab. After drying briefly, four 6-mm wells were made in each plate. 50 µL of the plant extract, 25 µL of neomycin, and DMSO as a negative control were added to separate wells. The plates were left for 30 minutes to allow diffusion, then incubated overnight at 37°C. After 24 hours, the zone of inhibition (ZOI) was measured, and antibacterial activity was expressed as the average diameter of the ZOI in millimeters. No ZOI indicated no activity, (Tagg & McGiven, 1971).

3.9.1.2 Antifungal activity

Ginger extracts from all districts were sent to the University of Hamburg, Germany, for antifungal testing. Antifungal activity was assessed using the agar well diffusion method, following Sabudak et al. (2013), with the zone of inhibition (ZOI) measured to evaluate antimicrobial properties.

3.9.1.2.1 Preparation of stock solution

A 50 mg/mL working solution was made by dissolving 100 mg of each crude extract in 2 mL of DMSO in a sterile vial. The solution was capped, sealed, and stored in the refrigerator (2-8 °C) until needed, (Dingle et al.,1953).

3.9.1.2.2 Collection of the test organism

The study's strains included *Fusarium oxysporium, Aspergillus flavus, Candida ablicans, Candida parapsilosis.*

3.9.1.2.3 Preparation of Standard Culture Inoculums

The test organisms were transferred from the primary culture plate to a test tube with 10 mL of sterile nutrient broth and incubated overnight at 37 ºC.

3.9.1.2.4 Preparation of media

The media used in the study were prepared according to the manufacturer's recommendation. The detailed procedure is given below.

3.9.1.2.4.1 Nutrient agar

Nutrient agar was prepared by dissolving 28 g in 1000 mL of distilled water, boiling with shaking, and autoclaving at 121 ºC for 15 minutes. After cooling to 50 ºC, 25 mL

of medium was aseptically poured into Petri dishes and left to solidify. After 24 hours of incubation, the plates were checked for fungal growth inhibition, indicated by a clear zone around the wells. The zone of inhibition (ZOI) was measured and recorded as the average diameter in millimeters, with no ZOI indicating no activity, (Sabudak et al., 2013).

3.9.1.1.4.2 Nutrient broth solution (HiMEDIA)

A nutrient broth was made by dissolving 0.65 g of powder in 50 mL of distilled water. The solution was cotton-plugged, sealed with aluminum foil, and cooled to room temperature. Then, 10 mL was transferred to a screw-capped bottle and sterilized by autoclaving at 121 ºC for 15 minutes, (Sabudak et al., 2013).

3.9.1.2.4.3 Muller Hinton agar (MHA) plates (HiMEDIA)

9.5 g of media was mixed with 250 mL of distilled water, boiled to dissolve, and sterilized by autoclaving at 121 ºC for 15 minutes. After cooling to 50 ºC, 20 mL of the medium was transferred into Petri dishes to solidify, (Sabudak et al., 2013).

3.9.1.2.5 Qualitative screening and evaluation of antifungal activity

Sterile Muller-Hinton Agar (MHA) plates were dried to remove excess moisture and labeled with the bacterial name and disc code. Bacterial inoculum was spread evenly on the plates using a sterile cotton swab. After drying, four 6 mm wells were created using a cork borer. 50 µL of plant extract, 25 µL of Amphotericin B (positive control), and DMSO (negative control) were added to separate wells. The plates were left for 30 minutes to allow diffusion, then incubated overnight at 37°C. After 24 hours, the zone of inhibition was measured, and antibacterial activity was expressed as the average diameter of the zone. No inhibition indicated no activity, (Sabudak et al., 2013).

3.9.2 Brine shrimp toxicity test:

The toxicity test was carried out using the brine shrimp assay as per Meyer et al. (1982), which is a simple, quick, and inexpensive method. Newly hatched Artemia salina larvae were exposed to crude plant extracts and their fractions. The toxicity was evaluated by determining the LC50 (µg/mL), with compounds having an LC50 below 1000 ppm suggesting potential pharmaceutical activity.

3.9.2.1 Required materials

- Eggs of brine shrimps
- Beaker for hatching eggs
- Test tubes (2×5 cm)
- Artificial seawater
- Disposal pipette
- Micropipette

3.9.2.2 Sterilization of apparatus

All the equipment were sterilized before using for bioassay.

3.9.2.3 Preparation of artificial seawater

The artificial seawater required for the bioassay was freshly prepared by dissolving specific chemicals in distilled water. The composition of the artificial seawater is provided in Table 2.

Table 2 Composition of artificial seawater (Janardan Lamichhane et al., 2009)

S. N.	Composition	Amount (g/L)
1.	NaCl	23.5
2.	Na_2SO_4	4
3.	KCl	0.68
4.	H_3BO_4	0.027
5.	$MgCl_2.2H_2O$	10.68
6.	$CaCl_2.2H_2O$	1.78
7.	$NaHCO_3$	0.197
8.	Na_2EDTA	0.0003

Percentage mortality (% M) = $\frac{Number\ of\ dead\ nauplii}{Total\ number\ of\ nauplii}$ x 100 %

In the present work, brine shrimp bioassay of different plants methanolic extracts was carried out and the lethal concentration value was calculated

3.9.2.4 Hatching of shrimps

To hatch the brine shrimp, approximately 10 mg of eggs were sprinkled into a beaker filled with artificial seawater. The beaker was positioned under a 60-watt table lamp for 48 hours, with the temperature kept at 30 ºC. The larvae, being phototropic, were drawn to the light throughout this time, (Meyer et al., 1982).

3.9.2.5 Preparation of samples and bioassay

To make the stock solution, 20 mg of the material was dissolved in 2 mL of methanol. To create concentrations of 1000, 100, and 10 µg/mL, particular stock volumes were evaporated into test tubes with 10 mL of seawater that had been sterilized, (Meyer et al., 1982).

Five milliliters of seawater were added and mixed, with triplicates prepared for each concentration and control. Ten mature brine shrimp were placed in each tube, and the test was conducted at 20°C under continuous light. After 24 hours, survivors were counted, and mortality rates were used to estimate the LC50 value. The same method was used for the ethyl acetate extract, (Meyer et al., 1982).

3.9.2.6 Data analysis

50% of the brine shrimp must be killed at a concentration known as the LC50 value. Probit analysis uses the following technique to determine it for three replicates (n = 3), x is the logarithm of the extract concentration (log 10, log 100, and log 1000 for three dose levels), and y is the average survival proportion across replicates. The LC50 is then calculated from this data

We have,

$$\alpha = \frac{1}{n} [\sum y - \beta \sum x] \ldots\ldots (1)$$

$$\beta = \frac{\sum xy - \sum x \sum y / n}{\sum x\, 2 - (\sum x)\, 2/n} \ldots\ldots (2)$$

Where,

From probit regression

$$Y = \alpha + \beta X \ldots\ldots (3)$$

$$X = \frac{Y - \alpha}{\beta} \ldots\ldots (4)$$

Where Y is constant having value 5 for calculating LC_{50} values

Thus LC_{50} = Antilog X……. (5)

The lethal concentration value was determined in the current study using a brine shrimp bioassay of various plant extracts.

3.10 Data analysis:

All experiments were conducted in triplicates, with results expressed as mean ± standard error. TPC, TFC, and antioxidant data were analyzed using Gen 5 Microplate Data Collection and Orion software, while the IC50 value was calculated using GraphPad Prism version 8.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Phytochemical extraction

The ginger extract yields varied between the samples collected from Arghakhanchi and Dang districts, depending on the solvent used (methanol, ethyl acetate, and dichloromethane). The highest yield was seen in the S1(MeOH) sample from Arghakhanchi, producing 18.2 g, while the lowest was from the S2 (DCM) sample from Dang, which gave only 3.4 g. Interestingly, all the extracts were in solid form. You can find a detailed breakdown of the yields for all ginger samples in Table 3.

Table 3 Yield of ginger extracts from all ginger samples

Ginger sample	**The dry weight of ginger (g)**	**Yield (g)**	**%Yield**
S1(MeOH)	400gm	18.2 g	4.55
S2(MeOH)	400gm	17.3g	4.32

Ginger sample	S1(EtOAc)	S1(DCM)	S2(EtOAc)	S2 (DCM)
Yield (g)	4.5 g	3.7 g	4.3 g	3.4 g

4.2 Phytochemical screening

Phytochemical screening was conducted on the selected ginger extracts, and the results obtained from the methanolic extracts, as well as those from the dichloromethane (DCM) and ethyl acetate extracts, have been compiled in a table 5.

Table 4 Phytochemical screening of ginger extracts of all ginger samples

Phytochemicals	**S1(EtAc)**	**S1 (MeOH)**	**S2(EtOAc)**	**S2 (MeOH)**
Basic alkaloids	-	+	+	++
Glycosides	+	++	+	++
Saponins	+	++	++	+++
Flavones	+	++	-	++
Tannin	++	+	++	+
Diterpenes	++	+	++	-
Steroids & terpenoids	++	_	+++	+
Cartenoids	++	+	++	-
Polyphenol	+	++	+	+
Carbohydrates	_	+	_	+
Quinone	+	-	+	+
Volatile oil	++	-	+++	+
Coumarin	+++	+	++	-

(++) test strongly positive

(+) weak positive test

(-) negative test (absence of required ppt/color)

Glycosides, Saponins, tannin, and polyphenol are present in all ginger extracts. Methanol (MeOH) extracts more polar compounds like glycosides, saponins, flavones, polyphenols, and carbohydrates, while ethyl acetate (EtAc) is more effective for non-polar compounds such as volatile oils, diterpenes, carotenoids, and coumarin, with both solvents showing varying levels of phytochemical presence across the S1 and S2 samples.

The phytochemical screening results for samples collected from various districts may vary due to factors such as plant altitude, environmental conditions, collection methods and timing, extraction techniques, as well as differences in laboratory setups and the

quality of chemicals used. As a result, the findings presented in the table above might slightly differ from data found in the literature for some plants. (Joseph et al., 2016).

4.3 Total phenolic content

TPC was measured using the Folin-Ciocalteu reagent, (Ainsworth & Gillespie, 2007). This method works by forming a phosphotungstic-phosphomolybdic complex when phenolic compounds are present. In an alkaline solution, the yellow reagent, a molybdotungstophosphoric heteropolyanion, reacts with the phenols, resulting in the formation of a blue complex. This complex can then be measured colorimetrically. The reaction between the phenolic compounds and the Folin-Ciocalteu reagent only occurs under basic conditions, which are created by adding sodium carbonate to the solution, (Masuda et al., 2004).

Ginger extract polyphenols interact with the Folin-Ciocalteu reagent to form a blue complex, and the absorption at 765 nm, measured via UV-Visible spectrometry, reflects the phenolic concentration. The total phenolic content (TPC) is quantified using a gallic acid calibration curve ($y = 0.265x$, $R^2 = 0.9992$) and expressed as mg GAE/g dry extract weight.

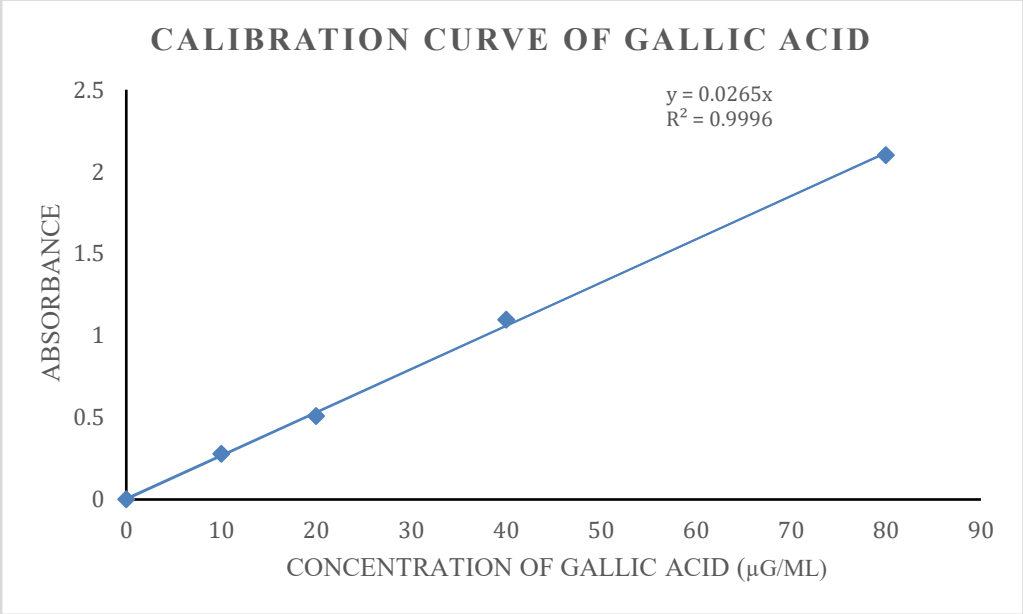


Figure 2 Calibration curve of gallic acid

Each test was conducted in triplicate, and the phenolic content, reported as gallic acid equivalents, is presented as the average ± standard deviation of the three trials.

Ginger extract of sample S2(MeOH) showed the highest TPC value of 141.57 ± 4.75mg

GAE/g and ginger extract of sample S1(DCM) showed the lowest 41.98 ± 4.63 mg GAE/g TPC value. TPC of all plants is shown in their bar diagram representation in Figure 4.

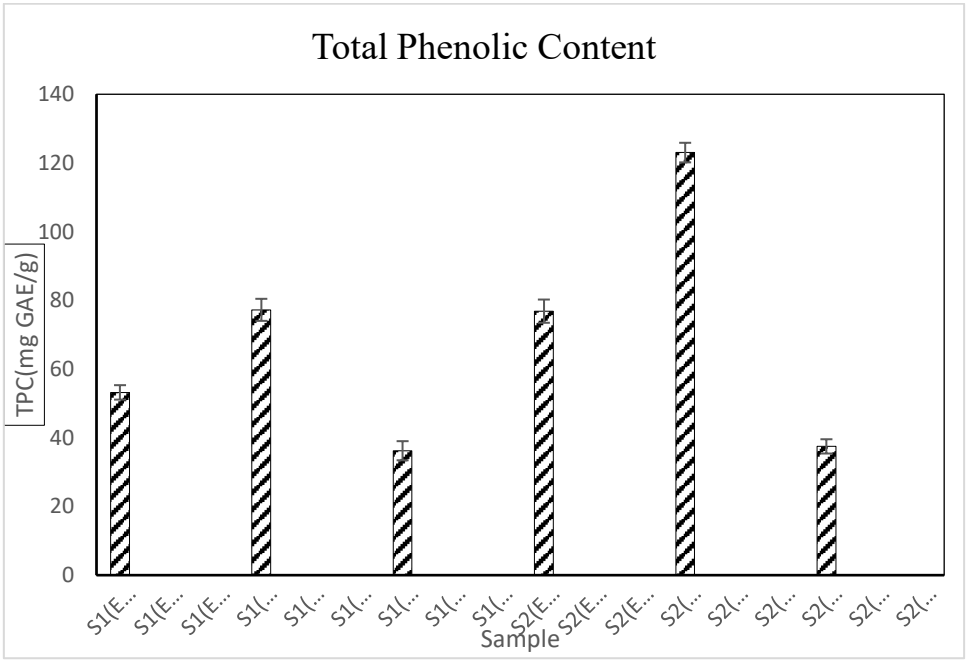


Figure 3 Total phenolic content of ginger extract of all ginger samples

The TPC value of methanolic dichloromethane (DCM) and ethyl acetate extracts extract of all samples collected from two districts follows the order as; S2(MeOH)> S2(EtOAc > S1(MeOH) > S1(EtOAc)> S2(DCM)> S1(DCM). Ginger extract of sample S2(MeOH) collected from Dang districts showed the highest TPC value of 123.0189 ± 2.84 mg GAE/g and extract of sample S1 (DCM) with the lowest TPC of 36.23 ± 3.45 mg GAE/g. Thus, the result indicates that the methanolic extract of ginger collected from the Dang district is rich in phenolic compounds as compared to other ginger samples. The total phenolic content of samples S1(EtOAc), S1(MeOH), S2(EtOAc) and, S2 (DCM) are 53.207 ± 2.1 mg GAE/g, 77.23 ± 3.20 mg GAE/g, 76.85 ± 2.07 mg GAE/g and 37.48 ± 2.42 mg GAE/g respectively. The result obtained in this study is comparable to the result of Ali et. al., (2018), which was 60.34 ± 0.43 mg GAE/g (Ali et al., 2018).

Research indicates that extracts rich in polyphenols have greater antioxidant activity. This is attributed to the strong free radical scavenging abilities of phenolic compounds. However, accurately quantifying phenolic compounds in plant extracts is challenging due to factors like structural complexity, diversity, the method of analysis, the choice

of standard, and interference from other substances.[31]

4.4 Total Flavonoid content

The TFC in the methanolic, dichloromethane (DCM), and ethyl acetate extracts of ginger was estimated using a standard procedure that employs quercetin as a reference. The intensity of light absorption at a wavelength of 415 nm correlates with the concentration of flavonoids, which exhibit a strong yellow fluorescence when analyzed with a UV spectrophotometer. TFC was analysed using the AlCl3 method, which relies on the formation of a flavonoid-AlCl3 complex. This interaction results in a bathochromic shift and a hyperchromic effect. AlCl3 creates acid-resistant complexes form with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavonoids.[89] The calibration curve of Quercetin is shown figure 5.

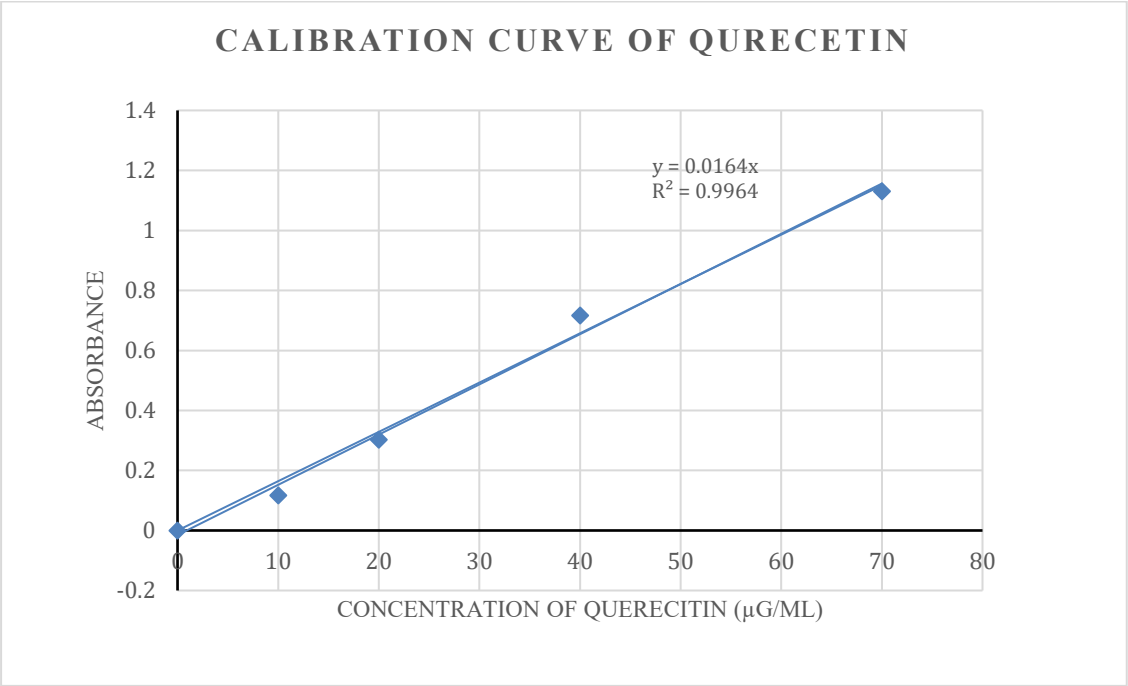


Figure 4 Calibration curve of quercetin

The reported TFC of the ginger extract is reported as quercetin equivalents (mg QE/g dry weight of extract), based on a quercetin calibration curve ($y = 0.0164x$, $R^2 = 0.9922$). The TFC data presented as mean ± standard error in Table 7 and their bar diagram in Figure 6.

Extract of ginger sample S2(MeOH) collected from Dang district exhibited the highest total flavonoid content (TFC) of 25.42 ± 1.69 mg QE/g and ginger extract of sample S1(DCM)showed the lowest 14.26 ± 1.58 mg QE/g TFC value. The TFC value of

sample S1(MeOH), S1(EtOAc), S2(EtOAc) and, S2 (DCM) are 20.91 ± 0.86 mg QE/g, 14.32 ± 1.74 mg QE/g, 22.70 ± 0.81 mg QE/g, 19.86 ± 1.13 mg QE/g respectively. The result from this study is somewhat lower than the findings of Ali et al. (2018), who reported a TFC value of 40.25 ± 0.21 mg QE/g. (Ali et al., 2018).

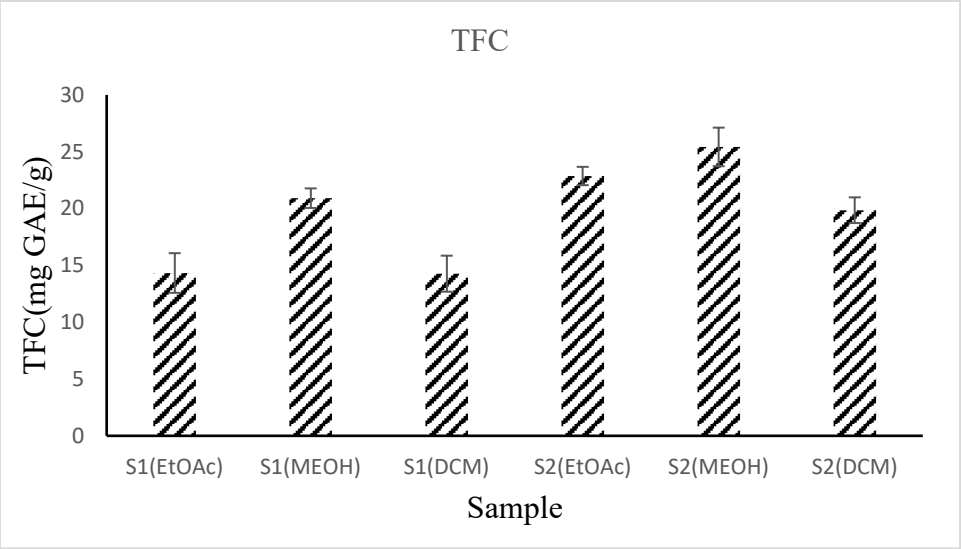


Figure 5 Total flavonoid content of ginger extract of all ginger samples

The TFC value of methanolic, dichloromethane (DCM) and ethyl acetate extract of all samples collected from two districts follows the order as; S2(MeOH) > S2(EtOAc) > S1(MeOH) > S1(EtOAc) > S2 (DCM) > S1(DCM). Quantitative determination of flavonoids in extracts depends on factors such as the molecular structure, especially the position of the hydroxyl group, the ability to donate electrons to a free radical, the choice of standard, the medium used, and the presence of interfering substances.

4.5 Antioxidant assay

When hydrogen atoms or electrons are donated by free radical scavenging secondary metabolites, the DPPH radical changes color from purple to yellow, indicating reduction, (Mishra et al., 2012), (Bag et al., 2015).

4.5.1. Antioxidant activity of a ginger extract

DPPH was employed to evaluate the antioxidant activity of ginger extracts, (Sabudak et al., 2013). The antioxidant capacity of the extracts is represented by the IC50 value, with a lower IC50 indicating stronger antioxidant potential. In other words, a smaller IC50 value corresponds to a higher ability of the extract to neutralize free radicals. Table 8 represents the antioxidant screening of methanolic ginger extract of all samples

and figure 7 represents the IC_{50} of potent crude extract along with standard quercetin. Table 9 shows the IC_{50} value of ginger extracts from all samples.

Table 5 The antioxidant screening ginger extract of all ginger samples

	Concentration (µg/mL)	**% inhibition**
S1(EtOAc)	500	72.58065
S1(MeOH)	500	68.92911
S1(DCM)	500	52.9908
S2(EtOAc)	500	75.92025
S2(MeOH)	500	73.6542
S2 (DCM)	500	53.14417

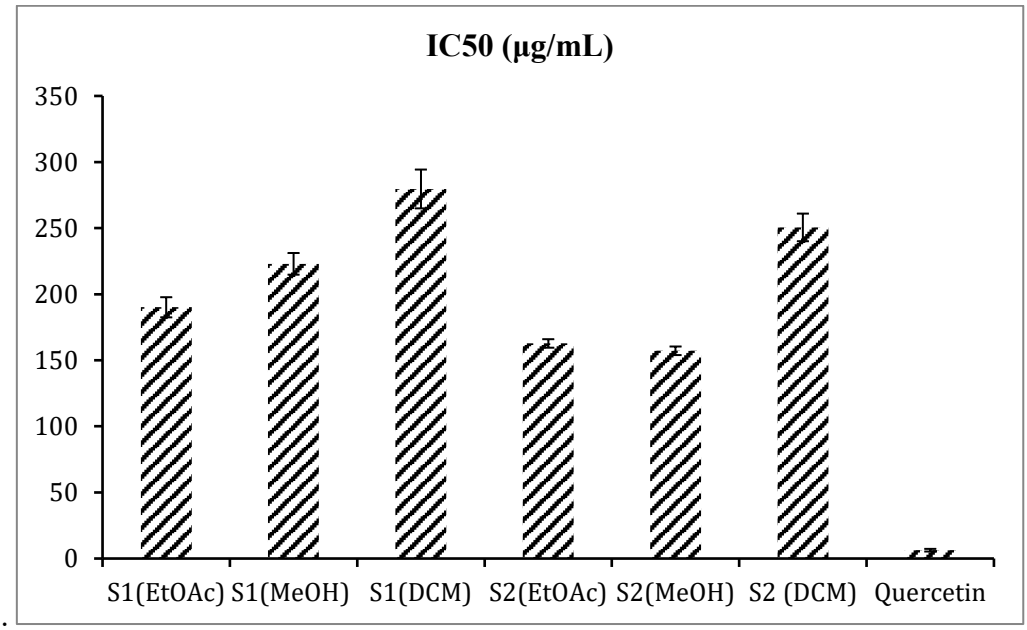


Figure 6 IC_{50} value of ginger extract of all ginger samples

The radical scavenging ability of methanolic extract of all ginger samples follows the order as; S2(EtOAc)> S2(MeOH)> S1(EtOAc)> S1(MeOH) > S2 (DCM)> S1 (DCM. Sample S2(EtOAc) which was collected from the Dang district has a good scavenging ability as compared with other samples. The results were compared to quercetin, which has an IC50 value of 6.3 ± 1.0 µg/mL. Sample S2 (EtOAc) displayed significant antioxidant activity, with an IC50 value of 162.73 ± 3.22 µg/mL. On the other hand, the ginger extract from sample S1 (DCM) showed weaker scavenging activity about

44-fold times less compared to standard with IC_{50} 279.63 ± 14.67 µg/mL. The free radical scavenging ability of extract of sample S2(EtOAc) is higher due to ethyl acetate extract provides either hydrogen or electron-donating secondary metabolites. The present result shows very few antioxidant activities as compared to about 19 to 30 folds less with Ali et. al., (2018), and they found a value of 8.29 ± 1.73 µg/mL.

The differences in results could be attributed to the presence of carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and other endogenous metabolites. Several factors influence the antioxidant levels in ginger rhizomes, including genetic variation, the stage of maturity, pre-harvest conditions such as climate, temperature, and light, as well as cultivation methods, post-harvest handling, and processing techniques.

4.6 Antimicrobial activity

The antimicrobial activity of ginger extracts was assessed by measuring the diameter of the zone of inhibition (ZOI) formed against specific bacteria and fungi. The antimicrobial action against various bacterial and fungal infections is thought to involve multiple mechanisms, such as disrupting bacterial protein and nucleic acid synthesis, blocking metabolic pathways, and compromising the integrity of bacterial membranes and cell wall production, (Izah, 2018).

4.6.1 Antibacterial activity

For antibacterial activity gram-positive bacterium (Salmonella typhi aureus) and a gram-negative bacterium (*Escherichia coli*, *Salmonella Typhi*, *Klebsiella pneumonia*) were used.

4.6.1.1 Antibacterial activity of a ginger extract

ZOI created by the plant extracts was measured, and the findings from the antibacterial screening of various extracts are presented in Table 12.

Table 6 Antibacterial activity of ginger extract from different districts

S. N.	Ginger sample	Bacteria	ZOI (mm) of extract at concentration 25 µg/mL	ZOI (mm) of neomycin as control
1	S1(EtOAc)	*E coli*	-	18
		Klebsiella pneumonia	7	20
		P. aeruginosa	-	16
		S. aureus	15.4	18
		Salmonella typhi	9	22
2	S1(MEOH)	*E coli*	-	18
		Klebsiella pneumonia	11	20
		P. aeruginosa	-	16
		S. aureus	15.8	18
		Salmonella typhi	12.3	22
3	S1(DCM)	*E coli*	-	18
		Klebsiella pneumonia	-	20
		P. aeruginosa	-	16
		S. aureus	11.2	18
		Salmonella typhi	10.5	22
4	S2(EtOAc)	*E coli*	-	18
		Klebsiella	9.6	20

S. N.	Ginger sample	Bacteria	ZOI (mm) of extract at concentration 25 µg/mL	ZOI (mm) of neomycin as control
		pneumonia		
		P. aeruginosa	-	16
		S. aureus	15.5	18
		Salmonella typhi	13.7	22
5	S2(MEOH)	*E Coli*	-	18
		Klebsiella pneumonia	7.6	20
		P. aeruginosa	6	16
		S. aureus	16.3	18
		Salmonella typhi		22
6	S2(DCM)	*E Coli*	-	18
		Klebsiella pneumonia	6	20
		P. aeruginosa	4	16
		S. aureus	12	18
		Salmonella typhi	9	22

Well size = 6 mm

Zone of inhibition of DMSO (negative control) = 6 mm

(-) the sign indicates the absence of antibacterial activity

Neomycin served as the positive control, administered at a concentration of 25 µg/mL.

Among the two strains, gram-positive bacteria were more effectively inhibited than gram-negative bacteria. This difference can be attributed to the unique outer membrane found in gram-negative bacteria, which prevents the extract from entering the cell, a feature not present in gram-positive bacteria, (Sivasothy et al., 2011), (Izah, 2018).

4.6.2 Antifungal activity

For antifungal activity, *Fusarium oxysporium, Aspergillus flavus, Candida ablicans, Candida parapsilosis* were used in the present study.

4.6.2.1 Antifungal activity of the ginger extract

The antifungal activity of the plant extracts was assessed by measuring the diameter of the zone of inhibition (ZOI) against specific fungi, with the results presented in Table 14.

Table 7 Antifungal activity of ginger extract of all ginger samples

S. N.	Ginger sample	Fungi	ZOI (mm) of extract at concentration 50 µg/mL	ZOI (mm) of Amphotericin B as control
1	S1(EtOAc)	*Fusarium oxysporum*	-	20
		Aspergillus flavus	8.4	20
		Candida ablicans	1.5	19
		Candida parapsilosis	14	16
2	S1(MEOH)	*Fusarium oxysporum*	-	20
		Aspergillus flavus	7	20
		Candida ablicans	13	19
		Candida parapsilosis	9	16
3	S1(DCM)	*Fusarium oxysporum*	8	20
		Aspergillus flavus	6.8	20
		Candida ablicans	-	19
		Candida parapsilosis	-	16
4	S2(EtOAc)	*Fusarium oxysporum*	-	20
		Aspergillus flavus	7.3	20
		Candida ablicans	8.2	19
		Candida parapsilosis	11	16
5	S2(MEOH)	*Fusarium oxysporum*	-	20
		Aspergillus flavus	9.3	20
		Candida ablicans	13	19
		Candida parapsilosis	9	16
6	S2(DCM)	*Fusarium oxysporum*	6	20

S. N.	Ginger sample	Fungi	ZOI (mm) of extract at concentration 50 µg/mL	ZOI (mm) of Amphotericin B as control
		Aspergillus flavus	-	20
		Candida ablicans	-	19
		Candida parapsilosis	-	16

Well size = 6 mm

Zone of inhibition of DMSO (negative control) = 6 mm

(-) a sign indicates the absence of antifungal activity

Amphotericin B was employed as the positive control at a concentration of 25 µg/mL.

Here zone of inhibition was measured. Ginger extracts of S1(EtOAc), S1(MEOH), S2(EtOAc), S2(MEOH) exhibited a zone of inhibition against *Candida albicans*, *Candida parapsilosis*, and *Aspergillus flavus*. S1(DCM), S2(DCM) samples show a zone of inhibition for *Fusarium oxysporum*. From this value, we can conclude that ginger extract can be used as antifungal activity. Extracts of samples S1(EtOAc) and S2(EtOAc) show the highest ZOI in *Candida parapsilosis* (14mm) and (11mm) respectively. Ginger extracts of samples S1(MEOH) and S2(MEOH) shows the same ZOI for *Candida ablicans* (13mm). This result is consistent with the findings reported by Rawal et al. (2016) and here different solvent extracts were used and antifungal activity increased with increasing concentration (Rawal & Adhikari, 2016).

The observed antifungal activity may be due to the presence of alkaloids and flavonoids. This study indicates that ginger rhizome extracts have potential for use in combating fungal infections. It was found that various factors, including environmental conditions (such as pH, temperature, water activity, oxygen levels, and nutrient availability), the solvent used, the source of the organisms, as well as the microbes' biochemistry, physiology, metabolism, and adaptation strategies, all influence the antifungal activity. Additionally, factors like the plant species, its biochemistry, age,

plant part used, the concentration of the extract, and the duration of exposure also affect the antifungal effectiveness.

4.7 Brine shrimp toxicity test

The toxicity of ginger extracts was assessed on newly hatched brine shrimp larvae using Mayer et al. (1982) method, with LC50 values determined at various concentrations, as shown in Table 16.

Table 8 Brine shrimp toxicity of the ginger extract

Ginger sample	**LC_{50} (**µg/mL)
S1(EtOAc)	1×10^5
S1(MeOH)	3×10^3
S2(EtOAc)	4.6×10^4
S2(MeOH)	3.5×10^4

The mortality percentage was determined by dividing the number of dead nauplii by the total number of nauplii used and then multiplying the quotient by 100%.

Extracts with LC50 values below 1000 µg/mL are considered pharmacologically active. Lethality was found to be positively correlated with the concentration of the extracts., with the highest mortality occurring at 1000 µg/mL and the lowest at 10 µg/mL. The highest LC50 value was observed in S1(EtOAc) at 1×10^5 µg/mL, while the lowest LC50 value was found in sample S1(MeOH) at 3.0×10^3 µg/mL.

In this study, the LC50 value exceeded 1000 µg/mL, indicating that ginger extracts from all districts are non-toxic and pharmacologically inactive, making them safe for use as spices. An LC50 value of 100 ppm was considered toxic. According to Meyer et al. (1982), an LC50 value below 1000 µg/mL indicates pharmacological activity, while a value above 1000 µg/mL suggests inactivity. Table 17 displays the number of surviving nauplii after treatment with ginger extracts, along with their corresponding mortality percentages.

Table 9 Number of survived nauplii after treating with ginger extract and their percentage mortality

S. N	Ginger sample	Concentration µg/ml	No. of nauplii taken	No. of survived nauplii	Mortality %
1.		10	10	6	40 %
2.	S1(EtOAc)	100	10	5	50 %
3.		1000	10	6	40 %
1.		10	10	9	10 %
2.	S1(MEOH)	100	10	8	20 %
3.		1000	10	5	50 %
1.		10	10	9	10 %
2.	S2(EtOAc)	100	10	6	40 %
3.		1000	10	3	70 %
1.		10	10	8	20 %
2.	S2(MEOH)	100	10	7	30 %
3.		1000	10	5	50 %

There is a variation in the LC50 value, which may be attributed to factors such as differences in the altitude where the plant was collected or variations in laboratory conditions. Biological factors like temperature and pressure can also influence the LC_{50} values. This method quickly screens bioactive compounds with minimal equipment and small samples, though it lacks detailed mechanistic insights and is often followed by more specific bioassays, (Sarah et al., 2017).

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Glycosides, Saponins, tannin, and polyphenol are present in all ginger extracts. Methanol (MeOH) extracts more polar compounds like glycosides, saponins, flavones, polyphenols, and carbohydrates, while ethyl acetate (EtAc) is more effective for non-polar compounds such as volatile oils, diterpenes, carotenoids, and coumarin, with both solvents showing varying levels of phytochemical presence across the S1 and S2 samples.

Ginger extract of sample S2(MeOH) collected from Dang district showed the highest TPC with value 123.01 ± 2.84 mg GAE/g and extract of sample S1(DCM) collected from Arghakhanchi district with the lowest TPC with value 36.23 ± 3.45 mg GAE/g TPC. This study shows that the methanolic extract of sample S2(MeOH) is rich in phenolic compounds as compared to other samples.

Ginger extract from sample S2(MeOH) of Dang district exhibited the highest total flavonoid content (TFC) of 25.42 ± 1.69 mg QE/g, while the ginger extract from sample S1(DCM) of Arghakhanchi district showed the lowest TFC of 14.26 ± 1.585 mg QE/g.

Ginger sample S2(EtOAc) collected from Dang district has a good scavenging ability as compared with other samples. The ginger extract from sample S2(EtOAc) collected from Dang demonstrated strong, with an IC50 value of 162.73 ± 3.22 µg/mL. In comparison, ginger extract from sample S1(DCM) of Arghakhanchi district exhibited lower neutralizing activity, with an IC50 value of 279.63 ± 14.67 µg/mL.

Extracts of all ginger samples did not show any zone of inhibition for *E. Coli*. Extract of S2(MeOH) shows maximum ZOI for S. *aureus,* and *P. aeruginosa.* Ginger *Extracts* of sample S1(EtOAc), S1(MEOH), S1(DCM), S2(EtOAc) and sample S2(DCM) show the highest ZOI for *S. aureus* (15.4 mm), (15.8 mm), (11.2 mm) (15.5 mm) and, (12 mm) respectively, and that of sample S2(EtOAc) shows the highest ZOI for *Salmonella typhi (*13.7mm).

All ginger extracts displayed a ZOI against *Candida parapsilosis*, *Candida albicans*, and *Aspergillus flavus*, but no inhibition was observed against *Fusarium oxysporum* a zone of inhibition is shown by S1(DCM), S2(DCM). Extracts of S1(EtOAc) and

S2(EtOAc)show the highest ZOI in *Candida parapsilosis* (14 mm) and (11 mm) respectively.

The highest LC_{50} value was found in S1(EtOAc) at 1×10^5 µg/mL, indicating lower toxicity. On the other hand, the methanolic extract (S1(MeOH)) showed the lowest LC_{50} of 3.0×10^3 µg/mL, reflecting higher toxicity. These findings from the brine shrimp bioassay indicate that MEOH extract of ginger is more unsafe than the ethyl acetate extract.

5.2 Recommendations

This study suggests further research to isolate and explore the active compounds in ginger, particularly its methanolic extract, which is rich in antioxidants and shows strong antibacterial properties. Exploring ginger's secondary metabolites could lead to new treatments for drug-resistant infections.

REFERENCES

Abozenadah, H., Bishop, A., Bittner, S., Lopez, O., Wiley, C., and Flatt, P.M. (2017) , CH105 – Chapter6, Consumer Chemistry: How Organic Chemistry Impacts Our Lives. CC BY-NC-SA. Available at: https://wou.edu/chemistry/courses/online-chemistry-textbooks/ch105-consumer-chemistry/

Abuajah, C. I., Ogbonna, A. C., & Osuji, C. M. (2014). Functional components and medicinal properties of food: a review. *Journal of Food Science and Technology*, *52*(5), 2522–2529. https://doi.org/10.1007/s13197-014-1396-5.

Adhikari, A., & Bhandari, T. (2022). Socio-economic analysis of ginger production in Terhathum district, Province no. 1, Nepal. *Archives of Agriculture and Environmental Science*, *7*(1), 61–69. https://doi.org/10.26832/24566632.2022.070109

Agriculture Development Strategy (ADS) | Ministry of Agriculture and Livestock Development. (2072). https://moald.gov.np/ads/

Aher, V., & Wahi, A. (2011). Immunomodulatory Activity of Alcohol Extract of *Terminalia chebula* Retz Combretaceae. *Tropical Journal of Pharmaceutical Research*, *10*(5). https://doi.org/10.4314/tjpr.v10i5.5

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

AITC (2020). Krishi Diary. Department of Agriculture, Agriculture Information and Training Centre, Government of Nepal.

Al-Fatimi, M., Wurster, M., Schröder, G., & Lindequist, U. (2007). Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *Journal of Ethnopharmacology*, *111*(3), 657–666. https://doi.org/10.1016/j.jep.2007.01.018

Ali, A. M. A., El-Nour, M. E. M., & Yagi, S. M. (2018). Total phenolic and flavonoid contents and antioxidant activity of ginger (Zingiber officinale Rosc.) rhizome, callus and callus treated with some elicitors. *Journal of Genetic Engineering*

and Biotechnology, *16*(2), 677–682. https://doi.org/10.1016/j.jgeb.2018.03.003

Ambu, G., Chaudhary, R. P., Mariotti, M., & Cornara, L. (2020). Traditional Uses of Medicinal Plants by Ethnic People in the Kavrepalanchok District, Central Nepal. *Plants*, *9*(6), 759. https://doi.org/10.3390/plants9060759

Aryal, K., Meghnath Dhimal, Pandey, A., & Mehta, R. Ku. (2016, November). *Knowledge Diversity and Healing Practices of Traditional Medicine in Nepal*. ResearchGate; unknown. https://www.researchgate.net/publication/313159390_Knowledge_Diversity_and_Healing_Practices_of_Traditional_Medicine_in_Nepal

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., Orhan, I. E., Banach, M., Rollinger, J. M., Barreca, D., Weckwerth, W., Bauer, R., Bayer, E. A., Majeed, M., Bishayee, A., Bochkov, V., Bonn, G. K., Braidy, N., Bucar, F., Cifuentes, A., D'Onofrio, G., Bodkin, M., . . . Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery*, *20*(3), 200–216. https://doi.org/10.1038/s41573-020-00114-z

Baba, S. A., & Malik, S. A. (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University Science, 9*(4), 449–454. c

Bag, G. C., Devi, P. G., & Bhaigyabati, T. (2015). Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three *Hedychium* species of Manipur Valley. *Pharmacognosy Journal, 30*(28), 6.

Bag, G. C., Grihanjali Dev, P., & Bhaigyabati, T. (2015). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry.* https://pubs.acs.org/doi/abs/10.1021/jf030723c (accessed April 5, 2021).

Baker, D. D., Chu, M., Oza, U., & Rajgarhia, V. (2007). The value of natural products to future pharmaceutical discovery. *Natural Product Reports*, *24*(6), 1225. https://doi.org/10.1039/b602241n

Beckman, C. H. (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiological and molecular plant pathology*, *57*(3), 101-110.

Bernardini, S., Tiezzi, A., Laghezza Masci, V., & Ovidi, E. (2017). Natural products for human health: an historical overview of the drug discovery approaches. *Natural Product Research*, *32*(16), 1926–1950. https://doi.org/10.1080/14786419.2017.1356838.

Bhalodia, N., & Shukla, V. (2011). Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. *Journal of Advanced Pharmaceutical Technology & Research*, *2*(2), 104. https://doi.org/10.4103/2231-4040.82956

Bhat, R. B., & Jacobs, T. V. (1995). Traditional herbal medicine in Transkei. *Journal of Ethnopharmacology*, *48*(1), 7–12. https://doi.org/10.1016/0378-8741(95)01276-j

Bhattacharjee, A., Anadón, J., Lohman, D., Doleck, T., Lakhankar, T., Shrestha, B., Thapa, P., Devkota, D., Tiwari, S., Jha, A., Siwakoti, M., Devkota, N., Jha, P., & Krakauer, N. (2017b). The impact of climate change on biodiversity in Nepal: Current knowledge, lacunae, and opportunities. *Climate*, *5*(4), 80. https://doi.org/10.3390/cli5040080

Bhattarai, K., Pokharel, B., Maharjan, S., & Adhikari, S. (2018). Chemical Constituents and Biological Activities of Ginger Rhizomes from Three Different Regions of Nepal. *J Nutri Diet Probiotics*, *2018*(1), 180005. https://www.academicstrive.com/JNDPS/JNDPS180005.pdf

Briskin D. P. (2000). Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant physiology, 124(2), 507–514. https://doi.org/10.1104/pp.124.2.507

Bucker, A., Falcao-Bucker, N. C., Nunez, C. V., De Souza Pinheiro, C. C., & Tadei, W. P. (2013). Evaluation of larvicidal activity and brine shrimp toxicity of rhizome extracts of Zingiber zerumbet (L.) Smith. *Revista Da Sociedade Brasileira De Medicina Tropical*, *46*(3), 377–380. https://doi.org/10.1590/0037-8682-1309-2013

C. Vitale-Brovarone, M. Miola, Balagna, C., & E. Verné. (2008). 3D-glass–ceramic scaffolds with antibacterial properties for bone grafting. *Chemical Engineering Journal*, *137*(1), 129–136. https://doi.org/10.1016/j.cej.2007.07.083

Campbell, D. L., Lawton, L. A., Beattie, K. A., & Codd, G. A. (1994b). Comparative assessment of the specificity of the brine shrimp and microtox assays to hepatotoxic (microcystin-LR-containing) cyanobacteria. *Environmental Toxicology and Water Quality*, *9*(1), 71–77. https://doi.org/10.1002/tox.2530090109

Chang, C.-C. ., Yang, M.-H. ., Wen, H.-M. ., & Chern, J.-C. . (2020). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, *10*(3). https://doi.org/10.38212/2224-6614.2748

Chen, I-Nan., Chang, C.-C., Ng, C.-C., Wang, C.-Y., Shyu, Y.-T., & Chang, T.-L. (2007). Antioxidant and Antimicrobial Activity of Zingiberaceae Plants in Taiwan. *Plant Foods for Human Nutrition*, *63*(1), 15–20. https://doi.org/10.1007/s11130-007-0063-7

Chibueze Izah, S. (2018). Some determinant factors of antimicrobial susceptibility pattern of plant extracts. *Research and Reviews: Insights, 2*(3). https://doi.org/10.15761/RRI.1000139

Chun, D. T. W., Foulk, J. A., & McAlister, D. D. (2009). Testing for antibacterial properties of cotton/flax denim. *Industrial Crops and Products*, *29*(2-3), 371–376. https://doi.org/10.1016/j.indcrop.2008.07.009

Colinge, J., Rix, U., Bennett, K. L., & Superti-Furga, G. (2012b). Systems Biology Analysis of kinase inhibitor protein target profiles in leukemia treatments. In *Lecture notes in computer science* (pp. 62–66). https://doi.org/10.1007/978-3-642-28792-3_9

Cowan, M. M. Plant Products as Antimicrobial Agents. 1999, 12, 20. - Bing. (2021). Bing. https://www.bing.com/search?q=Cowan%2C+M.+M.+Plant+Products+as+Antimicrobial+Agents.+1999%2C+12%2C+20.&cvid=915fcd3d0fc64efc88dfa795a2573293&gs_lcrp=EgRlZGdlKgYIABBFGDkyBggAEEUYOdIBCDEyNzZqMGo0qAIAsAIA&FORM=ANAB01&PC=U531

Demirkiran, O., Sabudak, T., Ozturk, M., & Topcu, G. (2013). Antioxidant and Tyrosinase Inhibitory Activities of Flavonoids fromTrifolium

nigrescensSubsp.petrisavi. Journal of Agricultural and Food Chemistry, 61(51), 12598–12603. https://doi.org/10.1021/jf403669k

Dingle, J., Reid, W. W., & Solomons, G. (1953). The enzymic degradation of -pectin and other polysaccharides. II—Application of the 'cup-plate' assay to the estimation of enzymes. *Journal of the Science of Food and Agriculture, 4*(3), 305–312. https://doi.org/10.1002/JSFA.2740040305

Fatemeh Jamshidi-Kia, Zahra Lorigooini, & Hossein Amini-Khoei. (2018). *Medicinal plants: Past history and future perspective*. ResearchGate; MaadRayan Publishing Company. https://www.researchgate.net/publication/322399333_Medicinal_plants_Past_history_and_future_perspective

Fitriady, M. A., Sulaswatty, A., Agustian, E., Salahuddin, & Aditama, D. P. F. (2017). *Steam distillation extraction of ginger essential oil: Study of the effect of steam flow rate and time process*. https://doi.org/10.1063/1.4973159

Fleming, A. (1929). On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. *British Journal of Experimental Pathology*, *10*(3), 226. https://pmc.ncbi.nlm.nih.gov/articles/PMC2048009/

Freire, J. C. P., Júnior, J. K. de O., Silva, D. de F., Sousa, J. P. de, Guerra, F. Q. S., & de Oliveira Lima, E. (2017). Antifungal Activity of Essential Oils againstCandida albicansStrains Isolated from Users of Dental Prostheses. *Evidence-Based Complementary and Alternative Medicine*, *2017*, 1–9. https://doi.org/10.1155/2017/7158756

Graf, B. A., Milbury, P. E., & Blumberg, J. B. (2005). Flavonols, flavones, flavanones, and human health: epidemiological evidence. *Journal of medicinal food*, *8*(3), 281-290.

Great Iruoghene Edo, Favour Ogheneoruese Onoharigho, Agatha Ngukuran Jikah, Gracious Okeoghene Ezekiel, Efeoghene, A., Helen Avuokerie Ekokotu, Ufuoma Ugbune, Oghroro, A., Oghenerume Lucky Emakpor, Irene Ebosereme Ainyanbhor, Patrick Othuke Akpoghelie, Aghoghoroghene Elizabeth Ojulari, Kennedy Ahamefula Okoronkwo, & Joseph Oghenewogaga

Owheruo. (2024). Evaluation of the physicochemical, phytochemical and anti-bacterial potential of Zingiber officinale (ginger). *Food Chemistry Advances*, *4*, 100625–100625. https://doi.org/10.1016/j.focha.2024.100625

Gupta, A., Malviya, R., Singh, T. P., & Sharma, P. K. (2010). Indian Medicinal Plants Used in Hair Care Cosmetics: A Short Review. *Pharmacognosy Journal*, *2*(10), 361–364. https://doi.org/10.1016/s0975-3575(10)80110-5

Hadi, M. Y., & Hameed, I. H. (2017). Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Chemical Compounds of Lepidium sativum: A Review. *Research Journal of Pharmacy and Technology*, *10*(11), 4039. https://doi.org/10.5958/0974-360x.2017.00732.6

Halliwell, B. (2007). Biochemistry of oxidative stress. *Biochemical Society Transactions*, *35*(Pt 5), 1147–1150. https://doi.org/10.1042/BST0351147

Handbook of Herbs and Spices | ScienceDirect. (2012). Sciencedirect.com. https://www.sciencedirect.com/book/9780857090393/handbook-of-herbs-and-spices

J, D., & D, D. (2010, September 1). *Origins and Evolution of Antibiotic Resistance*. Microbiology and Molecular Biology Reviews: MMBR. https://pubmed.ncbi.nlm.nih.gov/20805405/

Ji, H.-F., Li, X.-J., & Zhang, H.-Y. (2009). Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? *EMBO Reports*, *10*(3), 194–200. https://doi.org/10.1038/embor.2009.12

Ji, H.-F., Li, X.-J., & Zhang, H.-Y. (2009). Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? *EMBO Reports*, *10*(3), 194–200. https://doi.org/10.1038/embor.2009.12

Joseph, N., Anjum, N., & Tripathi, Y. (2016). Phytochemical screening and evaluation of polyphenols, flavonoids, and antioxidant activity of *Prunus cerasoides* D. Don leaves. *Journal of Pharmacy Research, 10*, 502–508.

Joshi, P., & Khanal, S. (2021). Production status, export analysis, and future prospects of ginger in Nepal. *Archives of Agriculture and Environmental Science*, *6*(2), 202–209. https://doi.org/10.26832/24566632.2021.0602012

Kamaliroosta, Z., Kamaliroosta, L., & Elhamirad, A. H. (2013). Isolation and identification of ginger essential oil. *Journal of Food Biotechnology, 8*.

Kanadea, R., & Bhatkhandeb, D. S. (2016). Extraction of ginger oil using different methods and effect of solvents, time, temperature to maximize yield. *International Journal of Scientific Research, 4*(2), 4.

Katz, L., & Baltz, R. H. (2016). Natural product discovery: past, present, and future. Journal of Industrial Microbiology & Biotechnology, 43(2-3), 155–176. https://doi.org/10.1007/s10295-015-1723-5

Khan, R. A. (2018). Natural products chemistry: The emerging trends and prospective goals. *Saudi Pharmaceutical Journal*, *26*(5), 739–753. https://doi.org/10.1016/j.jsps.2018.02.015

Kumar Sharma, P., Singh, V., & Ali, M. (2016). Chemical composition and antimicrobial activity of fresh rhizome essential oil of *Zingiber officinale* Roscoe. *Pharmacognosy Journal, 8*(3), 185–190. https://doi.org/10.5530/pj.2016.3.3

Kumar, C. Ganesh., & Anand, S. K. (1998). Significance of microbial biofilms in food industry: a review. *International Journal of Food Microbiology*, *42*(1-2), 9–27. https://doi.org/10.1016/s0168-1605(98)00060-9

Kumar, S., & Pandey, A. K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal*, *2013*(162750), 1–16. https://doi.org/10.1155/2013/162750

Malu, S. P., Obochi, G. O., Tawo, E. N., & Nyong, B. E. (2009). Antibacterial activity and medicinal properties of ginger (*Zingiber officinale*). *Global Journal of Pure and Applied Sciences*, *15*(3-4). https://doi.org/10.4314/gjpas.v15i3-4.48561

Mangram, A. J., Horan, T. C., Pearson, M. L., Silver, L. C., & Jarvis, W. R. (1999). Guideline for Prevention of Surgical Site Infection, 1999. *Infection Control & Hospital Epidemiology*, *20*(4), 247–280. https://doi.org/10.1086/501620

Mao, Q.-Q., Xu, X.-Y., Cao, S.-Y., Gan, R.-Y., Corke, H., Beta, T., & Li, H.-B. (2019). Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe). *Foods*, *8*(6), 185. https://doi.org/10.3390/foods8060185

Masuda, Y., Kikuzaki, H., Hisamoto, M., & Nakatani, N. (2004). Antioxidant properties of gingerol related compounds from ginger. *BioFactors*, *21*(1–4), 293–296. https://doi.org/10.1002/biof.552210157

Mayorga, P., Pérez, K. R., Cruz, S. M., & Cáceres, A. (2010). Comparison of bioassays using the anostracan crustaceans Artemia salina and Thamnocephalus platyurus for plant extract toxicity screening. *Revista Brasileira de Farmacognosia*, *20*(6), 897–903. https://doi.org/10.1590/s0102-695x2010005000029

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, J. (1982). Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Medica*, *45*(05), 31–34. https://doi.org/10.1055/s-2007-971236

Mian, S. S., Upadhayay, S., T, T., & Naqvi, S. N. (2019). Physicochemical analysis of ginger (*Zingiber officinale* Rosc.) rhizome along with its TLC, HPLC, and HPTLC profile. *Pharmaceutical Methods, 10*(1), 31–36. https://doi.org/10.5530/phm.2019.1.6

Mishra, K., Ojha, H., & Chaudhury, N. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: Critical review and results. *Food Chemistry, 130*, 1036–1043. https://doi.org/10.1016/j.foodchem.2011.07.127

Mohd Sahardi, N. F. N., & Makpol, S. (2019). Ginger (Zingiber officinale Roscoe) in the Prevention of Ageing and Degenerative Diseases: Review of Current Evidence. *Evidence-Based Complementary and Alternative Medicine*, *2019*, 1–13. https://doi.org/10.1155/2019/5054395

Mostafa, N. M. (2018). Antibacterial activity of ginger (*Zingiber officinale*) leaves essential oil nanoemulsion against the cariogenic *Streptococcus mutans*. *Journal of Applied Pharmaceutical Science, 8*(09), 8. https://doi.org/10.7324/JAPS.2018.8906

Mukherjee, P., Venkatesh, P., & Ponnusankar, S. (2010). Ethnopharmacology and

integrative medicine - Let the history tell the future. *Journal of Ayurveda and Integrative Medicine*, *1*(2), 100. https://doi.org/10.4103/0975-9476.65077

Nadeen Waleed Al-Areer, Azzam, A., Al, R. H., Ibrahim Al-Deeb, Lyazzat Bekbayeva, & El-Sayed Negim. (2023). Quantitative analysis of total phenolic and flavonoid compounds in different extracts from ginger plant (Zingiber officinale) and evaluation of their anticancer effect against colorectal cancer cell lines. *Pharmacia*, *70*(4), 905–919. https://doi.org/10.3897/pharmacia.70.e103936

Nader, M. I., Ali, S. A., Ghanima, K. K., & Azhar, D. A. (2010). Antibacterial activity of ginger extracts and its essential oil on some of pathogenic bacteria. *Baghdad Science Journal, 7*(3), 8.

Nader, M. I., Kais Kassim Ghanima, Ali, S. A., & Azhar, D. A. (2010). Antibacterial activity of ginger extracts and its essential oil on some of pathogenic bacteria. *Baghdad Science Journal*, *7*(3), 1159–1165. https://doi.org/10.21123/bsj.2010.7.3.1159-1165

Naik, L. S., P. Shyam, Marx, P., & Devi, C. V. R. (2015). Antimicrobial activity and phytochemical analysis of Ocimum tenuiflorum leaf extract. *International Journal of PharmTech Research*, *8*(1), 88–95. https://www.researchgate.net/publication/282379057_Antimicrobial_activity_and_phytochemical_analysis_of_Ocimum_tenuiflorum_leaf_extract

Najafian, M., Najafian, B., & Najafian, Z. (2016). The Effect of Aspalathin on Levels of Sugar and Lipids in Streptozotocin-Induced Diabetic and Normal Rats. *Zahedan Journal of Research in Medical Sciences*, *In Press*(In Press). https://doi.org/10.17795/zjrms-4963

Newman, D. J. (2008). Natural Products as Leads to Potential Drugs: An Old Process or the New Hope for Drug Discovery? *Journal of Medicinal Chemistry*, *51*(9), 2589–2599. https://doi.org/10.1021/jm0704090

Newman, D. J., Cragg, G. M., & Snader, K. M. (2000). The influence of natural products upon drug discovery (Antiquity to late 1999). *Natural Product Reports*, *17*(3), 215–234. https://doi.org/10.1039/a902202c

Omeke, J. N., Anaga, A. O., & Okoye, J. A. (2018). Brine shrimp lethality and acute toxicity tests of different hydro-methanol extracts of Anacardium occidentale using in vitro and in vivo models: a preliminary study. *Comparative Clinical Pathology*, *27*(6), 1717–1721. https://doi.org/10.1007/s00580-018-2798-y

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science, 5*(e47). https://doi.org/10.1017/jns.2016.41

Parr, A. J., & Bolwell, G. P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, *80*(7), 985–1012. https://doi.org/10.1002/(sici)1097-0010(20000515)80:7%3C985::aid-jsfa572%3E3.0.co;2-7

Quideau, S., Deffieux, D., Douat-Casassus, C., & Pouységu, L. (2011). Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angewandte Chemie International Edition*, *50*(3), 586–621. https://doi.org/10.1002/anie.201000044

Ravi Kiran, C., Chakka, A. K., Padmakumari Amma, K. P., Nirmala Menon, A., Sree Kumar, M. M., & Venugopalan, V. V. (2013). Essential oil composition of fresh ginger cultivars from North-East India. *Journal of Essential Oil Research*, *25*(5), 380–387. https://doi.org/10.1080/10412905.2013.796496

Rawal, P., & Adhikari, R. S. (2016). Evaluation of antifungal activity of *Zingiber officinale* against *Fusarium oxysporum* f.sp. *lycopersici*. *Advances in Applied Science Research, 7*(2), 5–9.

Sabudak, T., Demirkiran, O., Ozturk, M., & Topcu, G. (2013b). Phenolic compounds from Trifolium echinatum Bieb. and investigation of their tyrosinase inhibitory and antioxidant activities. *Phytochemistry*, *96*, 305–311. https://doi.org/10.1016/j.phytochem.2013.08.014

Sarah, Q. S., Anny, F. C., & Misbahuddin, M. (2017). Brine shrimp lethality assay. *Bangladesh Journal of Pharmacology, 12*(2). https://doi.org/10.3329/bjp.v12i2.32796

Shi, Q.-W., Li, L.-G., Huo, C.-H., Zhang, M.-L., & Wang, Y.-F. (2010). Study on natural medicinal chemistry and new drug development. *Chinese Traditional*

and Herbal Drugs, *41*(10), 1583–1589. https://www.researchgate.net/publication/286474432_Study_on_natural_medicinal_chemistry_and_new_drug_development

Shirin, A. P. R., & Jamuna, P. (2010). Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). *Journal of Medicinal Plants Research, 4*(24), 2674–2679. https://doi.org/10.5897/JMPR09.464

Shrestha, R., Shakya, A., & Shrestha, K. K. (2015). Phytochemical screening and antimicrobial activity of *Asparagus racemosus* wild. and *Asparagus curillus* Buch-Ham. Ex Roxb. *Journal of Natural History Museum, 29*, 91–102.

Shukla, A., Goud, V. V., & Das, C. (2019). Antioxidant potential and nutritional compositions of selected ginger varieties found in Northeast India. *Industrial Crops and Products, 128*, 167–176. https://doi.org/10.1016/j.indcrop.2018.10.086

Sivasothy, Y., Chong, W. K., Hamid, A., Eldeen, I. M., Sulaiman, S. F., & Awang, K. (2011). Essential oils of Zingiber officinale var. rubrum Theilade and their antibacterial activities. *Food Chemistry*, *124*(2), 514–517. https://doi.org/10.1016/j.foodchem.2010.06.062

Srinivasa Reddy, P., Jamil, K., Madhusudhan, P., Anjani, G., & Das, B. (2001). Antibacterial Activity of Isolates from Piper longum and Taxus baccata. *Pharmaceutical Biology*, *39*(3), 236–238. https://doi.org/10.1076/phbi.39.3.236.5926

STOILOVA, I., KRASTANOV, A., STOYANOVA, A., DENEV, P., & GARGOVA, S. (2007). Antioxidant activity of a ginger extract (Zingiber officinale). *Food Chemistry*, *102*(3), 764–770. https://doi.org/10.1016/j.foodchem.2006.06.023

Tohma, H. (2016). Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS. *Food Science & Technology, 12*. https://doi.org/10.1007/s11694-016-9423-z

Videla, H. A. (2002). Prevention and control of biocorrosion. *International Biodeterioration & Biodegradation*, *49*(4), 259–270. https://doi.org/10.1016/s0964-8305(02)00053-7

Wood, A. J. J., Friedland, I. R., & McCracken, G. H. (1994). Management of

Infections Caused by Antibiotic-Resistant Streptococcus Pneumoniae. *New England Journal of Medicine*, *331*(6), 377–382. https://doi.org/10.1056/nejm199408113310607

Yasuyuki, M., Kunihiro, K., Kurissery, S., Kanavillil, N., Sato, Y., & Kikuchi, Y. (2010). Antibacterial properties of nine pure metals: a laboratory study using Staphylococcus aureus and Escherichia coli. *Biofouling*, *26*(7), 851–858. https://doi.org/10.1080/08927014.2010.527000

Yasuyuki, M., Kunihiro, K., Kurissery, S., Kanavillil, N., Sato, Y., & Kikuchi, Y. (2010). Antibacterial properties of nine pure metals: a laboratory study using Staphylococcus aureus and Escherichia coli. *Biofouling*, *26*(7), 851–858. https://doi.org/10.1080/08927014.2010.527000

Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules, 21*(5), 559. https://doi.org/10.3390/molecules21050559

Zancan, K. C., Marques, M. O. M., Petenate, A. J., & Meireles, M. Angela. A. (2002). Extraction of ginger (Zingiber officinale Roscoe) oleoresin with CO2 and co-solvents: a study of the antioxidant action of the extracts. *The Journal of Supercritical Fluids*, *24*(1), 57–76. https://doi.org/10.1016/s0896-8446(02)00013-x

APPENDIX-1

Phytochemical screening

1. Test for reducing sugars (Fehling's Test)

The methanolic solution was mixed with distilled water first and then filtered. To each filtrate, a few drops of Fehling's solution A and B (1:1) were added and warmed over a water bath for 30 minutes. The appearance of an orange red precipitate confirmed the presence of reducing compounds. To this solution, 5 % (w/v) ferric chloride solution (3 drops) was added and observed. The formation of greenish-blue color indicated the presence of polyphenols.

2. Test for basic alkaloids

The methanol extract (10 mL) was concentrated to yield a residue and dissolved in 3 mL of 2 % (v/v) HCl. This solution was equally divided into two test tubes.

I. **Meyer's Test:** The first test solution was treated with three drops of Meyer's reagent. White precipitate indicated the presence of basic alkaloids.

II. **Dragendrof's Test:** The second test solution was treated with three drops of Dragendrof's reagent. White precipitate indicated the presence of basic alkaloids.

3. Test for glycosides

The methanol extract (8 mL) was concentrated to half the original volume and divided into two test tubes.

I) The first test solution (2 mL) was treated with 25 % (v/v) ammonium hydroxide solution (2 mL) and shaken vigorously. A cherry red color indicated the presence of glycosides.

II) **Molisch's Test:** The second test solution was treated with 5 mL Molisch's reagent and conc. H_2SO_4 were added dropwise from the side of the test tube without disturbing the solution. A violet ring at the junction of two liquid layers was observed which turned violet completely on shaking indicating the presence of glycosides or free sugars.

4. Test for quinones

To the methanolic extract (2 mL), freshly prepared ferrous sulphate solution (1 mL) and ammonium thiocyanate (few crystals) was added and treated with conc. H_2SO_4 drop by drop. The deep red color was persistent indicating the presence of quinones.

5. Test for saponins

The methanolic extract (4 mL) was concentrated and mixed with hot water (2 mL). The solution was shaken vigorously for 15 seconds. The formation of persistent foam indicated the presence of saponins.

6. Test for coumarins

Firstly, single pellets of KOH were taken and dissolved in 1 mL of ethanol. Then 1 mL of extract solution was added. The presence of precipitate indicates the presence of coumarins.

7. Test for flavonoids

5 mL of dilute ammonia solution was added to the aqueous filtered solution of each fraction followed by the addition of concentrated sulphuric acid. The appearance of yellow color indicated the presence of flavonoids. The yellow color disappeared after some time.

8. Test for terpenoids (Salkowsky's Test)

The small amount of extract was dissolved in chloroform and an equal volume of conc. H_2SO_4 was added. Reddish-brown coloration at the interface of two layers indicated the presence of terpenoids.

APPENDIX-2

Preparation of reagents

1. Meyer's reagent

Mercuric chloride (0.679 g) was weighed in a 50 mL volumetric flask and dissolved in a distilled water. To this solution, potassium iodide (2.5 g) was added. The scarlet red precipitate was dissolved by shaking and then diluted with distilled water up to the mark of the volumetric flask.

2. Dragendroff's reagent

Bismuth nitrate was dissolved in 5 N nitric acid (10 mL) to make solution A. next potassium iodide (13.5 g) was dissolved in distilled water (20 mL) to make solution B. These solutions A and B were mixed in a 50 mL volumetric flask.

3. Molisch's reagent

α-naphthol or thymol (5 g) was dissolved in methanol (50 mL).

4. Neutral ferric chloride solution

Ferric chloride (1 g) was dissolved in distilled water (100 mL). To this aqueous solution, sodium carbonate is added little by little with stirring until the slight turbidity persisted. The mixture was filtered, and the colorless filtrate was used as neutral ferric chloride.

APPENDIX 3

Total Flavonoid Content

Ginger sample	Absorption			Inhibition		
S1(EtAc)	0.078	0.115	0.094	13.3	16.4	14.3
S1(MEOH)	0.171	0.159	0.167	22.1	20.9	21.7
S1(DCM)	0.082	0.11	0.095	13.2	16	14.5
S2(EtAc)	0.195	0.187	0.192	24.2	23.7	23.2
S2(MEOH)	0.222	0.184	0.214	27.2	24.4	26.3
S2(DCM)	0.171	0.159	0.167	20.23	21.5	19.3

Total Phenolic Content

Ginger sample	Absorption			Inhibition		
S1(EtAc)	1.13	1.087	1.172	62.45	58.8911	63.3333
S1(MEOH)	1.577	1.694	1.553	86.8333	93.5333	85.5
S1(DCM)	0.845	0.728	0.808	45.1667	36.6667	44.1111
S2(EtAc)	2.406	2.56	2.685	87.3256	94.4444	85.2778
S2(MEOH)	1.59	1.732	1.495	136.889	141.444	146.389
S2(DCM)	1.323	1.426	1.527	42.0052	41.3777	46

Absorption and inhibition of ginger extract collected from Arghakhanchi district S1 (EtOAc)

Concentration	Absorption			Inhibition		
1000	0.077	0.061	0.073	82.28528	85.96626	83.20552
500	0.101	0.104	0.109	76.7638	76.07362	74.92331
250	0.201	0.194	0.19	53.75767	55.3681	56.28834
125	0.248	0.262	0.243	42.94479	39.72393	44.09509
62.5	0.292	0.287	0.286	32.82209	33.97239	34.20245

Absorption and inhibition of ginger extract collected from Arghakhanchi district S1(MEOH)

Concentration	Absorption			Inhibition		
1000	0.05	0.054	0.059	88.45266	87.52887	86.37413
500	0.068	0.069	0.069	69.23077	68.77828	68.77828
250	0.236	0.192	0.226	45.70552	55.82822	48.00613
125	0.304	0.291	0.292	30.06135	33.05215	32.82209
62.5	0.307	0.318	0.32	29.37117	26.84049	26.38037

Absorption and inhibition of ginger extract collected from Arghakhanchi district S1(DCM)

Concentration	Absorption			Inhibition		
1000	0.084	0.083	0.088	80.64516	80.87558	79.7235
500	0.189	0.207	0.217	56.5184	52.3773	50.07669
250	0.263	0.253	0.26	39.49387	41.79448	40.18405
125	0.289	0.294	0.294	33.51227	32.36196	32.36196
62.5	0.26	0.259	0.282	40.18405	40.41411	35.1227

Absorption and inhibition of ginger extract collected from Dang district S2(EtOAc)

Concentration	Absorption			Inhibition		
1000	0.05	0.054	0.05	88.24885	87.5576	88.01843
500	0.104	0.106	0.107	73.04147	72.58065	72.11982
250	0.185	0.187	0.192	57.60369	57.60369	55.52995
125	0.253	0.227	0.253	41.80791	47.45763	41.80791
62.5	0.347	0.352	0.37	20.96774	18.66359	13.13364

Absorption and inhibition of ginger extract collected from Dang district S2(DCM)

Concentration	Absorption			Inhibition		
1000	0.179	0.17	0.172	58.81902	60.88957	60.42945
500	0.208	0.192	0.211	52.14724	55.82822	51.45706
250	0.283	0.279	0.264	34.89264	35.81288	39.2638
125	0.32	0.31	0.313	26.38037	28.68098	27.9908

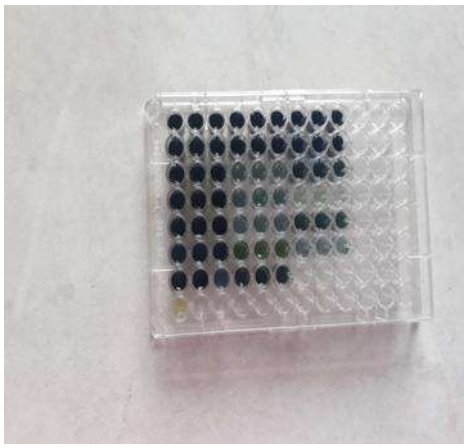
Brine Shrimp Toxicity Assay of all ginger samples extract

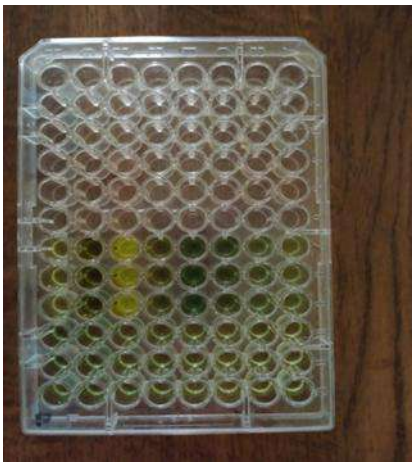
S.N	Ginger sample	Z	X	Y	XY	X^2	∑X	∑Y	∑XY	$\sum X^2$	B	A	X	LC_{50}
1.		10	1	4.75	4.75	1								
	S1						6	15	30.5	14	0.25	4.5	5.0	1×10^5
2.	(EtAc)	100	2	5	10	4								
3.		1000	3	5.25	15.75	9								
4.	S1	10	1	3.72	3.72	1								
	(MEOH)						6	12.88	27.04	14	0.64	3.013	3.50	3×10^3
5.		100	2	4.16	8.32	4								
6.		1000	3	5	15	9								
7.	S2	10	1	3.72	3.72	1								
	(EtAc)						6	13.99	29.78	14	0.9	2.863	4.65	4.6×10^4
8.		100	2	4.75	9.5	4								
9.		1000	3	5.52	16.56	9								
10.	S2	10	1	4.16	4.16	1								
	(MEOH)						6	13.64	28.12	14	0.42	3.706	4.54	3.5×10^4
11.		100	2	4.48	8.95	4								
12.		1000	3	5	15	9								

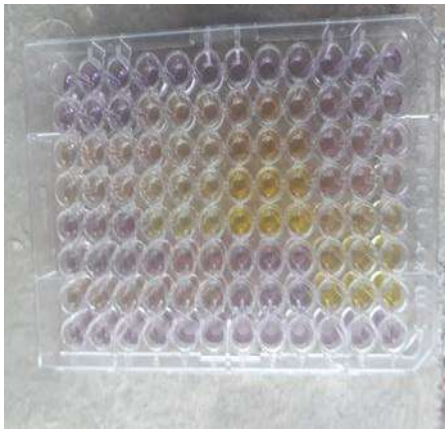
Number of survived nauplii after taking with ginger extract and their percentage mortality

S. N	Ginger sample	Concentration µg/ml	No. of nauplii taken	No. of survived nauplii	Mortality %
1.	S1(EtAc)	10	10	6	40 %
2.		100	10	5	50 %
3.		1000	10	6	40 %
1.	S1(MEOH)	10	10	9	10 %
2.		100	10	8	20 %
3.		1000	10	5	50 %
1.	S2(EtAc)	10	10	9	10 %
2.		100	10	6	40 %
3.		1000	10	3	70 %
1.	S2(MEOH)	10	10	8	20 %
2.		100	10	7	30 %
3.		1000	10	5	50 %

APPENDIX 4: PHOTOS







PLAGIARISM REPORT

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
वीरेन्द्र बहुमुखी क्याम्पस
BIRENDRA MULTIPLE CAMPUS
☎ : 056 {493253 493689 493159
भरतपुर, चितवन
Bharatpur, Chitwan

पत्रसंख्या :
च. नं.(Ref.) :

मिति :
Date : २०८१।१०।२३

जो जस सँग सम्बन्धित छ ।

तपाई PURNIMA BANJADE ले यस पुस्तकालयमा प्लेजारिजम परीक्षण गर्नका लागि हार्डकपी र सफ्टकपीको विषय वस्तुमा कुनै फरक छैन भनी स्वघोषणा गरी पेनड्राइभ/ईमेल मार्फत विभागमा पेश गर्नुभएकोले विभागबाट इमेल मार्फत प्राप्त सफ्टकपी ANTIOXIDENT AND BIOLOGICAL EVALUATION OF METHANOLIC, ETHYL ACETATE, AND DICHLOROMETHANE EXTRACT OF *Zingiber officinale* COLLECTED FROM TWO DIFEERENT DISTRICTS OF NEPAL शिर्षकको **M.Sc. in CHEMISTRY** तहको उपाधिका लागि तयार गरिएको Dissertation / Thesis मा प्लेजारिजम परीक्षण पछिको समानता सूची (Similarity Index) ८(आठ) प्रतिशत रहेको व्यहोरा प्रमाणित गरिन्छ ।

.....
महेन्द्रप्रसाद अधिकारी
पुस्तकालय प्रमुख

Library LP_5

PURNIMA BANJADE

ANTIOXIDENT AND BIOLOGICAL EVALUATION OF METHANOLIC, ETHYL ACETATE, AND DICHLOROMETHANE EX...

Tribhuvan University

Document Details

Submission ID
trn:oid:::3117:426755784

Submission Date
Feb 5, 2025, 11:19 AM GMT+5:45

Download Date
Feb 5, 2025, 12:16 PM GMT+5:45

File Name
3rd final-chem-thesis purnima.docx

File Size
236.0 KB

46 Pages

9,836 Words

54,678 Characters

iThenticate Page 1 of 50 - Cover Page Submission ID trn:oid:::3117:426755784

Submission ID trn:oid:::3117:426755784

8% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Small Matches (less than 8 words)

Match Groups

- **16** Not Cited or Quoted 5%
  Matches with neither in-text citation nor quotation marks
- **11** Missing Quotations 3%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

7% Internet sources
4% Publications
0% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

iThenticate Page 2 of 50 - Integrity Overview

Submission ID trn:oid:::3117:426755784