PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ANALYSIS OF *Tectaria coadunata* IN DHARAN, EASTERN NEPAL

A PROJECT WORK SUBMITTED TO THE

DEPARTMENT OF BIOLOGY

CENTRAL CAMPUS OF TECHNOLOGY

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUWAN UNIVERSITY

NEPAL

FOR THE AWARD OF

BACHELOR OF SCIENCE (B.Sc.) IN BOTANY

BY

PUSPA KARKI

SYMBOL No: 500080039

T.U. REGISTRATION No: 5-2-8-82-2018

[12, June,2023]

DECLARATION

This project work entitled "**PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF** *Tectaria coadunata* **IN DHARAN, EASTERN NEPAL**" is being submitted to the Department of Botany, Central campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the Partial Fulfillment of the requirement to the project work in Bachelor of Science (B.Sc.) degree in Botany. This project is carried out by me under the supervision of Asst. Prof. Sanju Parajuli in the Department of Botany, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

This work is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

Puspa Karki

Symbol No: 500080039

Registration No: 5-2-8-82-2018

[12, June, 2023]

LETTER OF FORWARD

[Date: 12/06/2023]

On the recommendation of **Asst. Prof. Sanju Parajuli**, this project work is submitted by **Puspa Karki**, Symbol No: 500080039, T.U. Registration No: 5-2-8-82-2018, entitled **"Phytochemical Analysis and Antimicrobial Activity of *Tectaria coadunata* in Dharan, Eastern Nepal"** is forwarded by the Department of Botany, Central Campus of Technology, for the approval to the Evaluation Committee, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

He/She has fulfilled all the requirements laid down by the Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the Project work.

Asst. Prof. Sanju Parajuli
Head of Department
Department of Botany
Central Campus of Technology
Tribhuvan University

[12, June, 2023]

BOARD OF EXAMINATION AND CERTIFICATE OF APPROVAL

This Project work (PRO-406) entitled **"PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF** *Tectaria coadunata* **IN DHARAN, EASTERN NEPAL"** by Puspa Karki (Symbol No: 500080039 and T.U. Registration No. 5-2-8-82-2018) under the supervision of Asst. Prof Sanju Parajuli in the Department Biology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), is hereby submitted for the partial fulfillment of the Bachelor of Science (B.Sc.) degree in Botany. This report has been accepted and forwarded to the Controller of Examination, Institute of Science and Technology, Tribhuvan University, Nepal for the legal procedure.

.....

Asst. Prof. Sanju Parajuli
Supervisor
Department of Biology
Central Campus of Technology
Tribhuvan university

.....

Assoc. Prof. Dr. Bhabindra Niroula
External
Department of Botany
Degree Campus Biratnagar
Tribhuvan University

.....

Asst. Prof. Mr. Pramod Sen Oli
Internal
Department of Biology
Central Campus of Technology
Tribhuvan University

.....

Asst. Prof. Sanju Parajuli
Head of Department
Department of Biology
Central Campus of Technology
Tribhuvan University

[12, JUNE, 2023]

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Ms. Puspa Karki

Symbol No: 500080039

Registration No: 5-2-8-82-2018

ABSTRACT

This study aims to evaluate phytochemicals and antimicrobial activity of medicinal ferns *Tectaria coadunata*. It was conducted from September 2022 to April 2023. Ferns which have been commonly used by local people for medicinal purpose *Tectaria coadunata* was collected from Panchakanya jungle, Dharan, Sunsari district. The collected ferns were shade dried, grind into powder and extracted with Methanol, Petroleum ether and aqueous extraction using Soxhlet extractor. The different solvents extract was evaluated and presence of phytochemicals like phenol, steroid, carbohydrates, glycosides, amino acid, alkaloids, flavonoids, terpenoids, saponins, and tannins. The TPC and TFC were also evaluated that ranged from 16.30 mg GAE/g to 57.48 mg GAE/g and 29.86 mg QE/g to 96.90 mg QE/g. Also tested for antimicrobial activity for two gram negative (*Escherichia coli* and *Salmonella typhi*) and two gram positive bacteria *(Bacillus subtilis* and *Staphylococcus aureus).* The antibacterial activity was done through disc diffusion method and Zone of inhibition (ZOI) was observed. The ZOI range from 8.5 to 23.5 mm. Overall result shows that the plant extracts have interesting antibacterial activity.

Keywords: *Tectaria coadunata*, Phytochemicals, Antimicrobial activity, Phenolic, Flavonoid compound.

शोधसार

यस अध्ययनले औषधीय फर्न टेकटेरिया कोआडुनाटाको फाइटोकेमिकल्स र एन्टिमाइक्रोबियल गतिविधिको मूल्याङ्कन गर्ने उद्देश्य राखेको छ। यो सेप्टेम्बर २०२२ देखि अप्रिल २०२३ सम्म सञ्चालन गरिएको थियो। स्थानीय मानिसहरूले औषधिको लागि प्रयोग गर्ने फर्नहरू टेक्टेरिया कोडुनाटा पञ्चकन्या जङ्गल, धरान, सुनसरी जिल्लाबाट सङ्कलन गरिएको थियो। सङ्कलन गरिएका फर्नलाई छायामा सुकाएर पाउडरमा पिसेर मिथेनोल, पेट्रोलियम ईथर र जलीय निकासी गरी सोक्सलेट एक्स्ट्रक्टरको प्रयोग गरी निकासी गरियो। विभिन्न सॉल्भेन्ट्स एक्स्ट्रयाक्टको मूल्याङ्कन गरिएको थियो र फाइटोकेमिकल्स जस्तै फिनोल, स्टेरोइड, कार्बोहाइड्रेट, ग्लाइकोसाइड्स, एमिनो एसिड, एल्कालोइड्स, फ्लेभोनोइड्स, टेरपेनोइड्स, सेपोनिन र ट्यानिन्सको उपस्थिति थियो। TPC र TFC लाई पनि मूल्याङ्कन गरिएको थियो जुन 16.30 mg GAE/g देखि 57.48 mg GAE/g र 29.86 mg QE/g देखि 96.90 mg QE/g सम्म थियो। दुई ग्राम negative(*Escherichia coli* र *Salmonella typhi*) र दुई ग्राम positive ब्याक्टेरिया (*Bacillus subtilis* र *Staphylococcus aureus*) को लागि एन्टिमाइक्रोबियल गतिविधिको लागि पनि परीक्षण गरियो। एन्टिब्याक्टेरियल गतिविधि डिस्क प्रसार विधि मार्फत गरिएको थियो र निषेध क्षेत्र (ZOI) अवलोकन गरिएको थियो। ZOI 8.5 देखि 23.5 मिमी सम्म हुन्छ। समग्र परिणामले देखाउँछ कि बिरुवाको राम्रो एन्टिब्याक्टेरियल गतिविधि छ।

Keywords: *Tectaria coadunata*, Phytochemicals, Antimicrobial activity, Phenolic, Flavonoid compound.

LIST OF ACRONYMS AND ABBREVIATIONS

AST:	Antimicrobial Susceptibility Test
AA	Aqueous Extract
DMSO:	Dimethyl Sulfoxide
GC-MS:	Gas Chromatography- Mass Spectrometry
GAE:	Gallic Acid Equivalent
ME:	Methanol Extract
MIC:	Minimum Inhibitory Concentration
MHA:	Mueller Hinton Agar
NA:	Nutrient Agar
NB:	Nutrient Broth
QE:	Quercetin Equivalent
PE:	Petroleum Ether
Spp.:	Species
TPC:	Total Phenolic Content
TPC:	Total Flavonoid Content
ZOI:	Zone of Inhibition

LIST OF SYMBOLS

+	Positive result
-	Negative result
%	Percentage
mm	Millimeter
&	And
Nm	nanometer
℃	Degree Celsius
µl	Microliter

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CHAPTER 1

1. INTRODUCTION

1.1 General Introduction

The seedless vascular plant known as pteridophytes were flourishing in this global village 400 million year ago (Thomas et al., 2021). Humans have relied on plants as a significant source of medicine from the dawn of mankind. Many rural and tribal groups still rely significantly on the natural materials derived from the surrounding forested areas to heal a variety of maladies and disorders. Indian traditional medicine is based on diverse systems used by distinct tribal people, including Ayurveda, Siddha, and Unani. Unfortunately, there have only been a few studies on the therapeutic potential of Pteridophytes, despite the fact that many have been conducted on the medicinal characteristics of plants, particularly angiosperms (Saha & Bikash Bhandari, n.d.). Pteridophytes includes ferns and their relatives. Because of our nation's enormous forests and lofty Himalayan peaks, they make up a sizable portion of plant biomass. The sporophyte and gametophyte phases of their sexual life cycle alternate between the two generations. While a gametophyte is very unnoticeable, fern sporophytes are quite frequent and recognizable plants in the vegetation (Kandel et al., 2021).Pteridophytes are frequently used as food and medicine in traditional cultures all over the world. Numerous ethnic groups have extensive knowledge of the usage of pteridophytes, and it is crucial for health care, food security, and conservation to record this information as well as the biodiversity of edible and medicinal pteridophytes. However proper use and documentation gathering information about the uses of these ferns have not been done (Ojha & Devkota, 2021).

Drugs made from the plants are simple to obtain, affordable, safe, and effective, and they have fewer side effects. In some regions, ferns have long coexisted with humans and have had a significant positive impact on people by serving as traditional medicines or remedies for a variety of ailments including ascarid illness, bleeding, trauma, burning, diarrhea, and colds (Mir et al., 2014). For many years, ferns coexisted with people and helped millions of people in several nations by serving as traditional medicines for ailments such ascarid disease, bleeding, trauma, burning, diarrhea, colds, and many more. Numerous beneficial phytochemicals or secondary metabolites, including as alkaloids, flavonoids, phenols, steroids, terpenoids, different amino acids, and fatty acids, have been found in ferns(Mir et al., 2014).The vast majority of ferns have enormous potential as antioxidants, according to

studies on the biological potential of ferns (Bajracharya & Bajracharya, 2022). *Diplazium esculentum* (Dauthe Niuro), *Diplazium maximum* (Shrawane Niuro), and *Tectaria coadunata* (Kalo Niuro), are three of Nepal's edible ferns that are frequently consumed and sold in markets (Kandel et al., 2021).

The change in the life style of human population has increased the rate of development of various life-threatening oxidative stress related diseases like cancer, diabetes, atherosclerosis, arthritis, Alzheimer's disease, other neurodegenerative disorder etc. Herbal remedies have also been utilized as antimicrobials, antivirals, anti-inflammatory, anti-rheumatic, antiallergic, etc. (Jaishee et al., 2016).According to studies, natural products with a high concentration of phenolic compounds and flavonoids have biological properties like anti-inflammatory, antioxidant, antibacterial, and anticancer properties. These active metabolites, especially those brought on by oxidative stress, can help treat and prevent the onset of age-related diseases. In complementary and alternative medicine, nutraceuticals, food supplements, and pharmaceutical bioactive metabolites of novel chemical entities, natural products are essential components of traditional knowledge systems. The usage of medicinal plants is also a key source of conventional pharmaceuticals, especially in light of the significant benefits of bioactive compounds in these plants and the pharmaceutical and nutraceutical businesses focus on research and development (Fonmboh et al., 2020).

The substances that are naturally present in plants are known as phytochemicals. These phytochemicals come from many different plant parts, including the leaves, flowers, seeds, barks, roots, and pulp. Due to their many medical applications, these phytochemicals are becoming more and more well-known nowadays. Asthma, arthritis, cancer, and other disorders are all greatly aided by phytochemicals. These phytochemicals don't have any negative side effects, unlike pharmaceutical compounds. The phytochemicals can also be referred to as "man-friendly medicines" because they treat illnesses without endangering humans (Sahira Banu & Cathrine, 2015).Fern like *T. coadunata* is used to cure a variety of conditions, including giardiasis, gastrointestinal problems, diarrhea, and jaundice, as well as to get rid of worms. Leaves are crushed and juice applied to the cut wounds to stop bleeding. The rhizome of *T. coadunata* is used for its anthelmintic activity and against stomach pains and gastrointestinal disorders(Shrestha et al., 2019).The *Tectaria coadunata* rhizome extract is used to cure leprosy and other skin conditions, while the leaf extract is used to treat asthma, bronchitis, honeybee stings, diarrhea, and blood ulcer disease (Fonmboh et al., 2020).

1.2 Rationale

Ferns are one of the underappreciated plant resources by both scientific and local people while having significant ethnomedicinal properties. Pharmacological studies show that ferns and fern-allies have a wide range of biological properties, including those that are antibacterial, antiviral, antifungal, antimalarial, antidiarrheal, anthelmintic, analgesic, anti-inflammatory, anticancer, neuroprotective, nephroprotective, hepatoprotective, etc. Important secondary metabolites found in ferns include terpenoids and flavonoids(Bajracharya & Bajracharya, 2022). Despite of diversity and plant resources carrying vast potential in medicinal field, proper documentation of fern species has been scarcely done. There are many fern species that has not been utilized which carry vast potential. The main aim of this study is that it may contribute to the identification of novel compounds with pharmacological properties that could serve as leads for the development of new medications.

1.3 Objectives

1.3.1 General Objectives

The general objectives of this project are to study the phytochemicals and antimicrobial activity present in fern *Tectaria coadunata* found in Dharan Eastern Nepal.

1.3.2 Specific Objective

i. To analyze the different phytochemicals of the fern *Tectaria coadunata* from its leaf and rhizome both qualitatively and quantitatively.
ii. To determine the total phenolic content (TPC) and total flavonoid content (TFC) of different extracts of *Tectaria coadunata* by using the Folin-Ciocalteu method and aluminum chloride Calorimetric method respectively.
iii. To perform antibacterial susceptibility test from prepared plant extract in *E. coli, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus.*
iv. To determine the minimum inhibitory concentration (MIC) of the most active extracts of *Tectaria coadunata* against *E. coli.*

CHAPTER 2

2. LITERATURE REVIEW

A significant source of medicine is plants. In several cultural societies, fern and fern allies have been utilized as medicine for ages. Different pteridologists occasionally conduct experiments to demonstrate the antibacterial activity of various plant components, such as the leaf or rhizome, which are used to treat infections(Saha, 2022). Plant-based phytochemicals are used as a model to create safer and more potent medications for preventing the growth of microorganisms(Khalid et al., 2016). Out of the 34 pteridophytes species verified among them, flavonoids are found in 27 (79.41%), phenolic compounds and tannins in 26 (76.47%), glycosides in 24 (70.58%), terpenoids in 23 (67.64%), saponins in 22 (64.70%), volatile oils in 18 (52.94%), alkaloids in 15 (44.11%), phlobatannins in 12 (35.29%), and resins in only three (8.35%). Similarly, *Diplazium maximum, Asplenium trichomanes,* and *Adiantum venustum* have been found to contain the highest amounts of phytochemicals, while *Polystichum discretum* and *Dryopteris blanfordii* had the lowest amounts(Mir et al., 2014).

According to the results of the phytochemical screening, there is a modest concentration of alkaloids, carbohydrates, flavonoids, saponins, terpenes, and steroids. Some of these chemical components have been linked to antioxidant activities and consequently have therapeutic potential. The inoculation in Mueller-Hinton agar led to the confirmation of bacterial growth suppression (Malviya et al., 2012). A review of the literature found that a total of 26 pteridophyte species were utilized as food and 43 species as traditional remedies. 14 of the 55 beneficial species were utilized both as traditional medicines and food. Common culinary species were *Diplazium esculentum*, *Diplazium maximum*, *Dryopteris cochleata,* and *Ophioglossum reticulatum,* while common medicinal pteridophytes included *Aleuritopteris albomarginata*, *Equisetum ramosissimum*, *Nephrolepis cordifolia,* and *Tectaria coadunate* (Ojha & Devkota, 2021).

It was discovered in the current investigation that methanolic extracts have higher antibacterial activity than ethanolic extracts. The inhibitory zone against Bacillus subtilis measured 12 mm in the 50% methanolic extract of *T. coadunata* leaves, compared to 18 mm in the positive control. *Davallia griffithiana* leaf extract at 80% methanol revealed a 10 mm inhibitory zone (Saha et al., 2019). The maximum inhibition zone against *E. coli* in 50%

methanolic extract of *T. coadunata* leaves, compared to a 21 mm inhibition zone for the positive control (Saha, 2022).

From the aforementioned trials that the *Tectaria coadunata* exhibit strong antibacterial activity against Bacillus subtilis and Escherichia coli. Plant extracts offer a lot of potential as microorganism-fighting antibacterial substances. Antibiotics and plant extracts work together synergistically to fight resistant bacteria, opening up new options for the treatment of infectious disorders. Although several beneficial substances have been identified as microbial metabolites, it's possible that many more molecules produced by soil microorganisms will still to be identified (Saha, 2022). The popularity of fern consumption is increasing due to its high nutritional content such as vitamin C, protein and iron (Chhetri, 2018).

Due to their significant antioxidant activity and partial target enzyme inhibition, the results suggested that *T. coadunata* extracts, which are a significant source of phenolics, could be taken into consideration for additional studies aimed at developing co-treatments for Alzheimer's disease using more complex in vitro and in vivo tests. Additionally, their strong antioxidant effects may be helpful in the treatment of metabolic illnesses (Shrestha et al., 2019).The results of *T. coadunata* antibacterial activity against the strains tested indicated a considerable impact. Plant extracts in ethyl acetate and methanol demonstrated excellent antibacterial activity, however petroleum ether extracts were ineffective. *T. coadunata* extracts showed increased sensitivity to *S. typhimurium*, *E. coli*, and *X. campestris*. The frond extracts significantly inhibited bacterial growth when rhizome and frond effects were compared. The corresponding pure solvents used as negative controls had no inhibitory effect(Thomas et al., 2021). The leaves and young shoots are a nutritious diet that can be advised to hypertension patients for frequent consumption because they have a low-fat content but a high crude fiber content. According to the results of the GC-MS analysis of *Tectaria coadunata* extracts, the rhizomes include the chemical compounds Decenediol<1,10>, Dodecanoic acid, and Methyl stearate while the leaves contain the most abundant chemical compound, Palmitic acid(Babu Marahatta et al., 2019).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Materials

The leaves and rhizomes of *Tectaria coadunata* were collected. Various materials used in this study are listed in Appendix A.

3.2 Laboratory Set up

The research was carried out in the laboratory of Department of Biology and Department of Microbiology, Central Campus of Technology (CCT), Hattisar, Dharan.

3.3 Study Area

The study area includes Dharan Sub- metropolitan city located in Sunsari district of the eastern Nepal. The area lies in the foothills of the Mahabharat range. Geographically, it is 192.32 square kilometers in size and has an altitude of 349m above sea level. Tropical and subtropical mixed forests are widespread in this region.

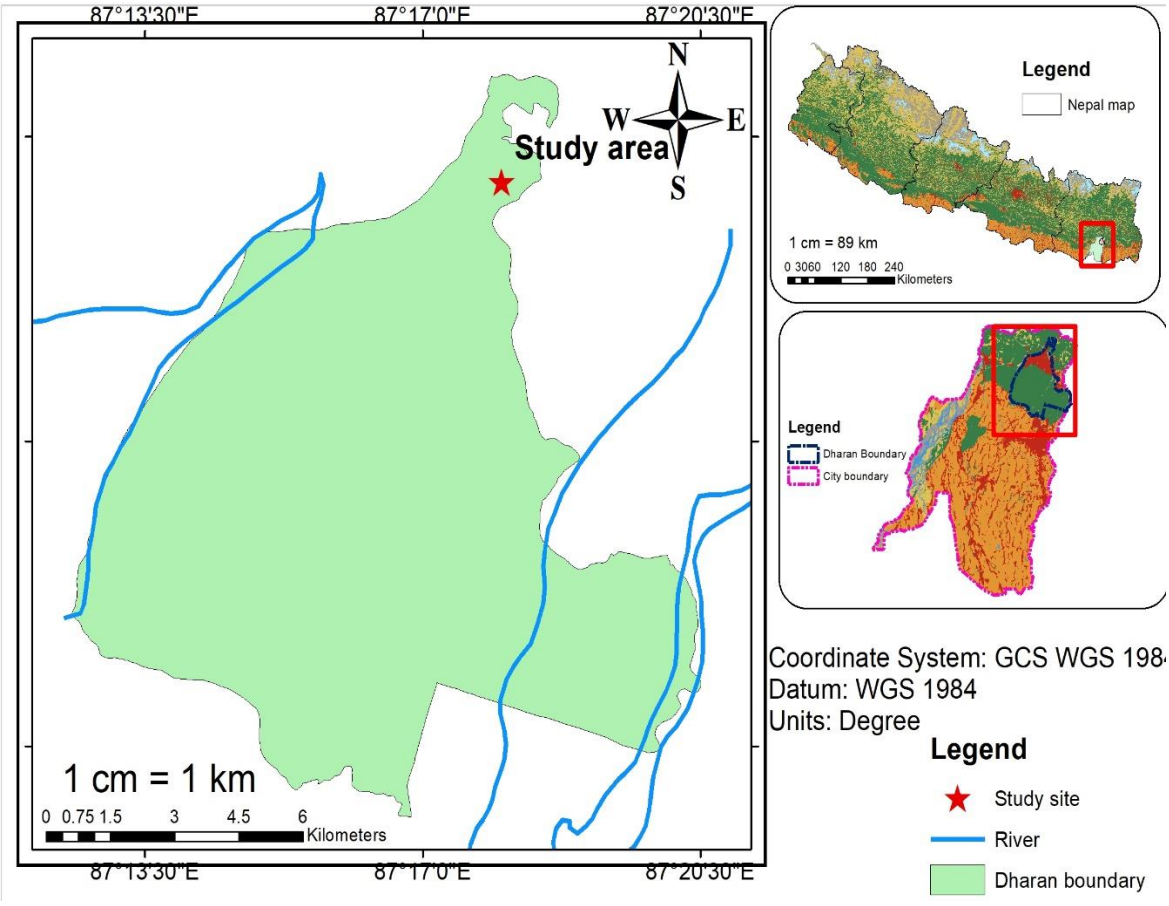

Figure 1: Map of the study area

3.4 Methods

3.4.1 Extraction

In order to remove undesired dirt particles from the surface of the freshly bought plant material, it was rinsed in tap water. After that the rhizome and leaf were cut off and preserved for 10 to 15 days and shade-dried in a dark on a newspaper and the newspaper were replaced every 3-5 days. The rhizome and leaves were then crushed into a powder, and stored in clean plastic bags till they were used again. Using the common Soxhlet extraction procedure, phytochemicals have been extracted from the plant material.

Use of the Soxhlet apparatus was made for the organic extraction. For the solvent extraction process, methanol, water, and petroleum ether were used. To carry out this extraction, 20 g of dried plant powder was placed in a glass thimble, and 200 ml of various solvents were used for each extraction. The extraction was continued in the Soxhlet equipment until siphon tube solvent turned colorless. The extracts were purified using a Rota-evaporator before being condensed, transferred, and sealed into brown-colored bottles. In order to use them in the future, the dried plant crude extracts must be stored at 4±2ºC in the refrigerator.

3.4.2 Phytochemical Analysis

To identify the main phytochemicals that were present in the extracts, we performed qualitative phytochemical screening. Following the guidelines provided, the plants aqueous was screened to determine its components (phenols, tannins, saponins, alkaloids, flavonoids, steroids, and terpenoids). For each sample, the procedure of experimentation was used three times to ensure accuracy. Readings were taken in three fixed patterns for each replication, then the average readings were collected.

Test for Phenols:

Ferric chloride test: 3-4 drops of ferric chloride solution was added to the extract. Appearance of bluish black or dark brown color formation indicates the presence of phenol and tannins.

Test for Flavonoids

Crude extract was combined with approximately 2ml of 2% NaOH solutions. An intense yellow color was form and when 2 drops of diluted acid were added, it turns colorless solution. This demonstrated the presence of flavonoids.

Saponins

In a boiling tube, 0.5 g of plant extract was dissolved in 2 ml of boiling water, then the mixture was allowed to cool and thoroughly mixed. The presence of saponin was indicated by the development of foam.

Steroid

Two milliliter of chloroform and concentrated H_2SO_4 will be mixed with the entire plant crude extract. In the lower chloroform layer produced red color that indicated the presence of steroids.

Test for Terpenoids

The plant extract was combined with 2ml of chloroform, dried on water bath and then heated with 2ml of pure H_2SO_4 was added carefully. Formation of reddish-brown color at interface indicates the presence of terpenoid.

Test for Alkaloid

2ml of Mayer's reagent was added to 1ml of the extract. A yellowish or white precipitate was formed indicating the presence of alkaloids.

Test for Carbohydrates

To 5ml of Benedict reagent, 8-10 drops extract was added and then it was heated for 5 minutes, then dark red ppt was observed which indicates the presence of carbohydrates.

Test for Glycosides

1ml of glacial acetic acid and 1ml of plant extract were combined in a solution, which was then cooled. Following the addition of 2 drops of ferric chloride, slowly conc.H_2SO_4 was added along the test tube's wall. Two layers forming suggest the presence of glycoside.

Test for Amino Acid

2ml of extract was treated with 1ml of Ninhydrin solution. The mixture was boiled on a water bath. Appearance of blue to purple color shows the presence of amino acids.

3.4.3 Quantitative Analysis of TPC and TFC

3.4.3.1 Estimation of Total Phenolic Content

Spectrophotometric analysis using the Folin-Ciocalteu (F-C) method was used to evaluate the total phenolic content of extracts from the whole plant with rhizome and leaves of the *Tectaria coadunata*(Ghorpade et al., n.d.).

In accordance with the standard technique, 1 mL of the sample is combined with 1.5 ml of 10% FC-reagent and 2 mL of 2% Na2CO3 solution. The final product, which was permitted an hour to react in a dark room after 4 ml of distilled water was added to the mixture, which was created. The solution's absorbance was calculated at 765 nm using a spectrophotometer. Each sample was subjected to an experimental procedure in three copies. Each replication included three readings taken in different fixed directions, and the average values were recorded.

3.4.3.2 Estimation of Total Flavonoid Content

Strong antioxidants, flavonoids have recently sparked a great deal of interest due to their ability to improve health and prevent disease. The molecular makeup of flavonoids determines how effective they are as antioxidants. The location of hydroxyl groups and other characteristics of flavonoids chemical structure play a crucial role in the antioxidant and free radical scavenging abilities of these compounds(Pallab et al., 2011).

According to standard practice, 1 ml of the sample should be mixed with 10% AlCl3 and 1 ml of 1M sodium acetate. After that, 4 ml of distilled water was added to the combination, which was then left alone for an hour in the dark. Finally, the absorbance was determined at 415nm. The experiment was performed three times on each sample. Readings were obtained during each replication in three different fixed orientations, and the average results were noted.

3.4.4 Antibacterial Susceptibility Test

Using the agar well diffusion method, extracts from various plants were tested for antimicrobial activity on Mueller Hinton Agar (MHA) plates. A final inoculum of 1.5 108 CFU/ml was obtained after the test organisms were inoculated in Nutrient broth and cultured overnight 24hrs at 37°C to adjust the turbidity to 0.5 McFarland standards. Standardized microbial culture broth was used to create culture MHA plates. Dimethyl Sulfoxide (DMSO) was used to create plant extracts at a concentration of 100 mg/ml. Use a sterile

cork-borer (6 mm) to drill five 6 mm wells into the infected medium. As a positive control (antibiotic discs for bacteria) and a negative/solvent control (DMSO), respectively, each well was filled with 10µl, 20µl, 30µl, and 40µl of extracts from various plants. The experimental approach for every sample were recorded.

It was incubated for 18 to 24 hours at 37°C after being allowed to diffuse for about 30 minutes at room temperature. After incubation, the test compounds antimicrobial activity was determined by looking at the plates for the development of a clear zone around the well. Inhibitory zones (ZOI) were observed and measured in millimeters.

3.4.5 Minimum Inhibitory Concentration (MIC) of Plant Extracts

The minimum inhibitory concentration (MIC) is widely used to evaluate the effectiveness of antimicrobial medications against certain pathogens. Test tube dilution method was used to evaluate the MIC values of *Tectaria coadunata* extracts(Wadhwani & Sc, 2012).

The test extracts are prepared for the 7 test tubes by a series of dilutions in a sterile medium in order to achieve different concentrations. 500µl of bacterial sample is added to each test tube containing 5ml diluted sample with 4.5ml nutrient broth and after that the test tubes are incubated at a temperature that is optimum for the bacterial strain being tested. After incubation, the lowest concentration of the extract at which bacterial growth is inhibited is used to determine the MIC. The MIC values of Methanol rhizome and aqueous rhizome extracts against the bacterial strain *Escherichia coli* were examined in this study.

CHAPTER 4

4. RESULTS AND DISCUSSION

The leaves and rhizome of *T. coadunata* were studied for the phytochemical analysis and antimicrobial susceptibility test. From the test performed it was found that different phytochemicals are present in the leaf and rhizome of *T. coadunata* in Table1&Table 2.

4.1 Qualitative Analysis of Phytochemicals

Table 1: Phytochemicals present in leaves of *T. coadunata*

S.N.	Phytochemicals	Observation	Aqueous	Ether	Methanol
1.	Phenol	Bluish Black	_	_	+
2.	Flavonoid	Intense Yellow	+	_	+
3.	Saponins	Steady Foam	+	_	+
4.	Steroid	Red color	+	_	+
5.	Terpenoid	Reddish brown color at the interface	+	_	+
6.	Alkaloid	Yellow white precipitate	_	_	+
7.	Carbohydrates	Brick Red ppt.	+	_	+
8.	Glycoside	Brown Ring	+	+	_
9.	Amino Acid	Purple Color	+	_	+

Table 2: Phytochemicals present in rhizome of *T. coadunata*

S.N.	Phytochemicals	Observation	Aqueous	Ether	Methanol
1.	Phenol	Blueish Black	+	_	+
2.	Flavonoid	Intense Yellow	+	_	+
3.	Saponins	Steady Foam	+	_	+
4.	Steroid	Red color	+	+	+
5.	Terpenoid	Reddish brown color at the interface	+	+	+
6.	Alkaloid	Yellow white precipitate	_	_	_
7.	Carbohydrates	Brick Red ppt.	_	_	+
8.	Glycoside	Brown Ring	+	+	_
9.	Amino Acid	Purple Color	+	_	+

(Aqueous- Aqueous Extraction, Ether-Petroleum Ether, Methanol- methanol extraction)

(+: Positive and -: Negative)

4.2 Quantitative Analysis of TPC and TFC

4.2.1 Total Phenolic Content

A complex class of naturally occurring secondary metabolites derived from plants are called phenolic compounds. They possess a wide variety of biological and pharmacological traits, such as anti-inflammatory, anti-microbial, anti-cancer, and antioxidant traits. The total phenol concentration was estimated as a milligram of Gallic acid equivalent using the calibration curve for Gallic acid. Using, a spectrophotometer, the triplet absorbance of each concentration was determined at 765 nm to create a calibration curve.

The following values were noted for each solution after the absorbance was measured.

Table 3: Absorbance of Gallic acid

Tube	Concentration	Absorbance at 765 nm			Average absorbance
1	10	0.705	0.701	0.718	0.708
2	20	0.933	0.936	0.9355	0.931
3	40	1.079	1.075	1.079	1.07
4	60	1.33	1.30	1.27	1.3
5	80	1.50	1.47	1.531	1.5
6	100	2	1.99	2.01	2

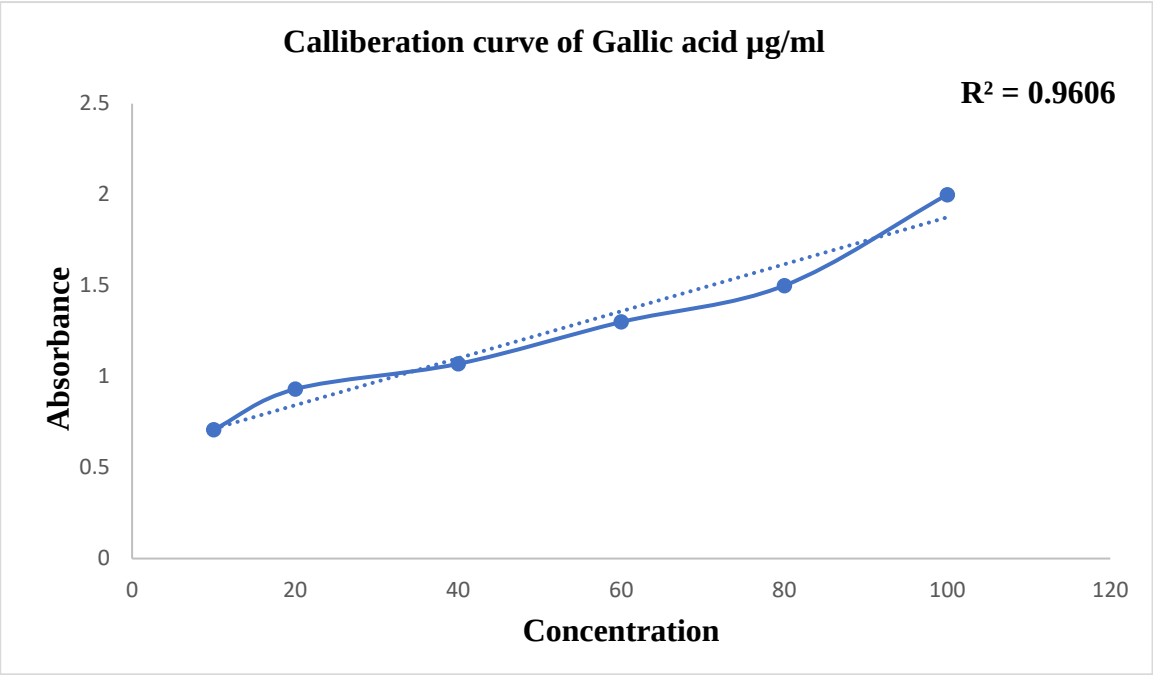

Figure 2: Calibration curve of Gallic acid for Determination of TPC

The amount of Gallic acid present in various *Tectaria coadunata* extracts was calculated using an equation developed from the usual Gallic acid curve represented in the figure.

The formula is given below:

$y = 0.0129x + 0.5845$

$R^2=0.9606$

Where, y = absorbance

x = Gallic acid concentration (GAC) (µg/ml)

m = slope = 0.0129

c = y–intercept= 0.5845

$$x = \frac{y - 0.5845}{0.0129}$$

The total phenolic content is shown in the table below along with the necessary details.

Table 4: Total Phenolic Content in different extracts of *T. coadunata* rhizome and leaf

S. N	Plant extract (100mg/ml)	Absorbance	TPC as GCE=$\frac{c \times v}{m} mg/g$
1	Methanol extract rhizome	1.326	57.48
2	Methanol extract leaf	0.81	17.46
3	Petroleum ether rhizome	0.85	20.56
4	Petroleum ether leaf	0.795	16.30
5	Aqueous rhizome	0.931	20.84
6	Aqueous leaf	1.127	42.02

The methanol extract rhizome has the highest TPC value i.e., 57.48 mg/g and petroleum ether leaf have lowest TPC value which is 16.30 mg/g.

4.2.2 Total Flavonoid Content

The calibration curve in this method was created using quercetin. After being dissolved in methanol, 10 mg of quercetin was diluted to 10, 20, 40, 60, 80, and 100 g/ml. Using, a spectrophotometer, the triplet absorbance of each concentration of the dilutions was determined at 415 nm to create a calibration curve.

Table 5: Absorbance for Quercetin

Tube	Concentration	Absorbance at 415nm			Average absorbance
1	10	0.213	0.217	0.217	0.215
2	20	0.305	0.314	0.304	0.35
3	40	0.71	0.66	0.73	0.7
4	60	0.901	0.903	0.903	0.903
5	80	1.25	1.20	1.21	1.22
6	100	1.49	1.50	1.52	1.5

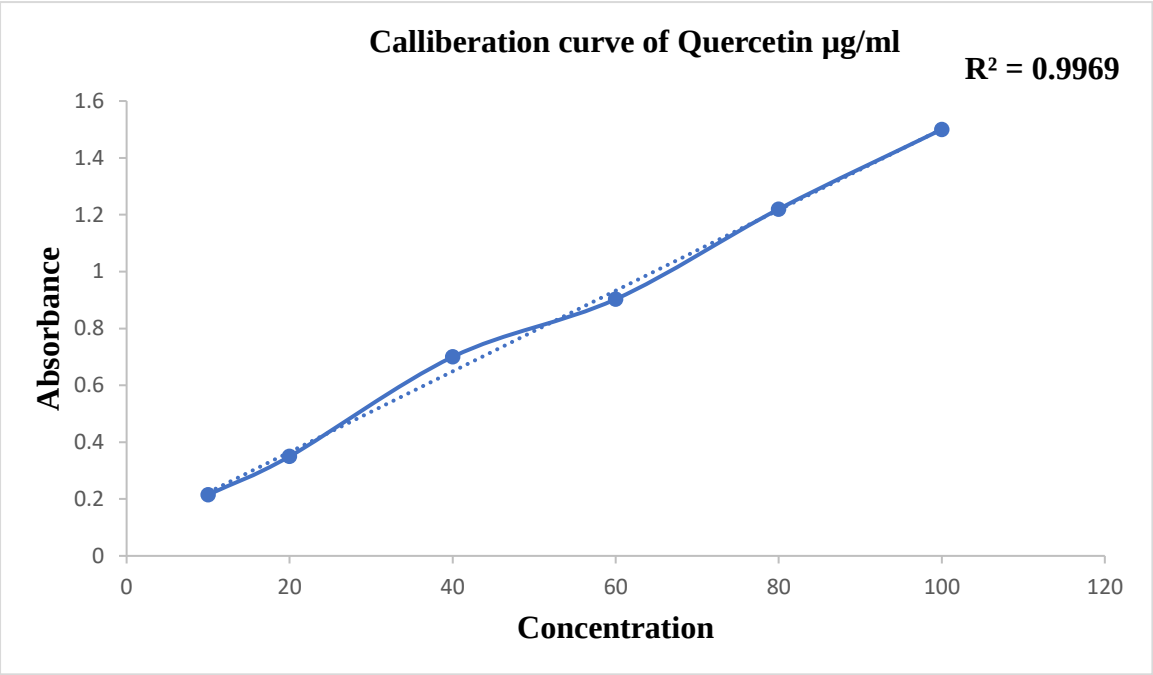

Figure 3: Calibration curve of quercetin for TFC determination

The amount of Quercetin present in various *Tectaria coadunata* extracts was calculated using an equation developed from the standard Quercetin curve represented in the figure.

The formula is given below:

y= 0.0142x+ 0.081

R^2= 0.9969

Where, y = absorbance

x = Quercetin concentration (µg/ml)

m = slope = 0.0142

c = y–intercept= 0.081

$$x = \frac{y-0.081}{0.0142}$$

The total flavonoid content is shown in the table below along with the necessary details

Table 6: Total flavonoid content in different extracts of *T. coadunata* rhizome and leaf

S. N	Plant extract (100mg/ml)	Absorbance	TFC as QE=$\frac{c \times v}{m} mg/g$
1	Methanol extract rhizome	1.4	96.90
2	Methanol extract leaf	1.31	86.61
3	Petroleum ether rhizome	1.126	73.63
4	Petroleum ether leaf	1.158	75.86
5	Aqueous rhizome	0.501	29.868
6	Aqueous leaf	0.665	41.173

The methanol extract rhizome has the highest TFC value i.e., 96.90 mg/g and aqueous rhizome has lowest TFC value which is 29.86 mg/g.

4.3 Antimicrobial Assay of Plant Extracts

Different diseases are caused by pathogenic micro-organisms in plants, animals and mostly human beings. Antimicrobial agents present in plant kill or inhibit the growth of micro-organisms. The antimicrobial activity of the plant was evaluated by calculating the zone of inhibition (ZOI), the area around the agar well where there is no growth of micro-organisms.

The ZOI values for the different bacteria species in methanol, petroleum ether and aqueous extractions of *Tectaria coadunata* rhizome and leaves are tabulated as below:

Table 7: Antimicrobial activity of different extracts of *T. coadunata* against *E. coli.*

S. N	Plant extracts	ZOI of different plant extracts (in mm) against E. coli					
		10µl	20µl	30µl	40µl	DMSO	Tetracycline
1	Methanol rhizome	14.5	15.5	17	19	0	22
2	Methanol leaf	0	0	0	8.5	0	
3	Aqueous rhizome	12	13	14.5	17.5	0	
4	Aqueous leaf	0	0	9	13	0	
5	Petroleum rhizome	0	0	0	0	0	
6	Petroleum leaf	0	0	0	0	0	

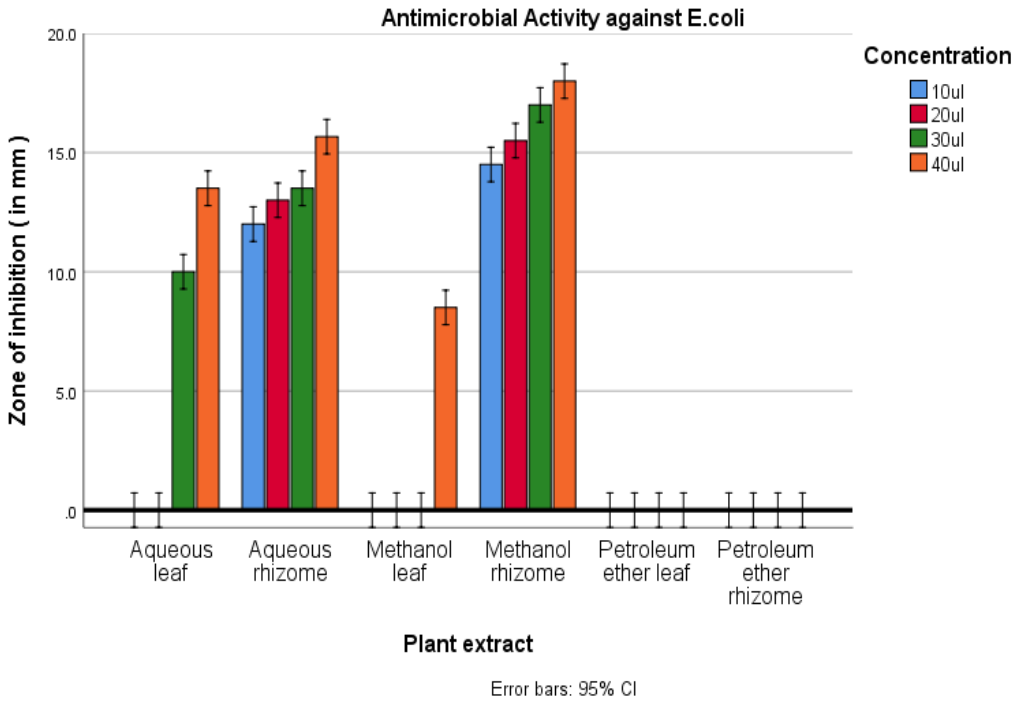

Figure 4: Antimicrobial activity of different extracts of *T. coadunata* against E. coli.

Table 8 : Antimicrobial activity of different extracts of *T. coadunata* against *S. typhi*

S. N	Plant extracts	ZOI of different plant extracts (in mm) against S.typhi					
		10µl	20µl	30µl	40µl	DMSO	Chloramphenicol
1	Methanol rhizome	12	14	15.5	18	0	30
2	Methanol leaf	0	0	0	0	0	
3	Aqueous rhizome	0	9.5	14	14	0	
4	Aqueous leaf	0	9	16	16	0	
5	Petroleum rhizome	0	0	0	0	0	
6	Petroleum leaf	0	0	0	0	0	

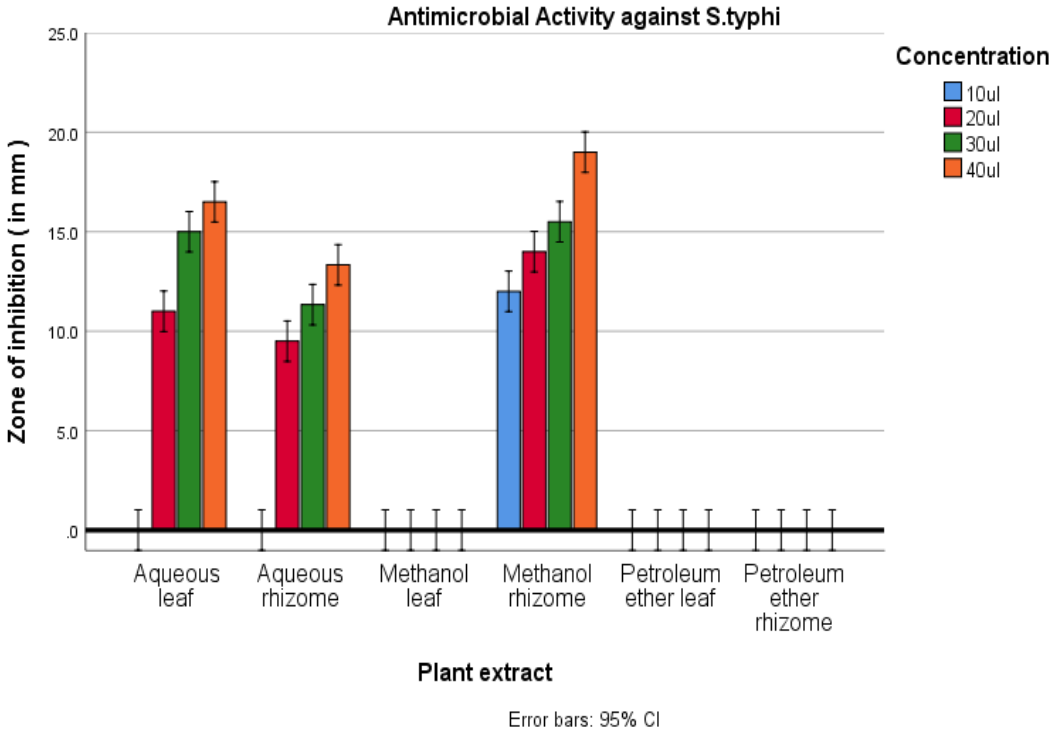

Figure 5: Antimicrobial activity of different extracts of *T. coadunata* against S. typhi

Table 9: Antimicrobial activity of different extracts of *T. coadunata* against *Bacillus subtilis*

S.N	Plant extracts	ZOI of different plant extracts (in mm) against Bacillus sps					
		10µl	20µl	30µl	40µl	DMSO	Ciprofloxacin
1	Methanol rhizome	15	16.5	21	23.5	0	44
2	Methanol leaf	8	10	12	16	0	
3	Aqueous rhizome	8.5	11.5	13.5	7.5	0	
4	Aqueous leaf	8	9.5	11	14	0	
5	Petroleum rhizome	0	0	0	0	0	
6	Petroleum leaf	0	0	0	0	0	

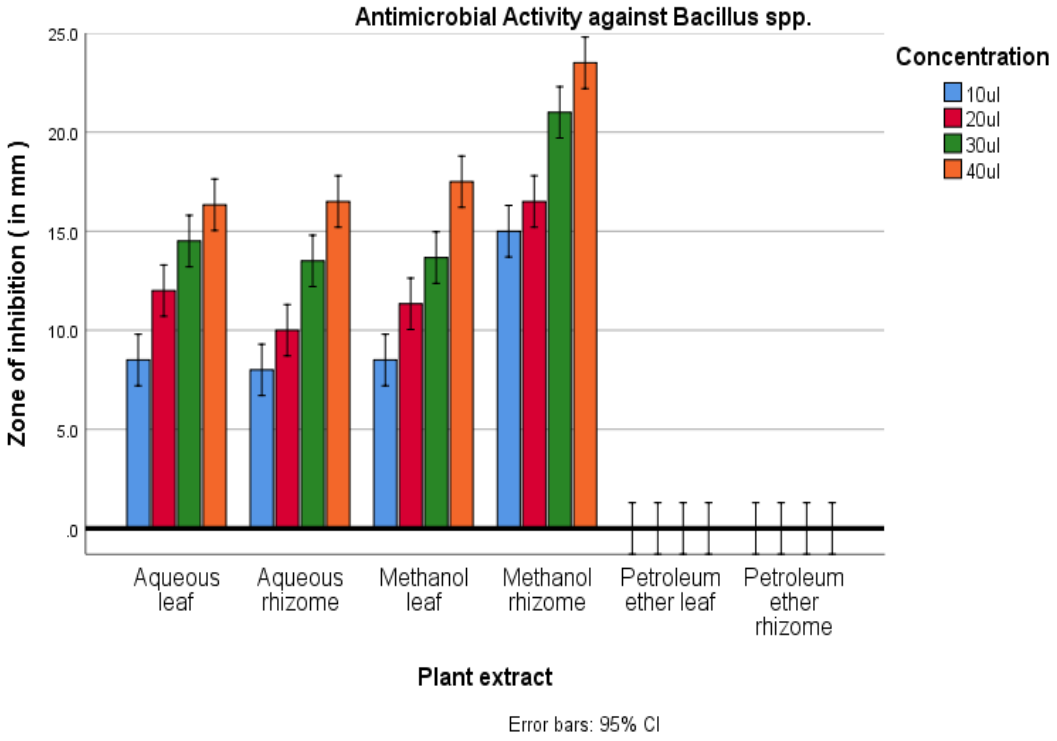

Figure 6*:* Antimicrobial activity of different extracts of *T. coadunata* against Bacillus subtilis

Table 10: Antimicrobial activity of different extracts of *T. coadunata* against *S. aureus*

S.N.	Plant extracts	ZOI of different plant extracts (in mm) against S. aureus					
		10µl	20µl	30µl	40µl	DMSO	Ciprofloxacin
1	Methanol rhizome	10	13	15	18	0	44
2	Methanol leaf	0	0	0	0	0	
3	Aqueous rhizome	0	9.5	13	14	0	
4	Aqueous leaf	0	9	14	16	0	
5	Petroleum rhizome	0	0	0	0	0	
6	Petroleum leaf	0	0	0	0	0	

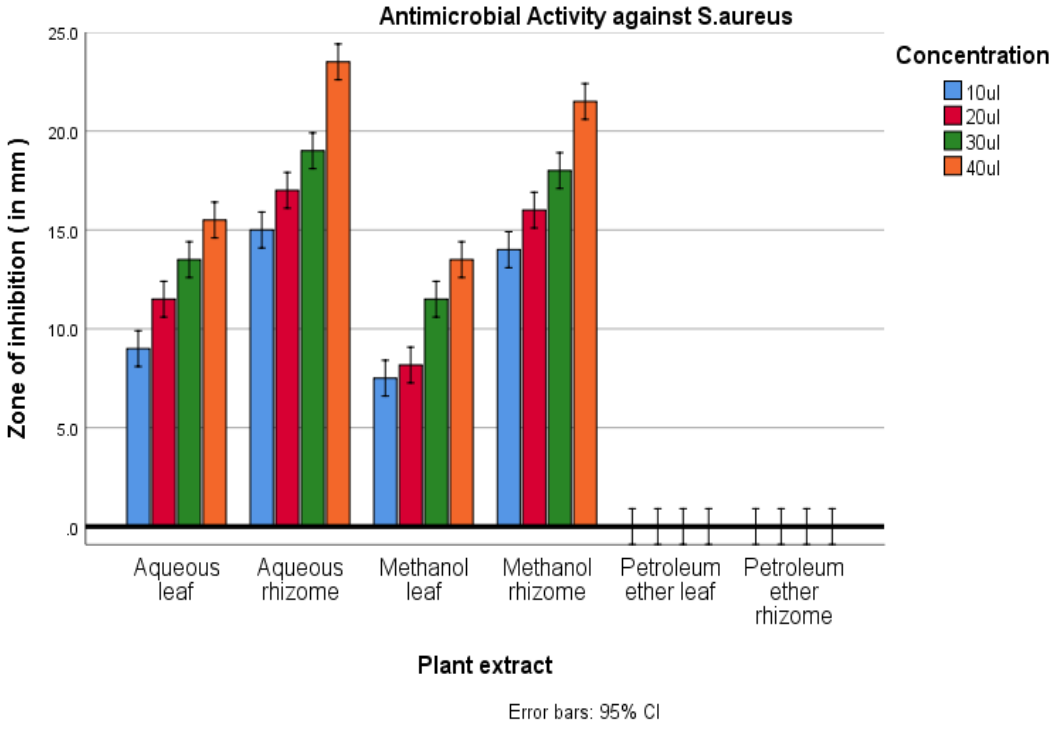

Figure 7: Antimicrobial activity of different extracts of *T. coadunata* against S. aureus

Investigations of the antibacterial effects of *Tectaria coadunata* extracts against 4 different bacterial strains were done independently. Methanol extracts rhizome was found to have greater antibacterial activity against *Bacillus subtilis* than *E. coli, Salmonella* typhi and *Staphylococcus aureus* when compared to these bacterial strains. The highest ZOI was against Bacillus subtilis i.e., 23.5 mm and lowest 8 mm. Methanol extracts rhizome and aqueous extracts rhizome has greater antibacterial activity. Petroleum ether failed to show any ZOI against bacterial strains.

ANOVA Table

Table 11: ANOVA Table of Comparison of Plant extracts

Multiple Comparisons							
Tukey HSD							
Dependent variable			Mean Difference (I-j)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
AA with E. coli	Aqueous leaf	Aqueous rhizome	-7.667*	0.2546	0.000	-8.422	-6.911
		Methanol leaf	3.750*	0.2546	0.000	2.994	4.506
		Methanol rhizome	-10.375*	0.2546	0.000	-11.131	-9.619
		Petroleum ether leaf	5.875*	0.2546	0.000	5.119	6.631
		Petroleum ether rhizome	5.875*	0.2546	0.000	5.119	6.631
	Aqueous rhizome	Aqueous leaf	7.667*	0.2546	0.000	6.911	8.422
		Methanol leaf	11.417*	0.2546	0.000	10.661	12.172
		Methanol rhizome	-2.708*	0.2546	0.000	-3.464	-1.953
		Petroleum ether leaf	13.542*	0.2546	0.000	12.786	14.297
		Petroleum ether rhizome	13.542*	0.2546	0.000	12.786	14.297
	Methanol leaf	Aqueous leaf	-3.750*	0.2546	0.000	-4.506	-2.994
		Aqueous rhizome	-11.417*	0.2546	0.000	-12.172	-10.661
		Methanol rhizome	-14.125*	0.2546	0.000	-14.881	-13.369
		Petroleum ether leaf	2.125*	0.2546	0.000	1.369	2.881
		Petroleum ether rhizome	2.125*	0.2546	0.000	1.369	2.881
	Methanol rhizome	Aqueous leaf	10.375*	0.2546	0.000	9.619	11.131
		Aqueous rhizome	2.708*	0.2546	0.000	1.953	3.464
		Methanol leaf	14.125*	0.2546	0.000	13.369	14.881
		Petroleum ether leaf	16.250*	0.2546	0.000	15.494	17.006
		Petroleum ether rhizome	16.250*	0.2546	0.000	15.494	17.006
	Petroleum ether leaf	Aqueous leaf	-5.875*	0.2546	0.000	-6.631	-5.119
		Aqueous rhizome	-13.542*	0.2546	0.000	-14.297	-12.786
		Methanol leaf	-2.125*	0.2546	0.000	-2.881	-1.369

		Methanol rhizome	-16.250*	0.2546	0.000	-17.006	-15.494
		Petroleum ether rhizome	0.000	0.2546	1.000	-0.756	0.756
	Petroleum ether rhizome	Aqueous leaf	-5.875*	0.2546	0.000	-6.631	-5.119
		Aqueous rhizome	-13.542*	0.2546	0.000	-14.297	-12.786
		Methanol leaf	-2.125*	0.2546	0.000	-2.881	-1.369
		Methanol rhizome	-16.250*	0.2546	0.000	-17.006	-15.494
		Petroleum ether leaf	0.000	0.2546	1.000	-0.756	0.756
AA with S. typhi	Aqueous leaf	Aqueous rhizome	2.083*	0.3576	0.000	1.022	3.145
		Methanol leaf	10.625*	0.3576	0.000	9.564	11.686
		Methanol rhizome	-4.500*	0.3576	0.000	-5.561	-3.439
		Petroleum ether leaf	10.625*	0.3576	0.000	9.564	11.686
		Petroleum ether rhizome	10.625*	0.3576	0.000	9.564	11.686
	Aqueous rhizome	Aqueous leaf	-2.083*	0.3576	0.000	-3.145	-1.022
		Methanol leaf	8.542*	0.3576	0.000	7.480	9.603
		Methanol rhizome	-6.583*	0.3576	0.000	-7.645	-5.522
		Petroleum ether leaf	8.542*	0.3576	0.000	7.480	9.603
		Petroleum ether rhizome	8.542*	0.3576	0.000	7.480	9.603
	Methanol leaf	Aqueous leaf	-10.625*	0.3576	0.000	-11.686	-9.564
		Aqueous rhizome	-8.542*	0.3576	0.000	-9.603	-7.480
		Methanol rhizome	-15.125*	0.3576	0.000	-16.186	-14.064
		Petroleum ether leaf	0.000	0.3576	1.000	-1.061	1.061
		Petroleum ether rhizome	0.000	0.3576	1.000	-1.061	1.061
	Methanol rhizome	Aqueous leaf	4.500*	0.3576	0.000	3.439	5.561
		Aqueous rhizome	6.583*	0.3576	0.000	5.522	7.645
		Methanol leaf	15.125*	0.3576	0.000	14.064	16.186
		Petroleum ether leaf	15.125*	0.3576	0.000	14.064	16.186
		Petroleum ether rhizome	15.125*	0.3576	0.000	14.064	16.186
	Petroleum ether leaf	Aqueous leaf	-10.625*	0.3576	0.000	-11.686	-9.564
		Aqueous rhizome	-8.542*	0.3576	0.000	-9.603	-7.480
		Methanol leaf	0.000	0.3576	1.000	-1.061	1.061
		Methanol rhizome	-15.125*	0.3576	0.000	-16.186	-14.064
		Petroleum ether rhizome	0.000	0.3576	1.000	-1.061	1.061

	Petroleum ether rhizome	Aqueous leaf	-10.625*	0.3576	0.000	-11.686	-9.564
		Aqueous rhizome	-8.542*	0.3576	0.000	-9.603	-7.480
		Methanol leaf	0.000	0.3576	1.000	-1.061	1.061
		Methanol rhizome	-15.125*	0.3576	0.000	-16.186	-14.064
		Petroleum ether leaf	0.000	0.3576	1.000	-1.061	1.061
AA with Bacillus subtilis	Aqueous leaf	Aqueous rhizome	0.833	0.4564	0.459	-0.521	2.188
		Methanol leaf	0.083	0.4564	1.000	-1.271	1.438
		Methanol rhizome	-6.167*	0.4564	0.000	-7.521	-4.812
		Petroleum ether leaf	12.833*	0.4564	0.000	11.479	14.188
		Petroleum ether rhizome	12.833*	0.4564	0.000	11.479	14.188
	Aqueous rhizome	Aqueous leaf	-0.833	0.4564	0.459	-2.188	0.521
		Methanol leaf	-0.750	0.4564	0.575	-2.105	0.605
		Methanol rhizome	-7.000*	0.4564	0.000	-8.355	-5.645
		Petroleum ether leaf	12.000*	0.4564	0.000	10.645	13.355
		Petroleum ether rhizome	12.000*	0.4564	0.000	10.645	13.355
	Methanol leaf	Aqueous leaf	-0.083	0.4564	1.000	-1.438	1.271
		Aqueous rhizome	0.750	0.4564	0.575	-0.605	2.105
		Methanol rhizome	-6.250*	0.4564	0.000	-7.605	-4.895
		Petroleum ether leaf	12.750*	0.4564	0.000	11.395	14.105
		Petroleum ether rhizome	12.750*	0.4564	0.000	11.395	14.105
	Methanol rhizome	Aqueous leaf	6.167*	0.4564	0.000	4.812	7.521
		Aqueous rhizome	7.000*	0.4564	0.000	5.645	8.355
		Methanol leaf	6.250*	0.4564	0.000	4.895	7.605
		Petroleum ether leaf	19.000*	0.4564	0.000	17.645	20.355
		Petroleum ether rhizome	19.000*	0.4564	0.000	17.645	20.355
	Petroleum ether leaf	Aqueous leaf	-12.833*	0.4564	0.000	-14.188	-11.479
		Aqueous rhizome	-12.000*	0.4564	0.000	-13.355	-10.645
		Methanol leaf	-12.750*	0.4564	0.000	-14.105	-11.395
		Methanol rhizome	-19.000*	0.4564	0.000	-20.355	-17.645
		Petroleum ether rhizome	0.000	0.4564	1.000	-1.355	1.355
		Aqueous leaf	-12.833*	0.4564	0.000	-14.188	-11.479
		Aqueous rhizome	-12.000*	0.4564	0.000	-13.355	-10.645

	Petroleum ether rhizome	Methanol leaf	-12.750*	0.4564	0.000	-14.105	-11.395
		Methanol rhizome	-19.000*	0.4564	0.000	-20.355	-17.645
		Petroleum ether leaf	0.000	0.4564	1.000	-1.355	1.355
AA with S. aureus	Aqueous leaf	Aqueous rhizome	-6.250*	0.3182	0.000	-7.194	-5.306
		Methanol leaf	2.208*	0.3182	0.000	1.264	3.153
		Methanol rhizome	-5.000*	0.3182	0.000	-5.944	-4.056
		Petroleum ether leaf	12.375*	0.3182	0.000	11.431	13.319
		Petroleum ether rhizome	12.375*	0.3182	0.000	11.431	13.319
	Aqueous rhizome	Aqueous leaf	6.250*	0.3182	0.000	5.306	7.194
		Methanol leaf	8.458*	0.3182	0.000	7.514	9.403
		Methanol rhizome	1.250*	0.3182	0.004	0.306	2.194
		Petroleum ether leaf	18.625*	0.3182	0.000	17.681	19.569
		Petroleum ether rhizome	18.625*	0.3182	0.000	17.681	19.569
	Methanol leaf	Aqueous leaf	-2.208*	0.3182	0.000	-3.153	-1.264
		Aqueous rhizome	-8.458*	0.3182	0.000	-9.403	-7.514
		Methanol rhizome	-7.208*	0.3182	0.000	-8.153	-6.264
		Petroleum ether leaf	10.167*	0.3182	0.000	9.222	11.111
		Petroleum ether rhizome	10.167*	0.3182	0.000	9.222	11.111
	Methanol rhizome	Aqueous leaf	5.000*	0.3182	0.000	4.056	5.944
		Aqueous rhizome	-1.250*	0.3182	0.004	-2.194	-0.306
		Methanol leaf	7.208*	0.3182	0.000	6.264	8.153
		Petroleum ether leaf	17.375*	0.3182	0.000	16.431	18.319
		Petroleum ether rhizome	17.375*	0.3182	0.000	16.431	18.319
	Petroleum ether leaf	Aqueous leaf	-12.375*	0.3182	0.000	-13.319	-11.431
		Aqueous rhizome	-18.625*	0.3182	0.000	-19.569	-17.681
		Methanol leaf	-10.167*	0.3182	0.000	-11.111	-9.222
		Methanol rhizome	-17.375*	0.3182	0.000	-18.319	-16.431
		Petroleum ether rhizome	0.000	0.3182	1.000	-0.944	0.944
	Petroleum ether rhizome	Aqueous leaf	-12.375*	0.3182	0.000	-13.319	-11.431
		Aqueous rhizome	-18.625*	0.3182	0.000	-19.569	-17.681
		Methanol leaf	-10.167*	0.3182	0.000	-11.111	-9.222
		Methanol rhizome	-17.375*	0.3182	0.000	-18.319	-16.431

Petroleum ether leaf	0.000	0.3182	1.000	-0.944	0.944

Based on observed means.

The error term is Mean Square (Error) = 608.

*. The mean difference is significant at the .05 level.

Different extracts of *Tectaria coadunata* have different antibacterial effects against *E. coli, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus*, according to the data analysis of variance (ANOVA). The mean difference is significant.

4.4 Minimum Inhibitory Concentration (MIC) of Plant Extracts

The highest dilution or lowest concentration of extracts necessary to stop an organism growth is known as the Minimum Inhibitory Concentration (MIC). In diagnostic labs, MIC determination is crucial because it tracks the action of new antimicrobial drugs and is useful in verifying a microorganism resistance to an antimicrobial agent. The MIC test is the simplest basic laboratory test for assessing if an antimicrobial agent is effective against an organism (Malviya et al., 2012).

Table 12: MIC performance of different extracts of *Tectaria coadunata* against E. coli.

S. N	Plant Extracts (100 mg/ml) MIC	Value
1	Methanol extraction rhizome	12.5
2	Aqueous extraction rhizome	6.25
3	Ciprofloxacin	1.78

The results of the study show that ciprofloxacin, a widely used antibiotic for the treatment of E. coli infections, had the lowest MIC value of all the compounds being studied, at 1.78. This demonstrates that ciprofloxacin exhibited the most potent inhibitory action against E. coli bacteria among all the extracts and compounds that were evaluated.

The MIC value of *Tectaria coadunata* in Methanol extraction rhizome is 12.5 and aqueous extraction rhizome is 6.25, which shows the minimum inhibitory concentration of the plant extract. The *Tectaria coadunata* Methanol extract, however, shown less inhibitory activity against E. coli bacteria, with a higher MIC value of 12.5 when compared to ciprofloxacin and the other extracts.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The Phytochemical screening of methanol, petroleum ether and aqueous extract of leaves and rhizome of *T. coadunata* revealed the presence of phenol, flavonoid, tannins, saponins, steroid, terpenoid, alkaloid, carbohydrates, glycoside and amino acid. Plant extract are excellent sources of antibacterial chemicals that are effective against pathogens. They can therefore be utilized to treat infectious disorders carried on by harmful microbes.

From the quantitative analysis of methanol extracts of leaves and rhizome of *T. coadunata* it is found that high amount of TPC and TFC are present and as well as total phenolic and flavonoid contents were also calculated. Thus, leaf and rhizome of *T. coadunata* are effective antioxidants. However, as per the result obtained TPC and TFC are found to be higher in rhizome extract than in leaves extract, rhizome show higher antimicrobial activity than that of leaves.

The greater ZOI values for the test organisms against Bacillus subtilis than Escherichia coli, Salmonella typhi and Staphylococcus aureus shown by the methanol extract of T. coadunate rhizome indicate their tremendous potential against illness caused by these bacteria. For the test organisms, rhizome extract serves as a more effective antibacterial agent than leaves.

5.2 Novelty and National Prosperity aspect of Project Work

Exploring the potential of these plants in terms of their chemical make-up and antibacterial qualities was the goal of the project study on phytochemical screening and antibacterial activity of *Tectaria coadunata* from Dharan, Sunsari, Nepal. *T. coadunata* is a wild edible fern that is used by different communities as both food and medicine. Proper phytochemical analysis and antimicrobial testing can result in the development of new medications that may be useful in the medical and pharmacological fields. The commercial production of unprocessed plant extracts can assist a country in obtaining significant royalties.

5.3 Limitation of the work

- All the phytochemicals test could not be carried out due to lack of time and unavailability of chemicals required to perform tests for the phytochemicals.
- Antioxidant analysis cannot be performed due to unavailability of equipment.

5.4 Recommendations for the further work

a. From the study it was found that *T. coadunate* is useful medicine for many diseases. It also acts as antioxidant and antimicrobial agent which helps in preventing various diseases. Despite being useful in many aspects study of this plant is scarcely done.
b. Despite having numerous uses, this particular plant is hardly ever studied. As a result, more extensive research is required across the country. Therefore, more detailed study is needed in different parts of nation. Detailed study regarding phytochemical composition, AST with other microbes, Antioxidant activity as well as antifungal and possible bioactivity is used to be done to know its medicinal values.

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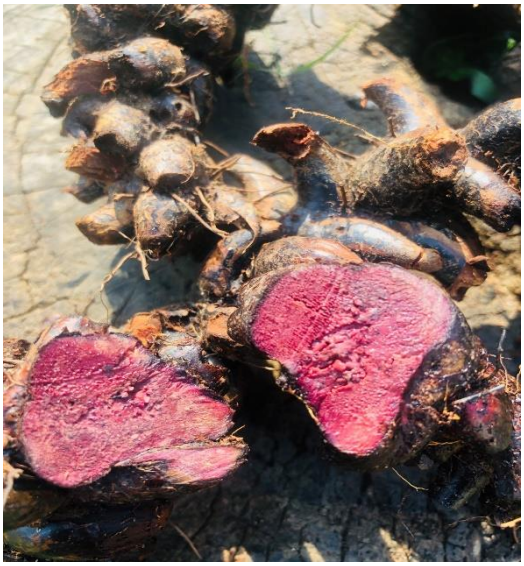
PHOTOGRAPHS

Photograph 1: *Tectaria coadunata* plant

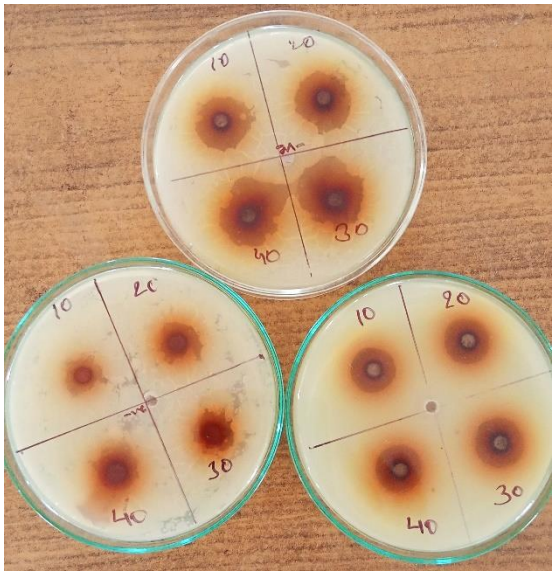
Photograph 2: *T. coadunata* rhizome



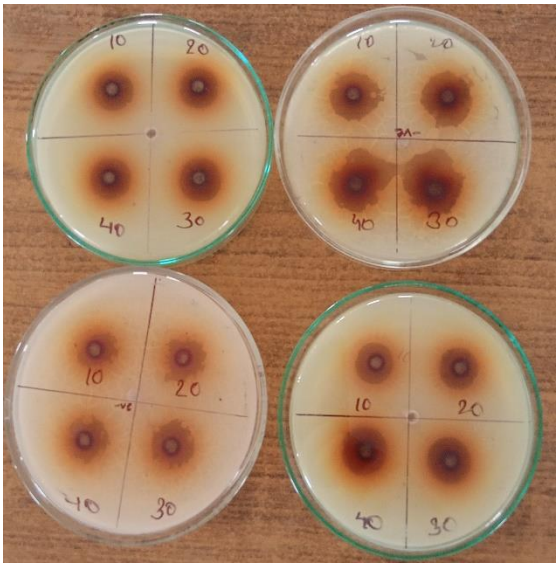
Photograph 3: Cut surface of Rhizome

Photograph 4: Soxhlet Extraction of plant

Photograph 5: MIC of *Tectaria Coadunata*

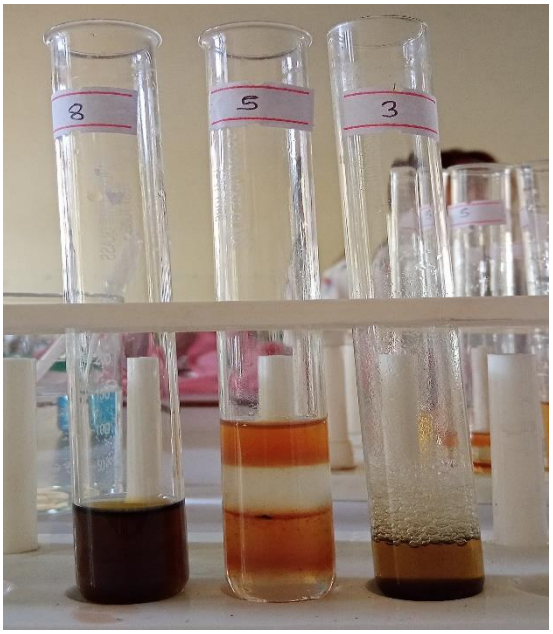


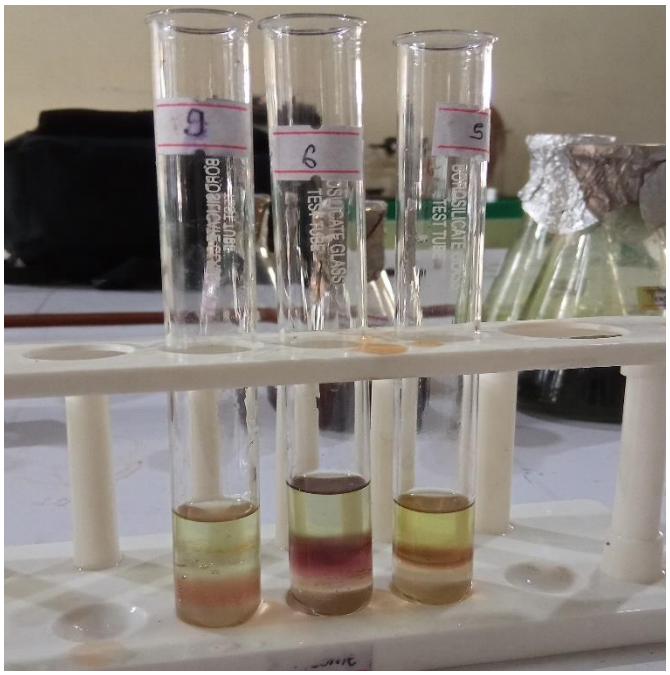
Photograph 6: ZOI of Methanol Rhizome



Photograph 7: ZOI of Aqueous Rhizome

Photograph 8: Lab work





Photograph 9: Phytochemical Screening of plant extracts

APPENDIX A

MATERIALS REQUIRED

Media

Nutrient Broth (NB)	HiMedia Laboratories Pvt. Ltd.
Muller Hinton Agar (MHA)	HiMedia Laboratories Pvt. Ltd.

Chemicals

Acetone	Sisco Research Laboratories Pvt. Ltd.
$AlCl_3$	Central Drug House (P) Ltd
Ammonia	Oxford Lab Fine Chemicals LLP
Benedict's reagent	Fizmerk India Chemicals
Benzene	Merck Specialities Pvt Ltd
Chloroform	Merck Life Science Pvt. Ltd.
Dehydrated alcohol	Mount Everest Industrial Works
DMSO	FINAR Limited
Dipotassium hydrogen phosphate	Merck Life Science Pvt. Ltd.
FC reagent	Oxford Lab Fine Chemicals LLP
Ferrous sulphate	REAL CHEMSYS PRODUCTS PVT. LTD.
Gallic Acid	Oxford Lab Fine Chemicals LLP.
HNO_3	Thermo Fisher Scientific India Pvt. Ltd.
H2SO4	Thermo Fisher Scientific India Pvt. Ltd.
Iso-amyl alcohol	Central Drug House (P) Ltd.
Methanol	Thermo Fisher Scientific India Pvt. Ltd.

Mercuric chloride	Thermo Fisher Scientific India Pvt. Ltd.
Na2Co3	SD Fine Chemical Ltd.
NaOH	HiMedia Laboratories Pvt. Ltd.
$NaNO_3$	Thermo Fisher Scientific India Pvt. Ltd.
Petroleum ether	Thermo Fisher Scientific India Pvt. Ltd.
Potassium iodide	Thermo Fisher Scientific India Pvt. Ltd.
Quercetin	HiMedia Laboratories Pvt. Ltd

Equipment

Autoclave	Accumax India
Electronic weighing machine	Electronic Precision Balance
Glass wares	Borosil
Grinder	CROMPTON
Hot water bath	Clifton
Hot air Oven	Faithful
Incubator	Accumax India
Refrigerator	LG
Soxhlet apparatus	Brosil
Water Bath shaker	Optics Technology

Test organisms	**Antibiotics**
Escherichia coli	Tetracycline
Salmonella typhi	Chloramphenicol
Bacillus subtilis	Ciprofloxacin
Staphylococcus aureus	Ampicillin

Antibiotics disc

Tetracycline

Ciprofloxacin

Chloramphenicol

Preparation of reagent used for phytochemical analysis

2% Ferric Chloride solution:

2gm of $FeCl_3$ Crystals were dissolved in 100ml of distilled water.

10% Ferric Chloride solution:

10gm of FeCl3 Crystals were dissolved in 100ml of distilled water.

2% NaOH solution:

2gm of NaOH pellets were dissolved in 100ml of distilled water.

10% NaOH solution:

10gm of NaOH pellets were dissolved in 100ml of distilled water.

Mayer's reagent:

1.36gm of Mercuric chloride and 5gm Potassium iodide were dissolved in 100ml water

APPENDIX B

Composition of media

Nutrient broth

Ingredients	**gm/ml**
Peptone	5
Sodium Chloride	5
Beef extract	1
Yeast extract	2
Final pH at 25^0C	7.4±0.2

Suspend 13.00 gm in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs. Pressure (121^0C) for 15 minutes.

Muller Hinton Agar

Ingredients	**gm/ml**
Beef infusion from (ex. Buffalo)	300.00
Acid hydrolysate of casein	17.50
Agar	17.00
Starch	1.50
Final pH at 25^0C	7.4±0.2

Suspend 39.00 grams in 1000ml Purified/distilled water. Heat to Bring to dissolve the medium completely. Sterilize by autoclaving at 15 lbs. pressure (121^0C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile petri plate.