PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *Artemisia vulgaris* (*L)* OF DHANKUTA NEPAL

A PROJECT WORK SUBMITTED TO THE

DEPARTMENT OF BIOLOGY

CENTRAL CAMPUS OF TECHNOLOGY

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

NEPAL

FOR THE AWARD OF

BACHELOR OF SCIENCE (B.Sc.) IN BOTANY

BY

SUJA KARKI

SYMBOL No: 500080041

T.U. REGISTRATION: 5-2-8-89-2018

JUNE, 2023

RECOMMENDATION

This is to recommend that **Suja Karki,** symbol no**.** 500080041, registration no. 5-2-8-89-2018, has carried out the project work entitled "**Phytochemical analysis and antimicrobial activity of *Artemisia vulgaris (L)* in Dhankuta, Nepal,"** as a requirement for the Bachelor of Science (B.Sc.) degree in Botany, under my supervision in the Department of Biology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

To my knowledge, this work has not been submitted for any other degree.

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal, for the submission of the project work for the partial fulfillment of the Bachelor of Science (B.Sc.) degree.

……

Mrs. Sabitri Shrestha

Supervisor

Department: Department of Biology

Campus: Central Campus of Technology

Tribhuvan University

DECLARATION

This project work, entitled "**Phytochemical Analysis and Antimicrobial Activity of *Artemisia vulgaris (L)* in Dhankuta, Nepal,"** is being submitted to the Department of Biology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal, for the partial fulfillment of the requirement for the project work in the Bachelor of Science (B.Sc.) degree in Botany. This project work is carried out by me under the supervision of **Mrs. Sabitri Shrestha** in the Department of Biology, Central Campus of Technology, Institute of Science and Technology, 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.

……
Suja Karki
Symbol No. 500080041
T.U. Registration No. 5-2-8-89-2018

[12, JUNE, 2023]

LETTER OF FORWARD

[Date: 27/04/2023]

On the recommendation of **Mrs. Sabitri Shrestha**, this project work is submitted by **Suja Karki**, Symbol No. 500080041, T.U. Registration No. 5-2-8-89-2018, entitled **"Phytochemical Analysis and Antimicrobial Activity of *Artemisia vulgaris(L)* in Dhankuta, Nepal," and** is forwarded by the Department of Biology, Central Campus of Technology (IoST), Tribhuvan University (T.U.), Nepal.

…….
Assist Prof. Ms. Sanju Parajuli
Head of Department
Central Campus of Technology
Tribhuvan University

BOARD OF EXAMINATION AND CERTIFICATE OF APPROVAL

This project work (PRO-406) entitled **"Phytochemical Analysis and Antimicrobial Activity of *Artemisia Vulgaris (L)* in Dhankuta, Nepal"** by Suja Karki (Symbol No. 500080041 and T.U. Registration No. 5-2-8-89-2018) under the supervision of Mrs. Sabitri Shrestha in the Department of Biology, Central Campus of Technology, Institute of Science and Technology, 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 Examinations, Institute of Science and Technology, Tribhuvan University.

……..…..
Asst. Prof. Sabitri Shrestha
Supervisor
Department of Biology
Central Campus of Technology
Tribhuvan University

……..….
Asst. Prof.Dr. Bhabindra Niroula
External Examiner
Department of Botany
Degree campus Biratnagar
Tribhuvan University

.....
Asst. Prof. Pramod Sen Oli
Internal Examiner
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

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Ms. Suja Karki
Symbol No. 500080041
T.U. Registration No. 5-2-8-89-2018
April, 2022

ABSTRACT

A. vulgaris (L), which is commonly called "mugwort," is a species of flowering plant in the daisy family Asteraceae. This plant is used as a medicinal plant to cure various diseases. Mugworts have also been used as culinary herbs. The leaves of *Artemisia vulgaris* were analyzed in the study for their phytochemical compounds and antimicrobial activity. The methanol extract of the plant contained important phytochemicals such as proteins, tannins, saponins, triterpenoids, and glycosides. Similarly, the petroleum ether extract revealed the presence of bioactive compounds like proteins, tannins, alkaloids, and flavonoids. A total of six microorganisms were used to find out the antibacterial activity of the plant extract. The methanol extract of plants showed strong antimicrobial activity against *Salmonella typhi and Streptococcus pyogenes,* with zones of inhibition of 20, 18.5, 15, 10 mm, and 20, 18, 16, and 14 mm, respectively. whereas the methanol extract of plants showed relatively weak antimicrobial activity against *MRSA* and *Staphylococcus aureus* with zones of inhibition of 17, 15, 13, 10mm and 17, 16, 14.5, 13 mm, respectively. No zone of inhibition was shown on any bacteria in plants petroleum ether extract. This finding concludes that the methanol extract has strong antibacterial activity and could be responsible for the presence of active compounds like flavonoids, tannins, saponins, alkaloids, and proteins. A further study should be done to investigate more bioactive compounds of *Artemisia vulgaris L,* which can be beneficial for the pharmacology field in the near future.

Keywords: Mugwort, Asteraceae, Methanol extract, Petroleum ether extract, bioactive compounds.

शोधसार

Artemisia vulgaris (L), जसलाई "mugwort" भनिन्छ, डेजी परिवार Asteraceae मा फूल फुल्ने बोटको एक प्रजाति हो। यो बिरुवा विभिन्न रोगहरु को उपचार को लागी एक औषधीय बिरुवा को रूप मा प्रयोग गरिन्छ। Mugworts पनि पाक जडिबुटी रूपमा प्रयोग गरिएको छ। आर्टेमिसिया भल्गारिसका पातहरूलाई तिनीहरूको फाइटोकेमिकल यौगिकहरू र antimicrobial गतिविधिको लागि अध्ययनमा विश्लेषण गरिएको थियो। बिरुवाको methanol एक्स्ट्र्याक्टमा protein, tannins, saponins, triterpenoids र glycosides जस्ता महत्त्वपूर्ण फाइटोकेमिकलहरू थिए। त्यसैगरी, petroleum ether एक्स्ट्र्याक्टमा, tannins, protein, alkaloids र flavonoids जस्ता bioactive यौगिकहरूको उपस्थिति पत्ता लाग्यो। प्लान्ट एक्स्ट्र्याक्टको antimicrobial गतिविधि पत्ता लगाउन कुल छवटा सूक्ष्मजीवहरू प्रयोग गरियो। बिरुवाहरूको methanol निकासीले *Salmonella typhi* र *Streptococcus pyogenes* विरुद्ध बलियो antimicrobial गतिविधि देखायो, क्रमशः 20, 18.5, 15, 10 mm, र 20, 18, 16, र 14 mm को निषेधित क्षेत्रहरू। जबकि बिरुवाको methanol निकासीले क्रमशः १७, १५, १३, १० mmर १७, १६, १४.५, १३ mm अवरोधका क्षेत्रहरू भएको *MRSA* र *Staphylococcus aureus* विरुद्ध अपेक्षाकृत कमजोर antimicrobial गतिविधि देखायो। बिरुवाका petroleum ether एक्स्ट्र्याक्टले कुनै पनि ब्याक्टेरिया विरुद्ध अवरोध गर्ने कुनै क्षेत्र देखाउँदैन। यो खोजले निष्कर्ष निकाल्छ कि methanol एक्स्ट्र्याक्टमा बलियो antibacterial गतिविधि हुन्छ र सक्रिय यौगिकहरू जस्तै flavonoids, tannins, saponins , alkaloids र protein उपस्थितिको लागि जिम्मेवार हुन सक्छ। अर्को अध्ययन *Artemisia vulgaris (L),* को अधिक जैव सक्रिय यौगिकहरु को अनुसन्धान गर्न को लागी गरिनु पर्छ, जुन निकट भविष्य मा pharmacology क्षेत्र को लागी लाभदायक हुन सक्छ।

Keywords:, Mugwort, Asteraceae, Methanol extract, Petroleum ether extract, Bioactive compounds.

LIST OF ACRONYMS AND ABBREVITIONS

AA : Antimicrobial Activity

ANOVA : Analysis of variance

AST : Antimicrobial Susceptibility Test

DMSO : Dimethyl Sulfoxide

MHA : Mueller Hinton Agar

MRSA : Methicilin-resistant staphylococcus aureus

NA : Nutrient Broth

PE : Petroleum Ether

Spp. : Species

WHO : World Health Organization

ZOI : Zone of Inhibition

LIST OF SYMBOLS

-	Negative result
+	Positive result
µl	Microliter
℃	Degree Celsius
mm	Millimeter

LIST OF TABLE

LIST OF FIGURES

LIST OF PHOTOGRAPHS

TABLE OF CONTENTS

Page No

CHAPTER 1

1. INTRODUCTION

1.1 General Introduction

Medicinal plants are significant species of plants that, according to both modern scientific research and traditional medical traditions, can be used to treat illnesses and improve human health. According to (Oladeji et al., 2020) these plants are thought to be abundant sources of components that can be employed in the synthesis and manufacture of medications. Phytoconstituents are a broad category of chemical components that make up plants (Gospel Ajuru, 2017). The secondary roles that phytoconstituents do for plants include promoting plant development, protecting them by triggering defense mechanisms, and providing them with color, flavor, and odor(Sharma et al., 2020).In the Asteraceae family, Artemisia vulgaris Linn. often known as mugwort pollen in Hindi and titipati in Nepali, is a significant perennial medicinal herb. The perennial plant A. vulgaris reaches a height of 50–150 cm, blooms in August, and sets seed in September. In addition to being an edible, A. vulgaris has long been utilized as a traditional treatment in public health plant, used largely as a spice (Anwar et al., 2015). It is an aggressive and invasive plant that can be seen growing wild and enormously in both temperate and cold temperate regions of the planet. A. vulgaris has been discovered to have many pharmacological actions and is somewhat poisonous(Robinson et al., 2003; El-Tantawy, 2015).

Due to the warming and drying properties of the mugwort plant, it was also suggested for the treatment of urological conditions such dysuria and nephrolithiasis. A. vulgaris, known as "mater herbarum" (the mother of herbs), was applied topically in the medicine of the Middle Ages for the treatment of wounds, prevention of gout, relief of leg tiredness, and treatment of fever(Stoll & Ohlmeyer, 1992).

Understanding the chemical components of plants is desirable since it may be useful in identifying new sources as well as in the search for medicinal treatments. Synthetic antibacterial and antioxidant medications that are typically available are frequently linked to unfavorable side effects and the problem of antibacterial drug resistance. When treating clinical infections and resistant strains, the use of phytochemicals with recognized antibacterial properties might be extremely important. According to reports, the Artemisia species have high levels of phenolic and flavonoids, which have

powerful antioxidant and radical-scavenging capabilities(Cha JD, Jung EK, Kil BS, 2007; Owlia et al., 2007; Shi et al., 2010). The world-wide distribution of Artemisia species makes them one of the most well-known plants used in both traditional and modern medical preparations. They are widely used to treat conditions like cancer, hepatitis, malaria, infections by fungi, bacteria, and viruses, and infections (Mohammed et al., 2022). Numerous pharmacological and phytochemical researches are being conducted on A. vulgaris right now.

Studies on phytochemistry have demonstrated the aerial sections of this plant's rich composition, which includes sesquiterpenoid lactones, flavonoids, and coumarins, as well as an essential oil with a range of qualitatively different constituents. In turn, pharmacological research has revealed previously undiscovered biological activities of A. vulgaris' raw materials, including antioxidant, hypolipemic, hepatoprotective, antispasmodic, analgesic, antihypertensive, estrogenic, cytotoxic, antibacterial, and antifungal effects (Ekiert et al., 2020). A phytochemical screening is a procedure in which the chemicals from a plant are extracted and tested to determine if they are biologically active. It is one of the methods through which new medications can be identified. Artemisia species have been reported to have a vast range of therapeutic plant constituents, including essential oils, terpenes, sesquiterpenes, and alkaloids, which have been linked to antiprotozoal, antibacterial, and antifungal activity (Valdés et al., 2008). However, a lot more research is still needed to understand the curative effects associated with conventional herbal remedies and to find simple manufacturing processes that could create affordable therapeutic agents for the treatment of infectious diseases that are widespread throughout the world (Mohammed et al., 2022).

1.2 Rationale

Asteraceae family belonging *Artemisia vulgaris* which common name is mugwort which is medically used as a traditional medicine for its therapeutic assets. It possesses strong antimicrobial, antioxidant and antifungal activities. Although it has been used in traditional medicine for a long period of time, there is insufficiency of detailed studies targeting on the phytochemical composition and its antimicrobial potential against pathogens of *Artemisia vulgaris* particularly in Dhankuta region of Nepal.

By performing phytochemical tests, we can obtain valuable knowledge regarding the compounds and concentrations of many bioactive compounds that are present in *Artemisia vulgaris,* such as flavonoids, tannins, phenols, alkaloids, carbohydrates, etc. Knowledge about the phytoconstituent is itself critical, as they are held responsible for the plant's biological activities. By assessing the antibacterial activity of plant extracts, it may help in the creation of naturally occurring medicines that are particular to a given area.

By performing this study in Dhankuta, Nepal, it may hold great importance as this particular region may retain different ecological and environmental surroundings that can determine the phytochemical and antimicrobial activities of *Artemisia vulgaris* against microorganisms. This research work can enlighten information about the local variation of plants' active compounds and their activities, which might help in providing valuable knowledge for the development of particular regions' natural remedies.

Hence, this research work is done to obtain the phytochemical compounds and antimicrobial potential of *Artemisia vulgaris* available in a specific geographical area of Dhankuta, Nepal.

1.3 Objectives

1.3.1 General Objectives

The general objective of this project is to analyze the phytochemicals and antimicrobial activity of *Artemisia Vulgaris* in Dhankuta, Nepal.

1.3.2 Specific Objectives

- To evaluate phytochemicals of *Artemisia Vulgaris* from its leaf qualitatively.
- To determine antibacterial susceptibility test from prepared plant extract in different microorganisms.
- To determine minimum inhibitory concentration test form prepared plant extraction.

CHAPTER 2

2. LITERATURE REVIEW

Medicinal plants are significant plant species that, in accordance with both modern scientific research and conventional medical practices, can be used to treat illnesses and improve human health(Sharma et al., 2020). According to(Liu et al., 2013) medicinal plants are a dependable source for developing non-toxic, effective oral alternative and complementary medicines. These medicines help maintain health and prevent the spread of disease. According to Goulletquer et al. (2014), cultural shifts, the purpose of collecting plants from their natural habitats, as well as biochemical and ecological factors, have all been taken into account while developing theoretical and practical understanding of medicinal plants. The manner in which medicinal plants are consumed has been planned in accordance with customs and ideas that predate the acceptance of so-called "scientific drug therapy" or "modern medicine (Abad et al., 2012). One of the most important sources of the phytochemical component known as secondary metabolites, which has been widely exploited in most pharmaceutical firms, is aromatic and medicinal plants (B. P. Pandey et al., 2017). As far as by WHO which is world health organization out of 100, 80% of this earth mass depend upon conventional medical knowledge, particularly that of medicinal plants, to support their healthcare (Ekor, 2014; Kumar et al., 2012).

Mugwort, or *Artemisia vulgaris (L.)*, is a member of the Asteraceae family(Hamad et al., 2018). It is made up of tough plants like shrubs and herbs that produce volatile oils. It is indigenous to temperate regions of Asia, northern Africa, but it is also a weed that has spread to North America (Ashok & Upadhyaya, 2013). The snakebite antidote medication "nagdaun" is derived from A. vulgaris in Ayurveda. Aerial components of A. vulgaris have historically been widely utilized as anthelmintic, antiseptic, antispasmodic, antidiabetic, antiepileptic, vermicides, and antidepressant (Nigam et al., 2019; (B. P. Pandey et al., 2017). Since ancient times, its crude extract has been used to treat malaria, and research has shown that artemisinin obtained from *A. vulgaris* has anticancer action (J. Pandey et al., 2021). Over skin conditions, a paste or powder made from the leaves is applied (Govindaraj et al., 2008).

In previous finding (Thangjam et al., 2020) the preliminary phytochemistry test disclosed the existence of saponins, glycosides, flavonoids, protein, and Titerpenoides. Phytochemicals in most plant extracts had a variety of biological properties, including pain killer, anti-cancer properties, anti-inflammation medication, and antioxidant properties; the residence of these properties also guaranteed the potential of this plant properties in medicine sector(Majid et al., 2015).

According to the previous investigation of (Research et al., 2021), all three extracts—the methanol, hexane, and chloroform extracts were subjected to phytochemical screening. While very few chemicals were discovered in the less polar extracts, many compounds were present in the more polar extracts. Phenols were present in methanol and chloroform extract. Whereas screening of the phytochemicals which was done confirmed the residence of flavonoids, glycosides as well as alkaloids and also a proteins in three liquid extracts. Quinones were absent in all three extracts; tannins and saponins were present in both chloroform and methanol extracts. For antimicrobial activity, chloroform showed antimicrobial activity against different microorganisms. In a volume of 80ul chloroform extract showed strong activity against *Micrococcus luteus with an* inhibition zone of 15mm; *Klebsiella pneumoniae* showed a zone of inhibition of 8mm; *Bacillus subtilis showed a* zone of inhibition of 6mm; and *Enterobacter* cloacae *subsp. disolvens* showed a zone of inhibition of 13mm. *Pseudomonas sp. and Staphylococcus aureus* showed no activity.

In previous work by (Karabegović et al., 2011), the antimicrobial properties of the Artemisia species extracts obtained by the two different extraction techniques were evaluated against four different microbial species, including two yeast species (*Sacc. cerevisiae and C. albicans*), two bacteria which are gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*), whereas in gram positive bacteria(*Staphylococcus aureus* and *Bacillus subtilis*), and one mold (*Aspergillus Niger*). None of the test microorganisms were inhibited by the methanol control treatment. The antibacterial activity of each studied extract was superior against yeasts compared to both gram-positive and gram-negative bacteria. The *A.* campestris extracts were discovered to be significantly more active than the *A. vulgaris* extracts (95% confidence interval) in all cases. The *A.* campestris extracts prepared using either extraction method had very potent antibacterial properties, particularly when used against the mold *A. niger*. The

extracts of *A. vulgaris* were ineffective against the mold, regardless of the extraction method used. This might imply that greater doses of the species' extract should be used.

A. vulgaris extracts produced by ultrasonic and conventional extraction did not exhibit any antibacterial activity that was significantly different (95% confidence interval). With substantial differences (95% confidence interval), the extracts of *A. campestris* obtained through classical extraction, however, had better activity than those obtained using ultrasonic extraction. These findings may help to explain why both Artemisia species have historically been used.

The effects of A. vulgaris essential oil on several bacteria were also researched in 2006 by (Blagojevic et al.) from Serbia and Montenegro. Steam distillation was used to separate the oil from the plant's above-ground and subsurface components. After 10- and 30-fold dilutions, the zone of pathogen growth inhibition on paper filters was studied. Due to its high concentrations of 1,8-cineole and -thujone, the oil extracted from the aerial parts exhibited inhibitory activity against a number of bacteria and fungi, including *C. albicans, Aspergillus niger, Escherichia coli, Salmonella enteritidis, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus.* However, due to the low quantity of antioxidants in the oil taken from the plant's subsurface areas, it only showed a minimal amount of effectiveness against the listed diseases(Blagojević et al., 2006).

In previous study of(Ahameethunisa & Hopper, 2012) A considerable level of inhibition was seen against *Erwinia sp.* and *X. campestris* in the study of methanol and chloroform extracts against phytopathogens. The considerable efficacy against *C. michiganense* and *P. syringae*, however, was demonstrated by extracts in ethanol and diethyl ether (p 0.05). The petroleum ether extracts also demonstrated an 11–12 mm zone of inhibition against *C. michiganense*. *A. nilagirica's* hexane extract, in contrast to other extracts, has the highest level of inhibitory action against all phytopathogens. Additionally, *Clavibacter michiganense* (13 mm), *Erwinia sp.* (13 mm), *Pseudomonas syringae* (12 mm), and *Xanthomonas campestris* (14 mm) all exhibited a strong inhibitory impact when exposed to hexane extracts. On clinical bacterial pathogens, the same examination of *A. nilagirica* leaf extracts was done. With regard to *P. aeruginosa, P. vulgaris, and S. typhi*, the extracts from hexane, methanol, and

petroleum ether showed significant high inhibitory zones. In comparison to other extracts (8 mm), the chloroform and diethyl ether extracts demonstrated the highest area zone of inhibition (10 mm) for *B. subtilis*. For E. coli, the ethanol extract showed a 14 mm zone, it is the highest in relation to the positive streptomycin standard. Methanol (12 mm), chloroform (13 mm), and diethyl ether (14 mm) extracts were then discovered to have antibacterial action against *Y. enterocolitica.*

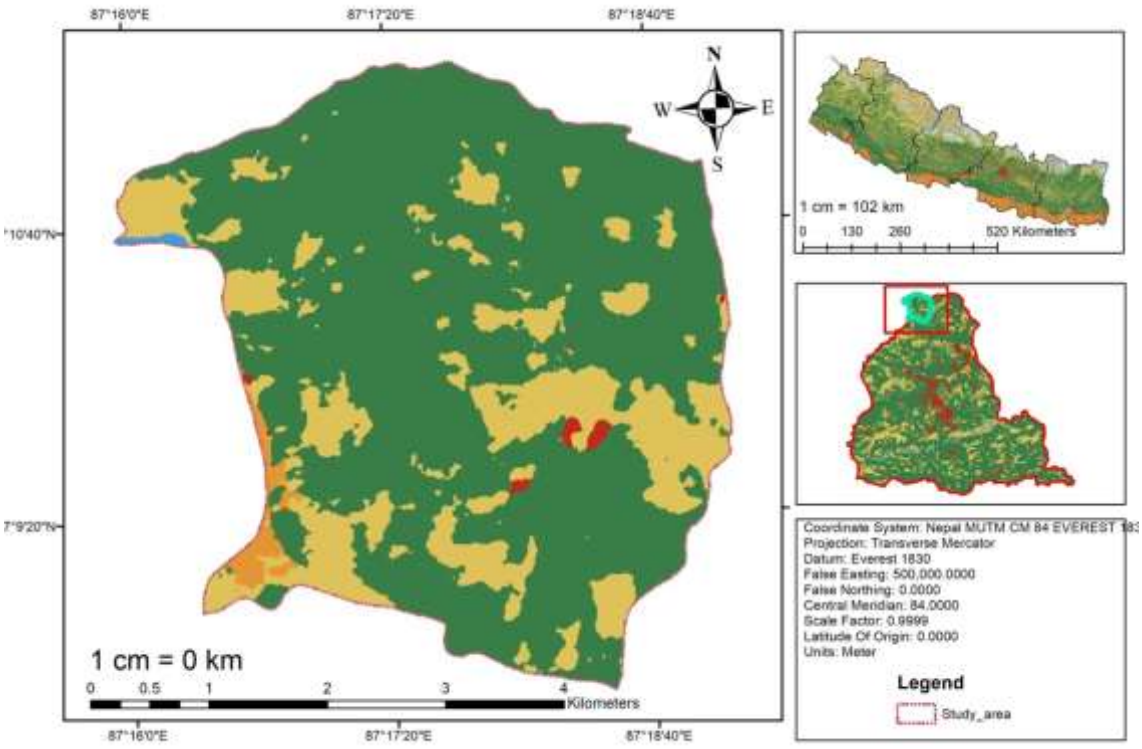
In previous study of(Ewunu, 2022) the author analyze that due to the characteristics of gram-positive and gram-negative bacteria's cell walls, the methanolic and chloroform extracts were potent against gram positive and gram negative bacteria. For the crude methanol extract, the maximum antibacterial activity against S. aureus was 200 mg/ml (11.77 ±0.86), whereas the minimum antibacterial activity against the same organism was 60 mg/ml and 130 mg/ml (both 0). *L. monocytogen* demonstrated antibacterial activity at concentrations which are of 60mg/ml, 130mg/ml, 200mg/ml (12.89± 0.62, 11.62 ±0.6, 11.22± 0.36, respectively, either .*K. pneumonia* has shown antibacterial activity at both concentrations of 60 mg/ml and 200 mg/ml (12.9±0.56 and 12.26±0.1, respectively) . According to(B. P. Pandey et al., 2017) results demonstrated that A. vulgaris's methanol extract has comparable antibacterial efficacy to the common antibiotics ampicillin and kanamycin against *Bacillus substilis* and *Enterococcus spp*., with zones of inhibition of 12.48 mm and 12.06 mm, respectively. In previous study of(Ahameethunisa & Hopper, 2010)Using the micro dilution technique, MIC assays of organic solvent extracts of *A. nilagirica* were conducted against 15 bacterial species. Six extracts had MIC values that ranged from 32 to 512 g/ml. For Erwinia sp. and X. campestris, chloroform extract demonstrated maximum activity with MIC 32 g/ml when considering phytopathogens. Diethyl ether extracts for P. syringae and C. michiganense revealed 32 g/ml MIC. In general, all extracts had MICs for the investigated phytopathogens of less than 128 g/ml. Interestingly, even at a low concentration of 32 g/ml, hexane extract demonstrated stronger activity in all the phytopathogens. Hexane and methanol extracts are significantly effective inhibitors of phytopathogens and clinical pathogenic bacteria, respectively, according to the findings of zone of inhibition and MIC investigations.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Study Area

The study area includes Mahalaxmi municipality, Dandagaun in Eastern Nepal located in Dhankuta District. Geographically it covers an area of 126.3 sq. km. with altitude ranging from 250m to 2696m above sea level.



3.2 Materials

The fresh leaves of A. vulgaris were collected from hilly region in Dhankuta, Nepal (Province no. 1) during autumn season 2022. The taxonomic identification of the plant was done at the Central Campus of Technology located at Dharan by professor of the campus.

3.3 Methods

3.3.1 Extraction

The collected fresh plant material was washed with tap water in order to remove unwanted dirt particles from the surface of plant material. The leaves were then shade dried and grinded to powder form and stored in clean zipper bag until further use. The

extraction of the plant materials was done by using Soxhlet extraction method. The powdered sample was taken in two separate thimbles of Soxhlet extractor. 10gm each sample was taken on 200ml methanol and petroleum ether respectively. The round bottom flask was filled with methanol and petroleum ether and adjusted to extractor. The solvent methanol and petroleum ether was then heated at 70℃ and 60℃ respectively until the clear solvent was seen at apparatus. These two samples methanol and petroleum ether was used for qualitative analysis of phytochemicals(Dong, 2012).

3.3.2 Phytochemical Analysis

Tests for different phytochemicals were done by following procedures.

A. Test for Proteins

a) Xanthoproteic test:

In 2 ml of extract, 2ml of concentrated HNO3 was added. The formation of orange color indicated the presence of proteins (Godghate et al., 2012).

B. Test for Carbohydrates

a) Benedicts Test:

Benedict's reagent was heated after combining to 2 ml of extract. The residence of carbohydrates was indicated by the formation of orange-red precipitate (Godghate et al., 2012).

C. Test for Alkaloid

a) Mayer's Test:

In 2 ml of the extract Mayer's reagent was combined drop wise, and when a precipitate that was white or creamy formed on test tube side, it was determined that the extract contained alkaloids(kumar et al., 2012).

D. Test for Flavonoids

a) Alkaline Reagent Test:

In 2milliliter plant extract 2ml of 2% sodium hydroxide was merged. Precipitation that is yellow in color suggested the presence of flavonoids(kumar et al., 2012).

E. Test for tannins

a) Ferric Chloride Test:

When 2milliliter of the plant extract was merged to a solution of 10% ferric chloride drops, the color changed to a bluish dark to green hue, indicating the residence of catechol tannins and gallic(Auwal et al., 2014).

F. Test for Saponins

a) Foam Test:

Distilled water of about 10ml was merged in 2ml of extract and vigorously shaken for 5 minutes in a test tube, Presence of saponins is confirmed by observing foam(Auwal et al., 2014).

G. Test for Glycosides

a) Keller-killani Test:

To 10 milliliter plant concentrate, 1 milliliter of concentrated H2SO4 was added after adding a combination of a drop of 2% FeCl3 as well as 4 milliliter of glacial acetic acid. The occurrence of cardiac glycosides is shown by the brown ring that forms between the layers (Gul et al., 2017).

H. Test for Terpenoids

a) Salkowski's Test:

In extract of about 2ml,1 ml of chloroform was added after that few drops of H2SO4 was also added on test tube side and well agitated. Titerpenoides presence is indicated by the creation of a yellow color at the lower layers (kumar et al., 2012).

3.3.4 Antibacterial Susceptibility Test

A. Preparation of Bacterial culture

The bacterial culture required for this study was obtained from the preserved bacterial culture at CCT Microbiology Laboratory, Dharan and maintained in NB. Before using the prepared bacteria, turbidity was maintained as McFarland standard by adding Normal saline in the bacterial culture (HiMedia Laboratories Pvt.Ltd).

B. Preparation of MHA Agar

For the preparation of MHA Agar 19gm of agar was taken for 500 ml of distilled water and in volumetric flask it was stirred and then heated on Bunsen burner up to boiling point. After that it was autoclaved at 121℃ in 15lbs for 15mintues (HiMedia Laboratories Pvt.Ltd).

C. Preparation of Nutrient Broth

For the preparation of Nutrient Broth 1.4gm of NB was taken for 50ml distilled water and it was heated for some time. After that it was autoclaved for 15 minutes, on 15lbs at 121℃ (HiMedia Laboratories Pvt.Ltd).

D. Plating and Inoculating Bacteria and Extract

Using the agar-well diffusion method, the extract's antibacterial activity was evaluated separately against harmful bacteria such as Staphylococcus aureus, E. coli, Shigella dysenteriae, Salmonella typhi, Streptococcus pyogenes, and MRSA (Azoro, 2000). Using sterilized cotton swabs and a 4-hour-old broth culture containing the appropriate microorganisms, MHA plates were swabbed. The dried extract was combined with 90 ul of distilled water and 10 ul of DMSO for sample preparation. Each Petri plate had a 6-mm well cut into it using a sterile cork borer. Separately, 50 ul of methanol and petroleum ether extracts of Artemisia vulgaris leaf material were added to the well. The plates were then incubated for 16–24 hours at 37°C. Based on the measurement of the inhibition zone that formed around the well, the extract's antibacterial activity was determined. To compare the effectiveness of the extracts,

ampicillin was employed as a positive control and DMSO as a negative control(Rakkimuthu et al., 2018).

3.3.5: Minimum Inhibitory Concentration of Plant Extract

For the MIC determination, a variation of the dilution approach was applied. Azithromycin was diluted into a range of concentrations in test tubes filled with sterile nutrient broth, and plant extract was diluted on the range of concentrations in test tubes filled with sterile nutrient broth including 100, 50, 25, 12.5, 6.25, 3.125, 1.56 mg/ml. A loopful (10ul) of 0.5 McFarland standard *Escherichia coli* culture (Eucast, 2003) was inoculated into test tubes nutrient broth using a standard wire loop. The same procedure was carried out for *St. Pyogenes*. The tubes were incubated at 37°C for 18 to 24 hours, following which growth or turbidity was checked.

CHAPTER 4

4. RESULT AND DISCUSSION

The leaves of *A. vulgaris* were studied for the phytochemical analysis. From the tests performed, it was found that different phytochemicals were present in leaves of *A. vulgaris* in methanol petroleum ether.

The results of the Phytochemical Screening have been summarized in Table 1.

4.1 Qualitative Analysis of Phytochemicals

Table 1: Results of the Phytochemical Screening of Methanol Extract (ME): Petroleum Ether Extract (PE) extract of *A. Vulgaris* leaves.

S.N	Detection	Reference	Results	
			Leaf extract	
			ME	PE
1	Proteins	Orange color appeared	+	+
2	Carbohydrates	Orange red ppt	-	-
3	Alkaloids	White creamy ppt	-	+
4	Flavonoid	Yellow ppt	-	+
5	Tannins	Change in color dark green	+	-
6	Glycosides	Brown rings	+	-
7	Saponins	Liquid Foamed	+	-
8	Triterpenoids	Yellow ring on bottom	+	+

The result of phytochemicals revealed the presence of proteins, alkaloids, saponins, triterpenoids and glycosides in methanol extract Whereas according to the result of (Thangjam et al., 2020), the screening of phytochemicals revealed the presence of triterpenoids, proteins, glycosides, flavonoids, and saponins in methanol extract. Similarly bioactive compound like protein, Alkaloids, flavonoids and triterpenoids has been found on petroleum ether extract.

According to the previous finding by(Research et al., 2021), The numerous phytoconstituents including flavonoids, phenols, saponins, tannins, glycosides, carbohydrates, protein, and emolins were identified from the early phytochemical screening of the methanol extract but only few of bioactive compounds were found in current study of phytochemicals.

4.2 Antibacterial Susceptibility Test

The antimicrobial activity of the plant was evaluated by calculating the zone of inhibition (ZOI). The ZOI values for the different bacterial species in methanol and petroleum ether extract are tabulated below.

Table 2: Antimicrobial activity of methanol and petroleum ether extract of *A. vulgaris* leaves.

S. N	Bacteria	Zone of Inhibition (in mm)									
		Methanol				Petroleum Ether				Antibiotics	
		10 ul	20 ul	30 ul	40 ul	10ul	20ul	30ul	40ul	Name	
1	*Salmonella typhi*	11	15	18.5	20	-	-	-	-	Chloramphenicol	18
2	*Shigella dysenteriae*	14.5	16	17.5	19.5	-	-	-	-	Ciprofloxacin	53
3	*Streptococcus pyogenes*	14	16	18	20	-	-	-	-	Ciprofloxacin	14.5
4	*Escherichia coli*	12	14.5	16.5	19	-	-	-	-	Tetracyclin	17
5	*Staphylococcus aureus*	13	14.5	16	17	-	-	-	-	vancomycin	13.5
6	*MRSA*	10	13	15	17	-	-	-	-	vancomycin	22

The results of my investigation showed that the methanol extract had antibacterial properties against several infections. The highest zone of inhibition among the tested strains was found for two pathogens, reaching 20mm when using a volume of 40 ul. Shigella has also shown notable sensitivity to the methanol extract, with a 19.5mm zone of inhibition. Additionally, the extract showed notable effectiveness against E. coli, with a 19-mm zone of inhibition. Additionally, the extract inhibited Staphylococcus aureus and MRSA, with zones of inhibition

measuring 17 mm each. The antimicrobial activity using a volume of 30 ul was found to be highest against *Salmonella typhi with a* zone of inhibition measuring 18.5mm, followed by *Streptococcus pyogenes with a* zone of inhibition measuring 18 mm, while *Staphylococcus aureus* exhibited a zone of inhibition of 16 mm, and *E. coli, MRSA, and Shigella dysenteriae* showed inhibition zones of 16.5mm, 15 mm, and 17.5 mm, respectively. Further, the antimicrobial activity using a volume of 20 ul was found to be highest against *Shigella dysenteriae and Streptococcus pyogenes,* with zones of inhibition of 16mm each. And it was found to be lowest against *MRSA,* which had a 13-mm zone of inhibition. The zones of inhibition shown by E. coli *and Staphylococcus aureus* were 14.5mm each, and the bacteria *Salmonella typhi showed an* inhibition zone of 15mm. Similarly, the antimicrobial activity using volume 10 ul was found to be highest against the bacteria *Shigella dysenteriae* with an inhibition zone of 10mm and lowest against *MRSA with an* inhibition zone of 10mm. Antimicrobial activity against *Salmonella typhi, Streptococcus pyogenes, E. coli, and Staphylococcus aureus* was found to be 11mm, 14mm, 12 mm, and 13 mm, respectively. On the other hand no antimicrobial activity was determined in the petroleum ether extract whereas according to the result of (Ahameethunisa & Hopper, 2010) the zone of inhibition was found to be 12mm against *Salmonella typhi*, 8mm against *Escherichia coli*, 8mm against *Bacillus subtilis* and no zone of inhibition was shown against *Staphylococcus aureus, Klebsiella pneumoniae* and *Enterococcus faecalis* in petroleum ether extract on *Artemisia nilagirica.*

The present study revealed that *Artemisia vulgaris* methanol extract has comparable antibacterial efficacy to the common antibiotics chloramphenicol, tetracyclin, ciprofloxacin and vancomycin against *Salmonella typhi, Escherichia coli, Streptococcus pyogenes* and *Staphylococcus aureus* respectively.

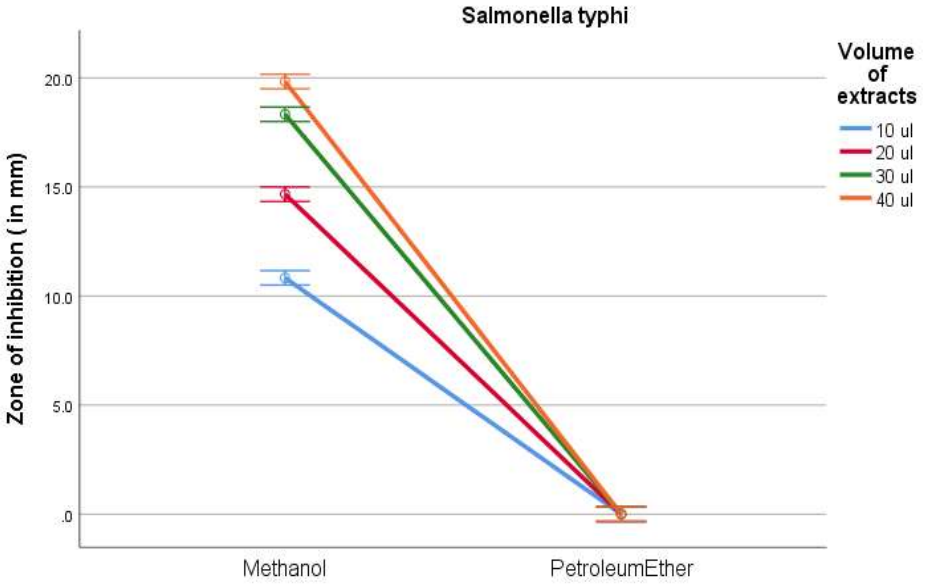


Figure 1: Antimicrobial activity against *Salmonella typhi.*

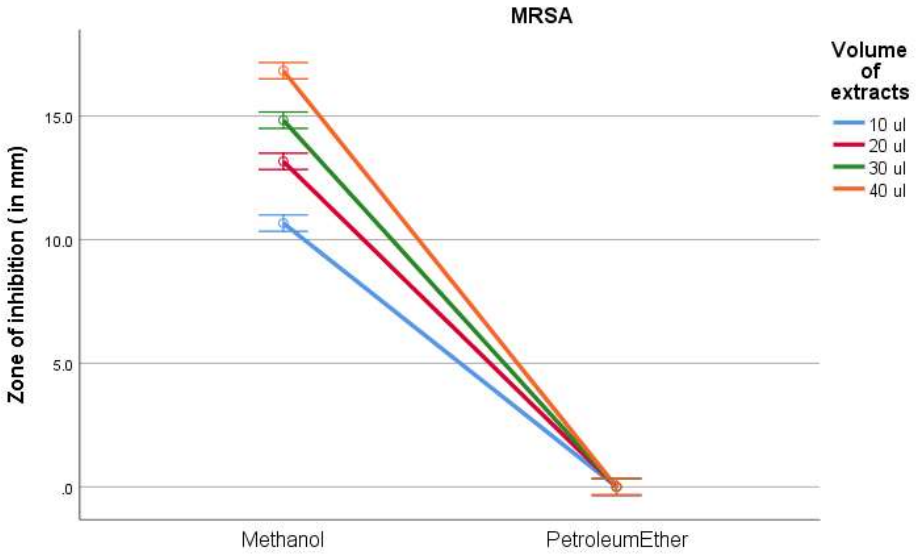


Figure 2: Antimicrobial activity against *MRSA.*

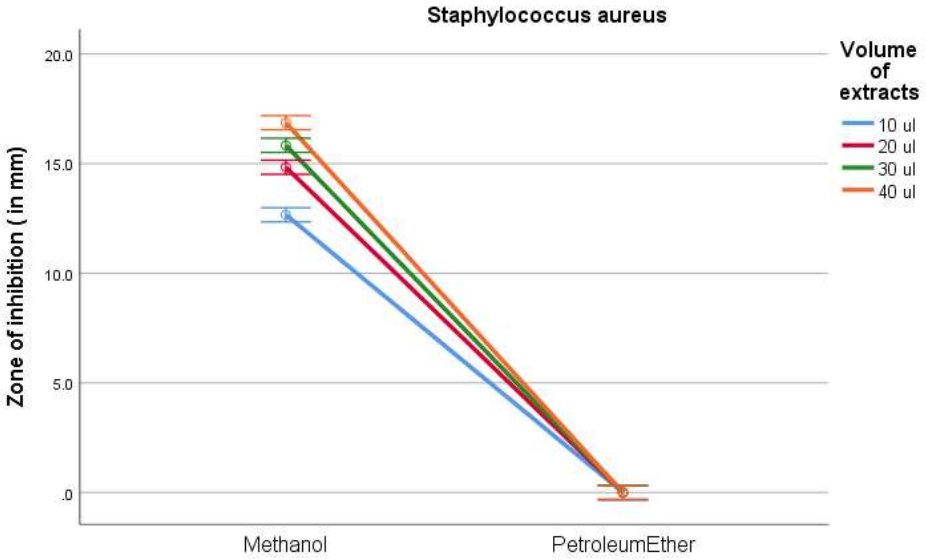


Figure 3: Antimicrobial activity against Staphylococcus aureus.

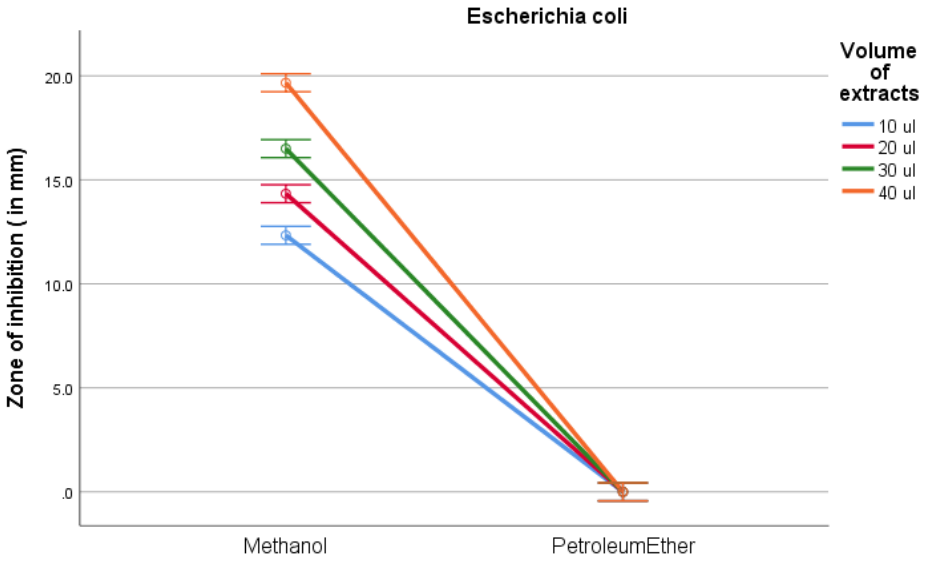


Figure 4: Antimicrobial activity against *Escherichia coli.*

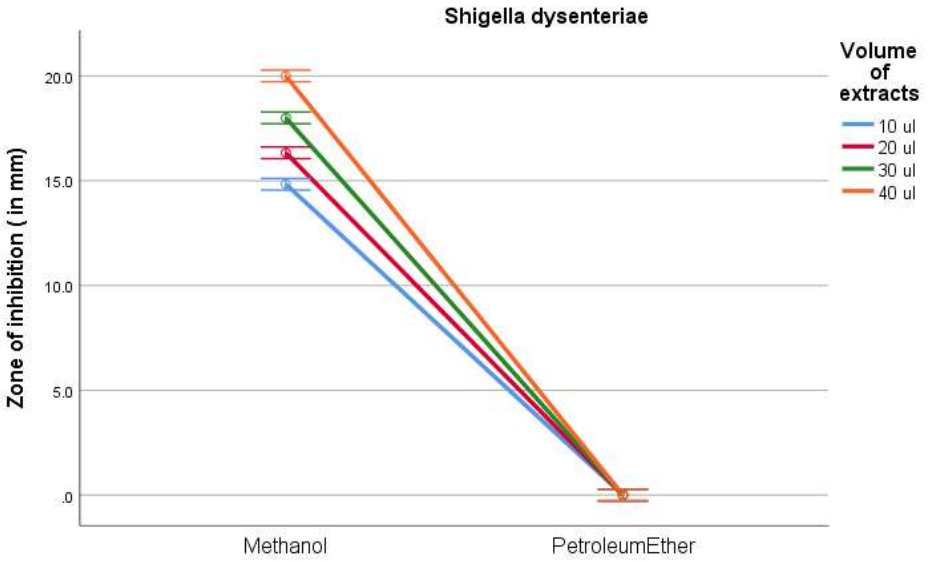


Figure 5: Antimicrobial activity against *Shigella dysenteriae.*

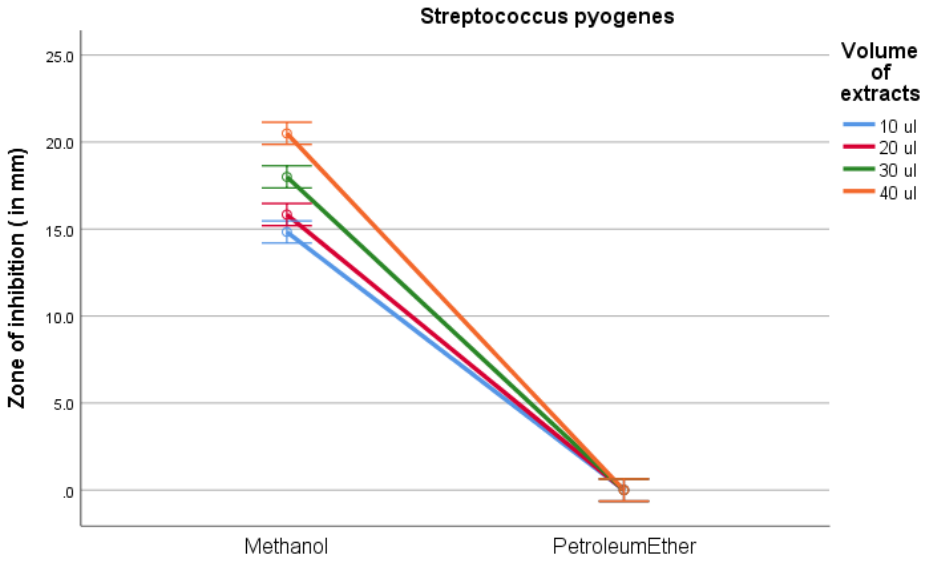


Figure 6: Antimicrobial activity against *Streptococcus Pyogenes*.

Table 3: Anova table

Levene's Test of Equality of Error Variances[a]

		Levene Statistic	df1	df2	Sig.
AA with Salmonella typhi	Based on Mean	11.145	7	15	0.000
	Based on Median	0.757	7	15	0.630
	Based on Median and with adjusted df	0.757	7	4.000	0.650
	Based on trimmed mean	8.803	7	15	0.000
AA with Streptococcus pyogenes	Based on Mean	6.053	7	15	0.002
	Based on Median	3.018	7	15	0.034
	Based on Median and with adjusted df	3.018	7	5.158	0.117
	Based on trimmed mean	5.827	7	15	0.002
AA with Shigella dysenteriae	Based on Mean	4.133	7	15	0.010
	Based on Median	1.398	7	15	0.276
	Based on Median and with adjusted df	1.398	7	6.000	0.350
	Based on trimmed mean	3.919	7	15	0.013
AA with Escherichia coli	Based on Mean	5.643	7	15	0.002
	Based on Median	1.025	7	15	0.454
	Based on Median and with adjusted df	1.025	7	4.000	0.522
	Based on trimmed mean	5.106	7	15	0.004
AA with Staphylococcus aureus	Based on Mean	11.180	7	15	0.000
	Based on Median	0.699	7	15	0.673
	Based on Median and with adjusted df	0.699	7	4.000	0.682
	Based on trimmed mean	8.806	7	15	0.000
AA with MRSA	Based on Mean	11.145	7	15	0.000
	Based on Median	0.757	7	15	0.630
	Based on Median and with adjusted df	0.757	7	4.000	0.650
	Based on trimmed mean	8.803	7	15	0.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Nameofextracts * Volumeofextracts + Nameofextracts + Volumeofextracts

Based on the analysis of variance (ANOVA) the potential antibacterial activity of methanol as well as petroleum ether concentrations of *Artemisia vulgaris,* there is a significant difference in the activity against bacteria (*Salmonella typhi, Escherichia coli, MRSA, Staphylococcus aureus, Shigella dysenteriae, and Streptococcus pyogenes*).

The previous investigation of antimicrobial activity by(Research et al., 2021), was found to have an 8mm zone of inhibition against *Klebsiella pneumonia*, 6mm against *Bacillus subtilis,* 15mm against *Micrococcus luteus, and 15* mm against *Enterobacter cloaceae subsp. disolvens KACC 13002* and there was no zone of inhibition shown against *Pseudomonas sp*. and *Staphylococcus aureus in KACC 10768*. In another study of(B. P. Pandey et al., 2017),results demonstrated that A. vulgaris's methanol extract has comparable antibacterial efficacy to the common antibiotics ampicillin and kanamycin against Bacillus substilis and Enterococcus spp., with zones of inhibition of 12.48 mm and 12.06 mm, respectively.

4.3 Minimum Inhibitory Concentration Test

The result of MIC is summarized in table:

Table 4: MIC of the methanol extract.

S.N.	Bacteria name	MIC value
1	*Escherichia coli*	6.25mg/ml
2	*Streptococcus pyogenes*	12.5mg/ml
3	Azithromycin	0.764335mg/ml

The lowest MIC value of methanol extract against *Escherichia coli* was found to be 6.25 mg/ml. Similarly, the MIC value of methanol extract against Streptococcus pyogenes was found to be 12.5 mg/ml, which means that these are the concentrations that have the potential to inhibit bacterial growth. The MIC value of antibiotics was found to be 0.764335 mg/ml, which is comparable to our methanol extracts whereas according to the result of (Chikezie, 2017) After being incubated in nutritional broth with varying gentamicin concentrations, E. coli and K. pneumonia displayed turbidity at 5 and 6 g/ml, but not at higher concentrations. Staphylococcus aureus displayed turbidity at 9.5 g/ml, but not at 9.8 g/ml or higher gentamicin concentrations. According to (Mamatova et al., 2019) Regardless of the chloroform and ethanol

extract of Artemisia gmelinii, gram-positive bacteria such as staphylococci, Micrococcus luteus, and Bacillus spp. (MIC = 1.25–5 mg/ml) and yeasts such as Candida spp. (MIC = 2.5–5 mg/ml) were the most resistant. Against gram-negative rods, both extracts' biological activity was at its lowest.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusions

The present study of screening of phytochemicals in petroleum ether and methanol plants extract of *Artemisia vulgaris* leaves revealed the presence of bioactive compounds like proteins, tannins, saponins, flavonoids. Thus this plant can be used as good therapeutic agent in modern era and it can be used as home remedy to cure multiple diseases.

The present investigation of antimicrobial activity of *Artemisia vulgaris* revealed that the leaves of this plant part acquire significance potential as antimicrobial agent. The methanolic extract of this plant showed great antimicrobial properties against *Salmonella typhi* as well as *Streptococcus pyogenes* followed by bacteria *Shigella dysenteriae, Escherichia coli, Staphylococcus aureus and MRSA* whereas petroleum ether extract of leaves did not show any zone of inhibition against any microoraganisms. These finding suggest that *A. vulgaris* antimicrobial activity is very promising which may help to discover new resources of new drugs.

5.2 Novelty and National Prosperity aspect of project work

1. The research on phytochemical analysis and antibacterial activity in this particular geographical area is hardly ever done, making it by far the most original investigation.
2. The phytochemical screening of A. vulgaris in Dhankuta has not been covered in any national literature before.
3. The antimicrobial activity of this plant in petroleum ether extract has not been done.
4. This study has an impact on the use of invasive plants in medicine as well as their usage as a natural home treatment.
5. Overall, the production of crude plant extract for commercial purposes can assist a country in obtaining royalties.

5.3 Limitation of work

- The main limitations of this investigation are that all phytochemical tests and antimicrobial assays could not be completed due to a lack of time and the inability to obtain the chemicals required.
- TPC and TFC could not be completed.
- Antioxidant activity could not be completed.

5.4 Recommendation

According to this study, the plant has a variety of phytochemicals that can be used to treat various disorders. The presence of several phytoconstituents demonstrates a considerable advantage in the medical area. It also functions as an antibacterial agent, helping in the prevention of sickness caused by numerous pathogens and delaying the spread of microbes. Despite being beneficial in numerous ways, this particular plant's study is inadequate. To determine this plant's therapeutic potential, a thorough investigation should be conducted with a focus on phytochemical screening and antibacterial activity. Additionally further research should be done to analyze its total phenolic and flavonoid content and antioxidant activity.

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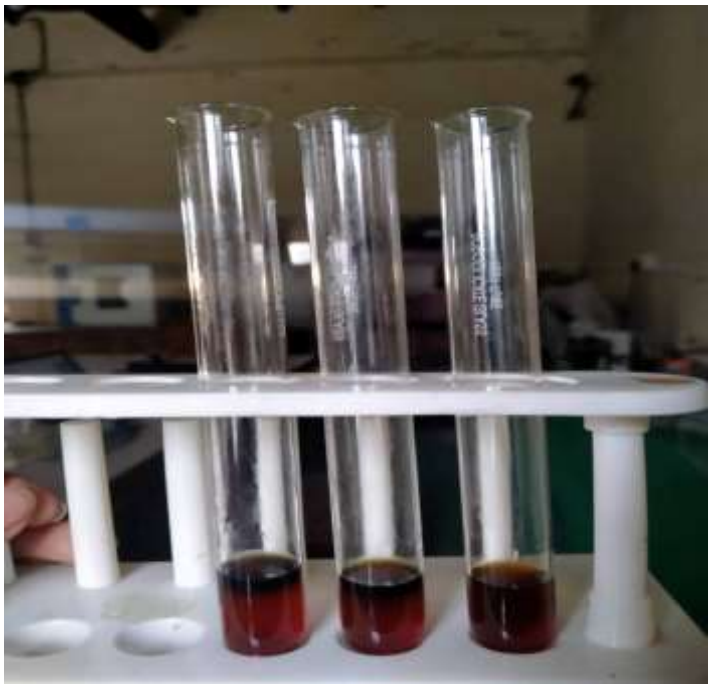
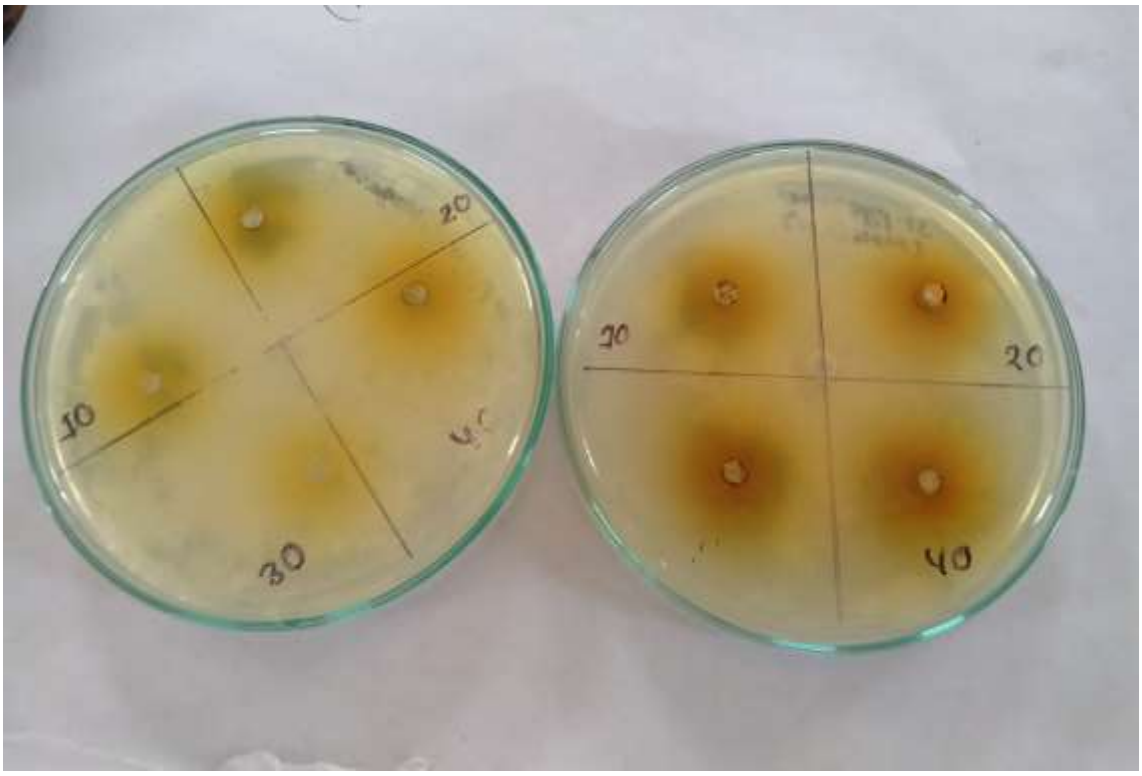
APPENDIX

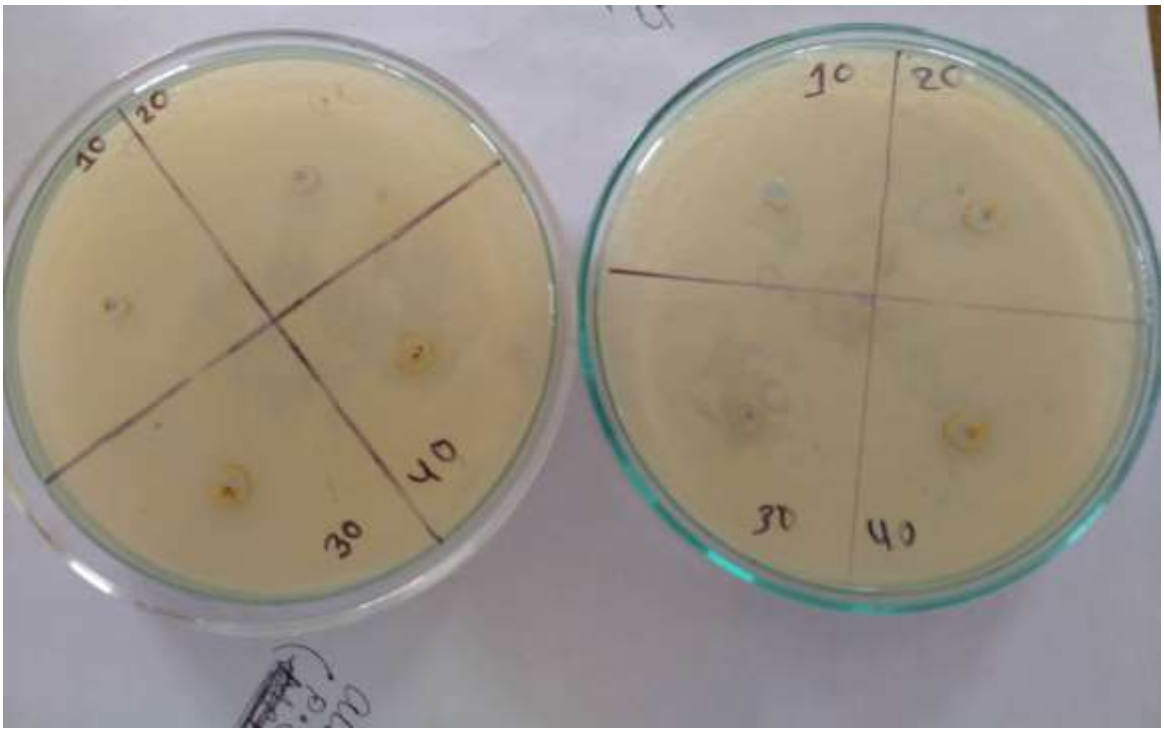
Photographs 1: Showing positive result of Alkaloids in P.E.



Photographs 2: Showing positive result of Proteins in M.E.



Photographs 3: ZOI shown by M.E. against *Streptococcus pyogenes*.



Photographs 4: No ZOI shown by P.E. against *MRSA*.

Photographs 5: Soxhlet extraction of *Artemisia vulgaris (L)*.

Photographs 6: Photo during laboratory work.