Antibacterial Activity of Selected Orchid Species of Nepal

A Dissertation Submitted for Partial Fulfillment of the Requirement for Master's Degree in Biodiversity and Environmental Management (BEM)

Submitted By

Sajan Dulal

Exam Roll No.: 309/075

T.U. Regd. No. : 5-2-37-1949-2013

Batch: 2075

Submitted to

Central Department of Botany

Institute of Science and Technology

Tribhuvan University,

Kirtipur Kathmandu, Nepal

October, 2023

RECOMMENDATION

This is to certify that the dissertation work entitled **"Antibacterial Activity of Selected Orchid Species of Nepal"** submitted by Mr. Sajan Dulal was accomplished under my supervision. It is original research carried out by the candidate and to the best of my knowledge the work has not been submitted elsewhere for any academic purpose. I hereby recommend for the acceptance of this dissertation as a partial fulfillment of the requirement of Master's Degree in Biodiversity and Environmental Management at Tribhuvan University.

…….

Supervisor

Dr. Bijaya Pant, PhD
Professor
Central Department of Botany
Tribhuvan University
Kirtipur, Kathmandu,Nepal

Date: 8th October, 2023

LETTER OF APPROVAL

The M.Sc. dissertation entitled **"Antibacterial Activity of Selected Orchid Species of Nepal"** submitted by Mr. Sajan Dulal has been accepted as partial fulfilment of the requirement of Master of Science in Biodiversity and Environmental Management (M.Sc. BEM).

EXAMINATION COMMITTEE

……

(Supervisor)

Dr. Bijaya Pant, PhD

Professor

Central Department of Botany

Tribhuvan University, Nepal

……..

(Head of Department)

Dr. Sangeeta Rajbhandari, PhD

Professor

Central Department of Botany

Tribhuvan University, Nepal

……..

(External Examiner)

Dr. Anjana Singh, PhD

Professor

Cantral Department of Microbiology

Tribhuvan University, Nepal

……

(Internal Examiner)

Dr. Muktiram Paudel

Assistant Professor

Central Department of Botany

Tribhuvan University, Nepal

Date: 8th October, 2023

DECLARATION

I, Sajan Dulal, M. Sc student of BEM certify that this dissertation entitled **"Antibacterial Activity of Selected Orchid Species of Nepal"** submitted to the Institute of Science and Technology, Tribhuvan University, for completion of Master's Degree in BEM is a record of genuine work carried out by me under the supervision of Dr. Bijaya Pant, Professor, Central Department of Botany. I further declare that the work reported in this research has not been previously submitted either in part or in full for the award of any degree, in this or any other institute or University.

……

Sajan Dulal

Biodiversity and Environmental Management (BEM)

Central Department of Botany

Date: 8th October, 2023

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my venerable supervisor Professor Dr. Bijaya Pant, Professor of Central Department of Botany, Tribhuvan University for her continuous encouragement, support, patience, belief and guidance during the completion of research work.

I am thankful to Professor Dr. Sangeeta Rajbhandari, Head of Central Department of Botany, Tribhuvan University as well as other faculty members for their support. I am equally thankful to Prof. Dr. Suresh Kumar Ghimire, Coordinator of BEM for his support and encouragement.

I would like to extend my thanks to Department of Forest, Babarmahal, Kathmandu for granting permission for the collection of plants samples from various parts of Nepal. Likewise, I am in debt to the National Youth Council (NYC), Bhaktapur, Nepal for their financial support. I am much obliged to National Public Health Laboratory (NPHL), Teku for providing me bacterial strains for this research.

I would also like to convey my warm regards to PhD Scholar Pushpa Raj Joshi for his guidance, suggestions and encouragement throughout the research work

I am thankful to Annapurna Reseaech Center, Maitighar, for providing laboratory facilities to carry out plant extraction, phytochemical screening and antibacterial work. I am grateful to Anil Kumar Shah, and thanks to all members of Annapurna research center for their suggestions and support during the research work.

Finally yet importantly, I owe a lot to my father, mother, and my friends who helped me get to where I am today and whose support inspires me to keep moving in the right direction.

Sajan Dulal

8th October, 2023

Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

ATCC - American Type Culture Collection

DMSO - Dimethyl Sulfo Oxide

MBC - Minimum Bactericidal Concentration

µg - microgram

MHA - Mueller Hinton Agar

MIC - Minimum Inhibitory Concentration

NA - Nutrient Agar

NB - Nutrient Broth

ZOI - Zone of Inhibition

E. coli - *Escherichia coli*

Fig. - Figure

Gram –ve - Gram negative

Gram +ve - Gram positive

WHO - World Health Organization

wt. – Weight

L-leaf

R- Root

gm – grams

^{o} C – degree Celsius

mg –milligram

ml – milliliter

H_2So_4 – Sulphuric Acid

NaOH – Sodium Hydroxide

HCL – Hydrochoric Acid

$FeCl_3$ – Ferric Chloride

lbs – Pounds

cm –Centimeter

Abstract

Orchidaceae, a highly advanced and widely spread family of monocotyledonous plants. Orchids have been found to contain a rich source of natural compounds with significant therapeutic activities against various disease. So this study mainly focused on antibacterial activity to generate natural medicines to substitute synthetic drugs. The orchid plants were collected from various regions of Nepal and subsequent extract preparation and laboratory work conducted at the Annapurna Research Center.

A total of fifteen orchid species were selected for the purpose of this study. The different parts of orchids were used for extraction using methanol through a combination of the Percolation and intermittent sonication methods. After that in vitro antibacterial evaluation was conducted using the agar well diffusion technique with different concentration.

Out of 15 species, the highest yield was obtained from *Habenaria marginata* 15%, and the lowest was from *Coelogyne stricta* 1%. In the qualitative analysis, various reagents were used to quantify tannins, flavonoids, glycosides, phenols and alkaloids. A qualitative phytochemical screening indicate that alkaloids and flavonoids were present in the extracts of all the orchid species that were tested. Both Phenol and Carbohydrate were absence in *Coelogyne stricta* and *Eria graminifolia*. The antibacterial activity of the methanol extracts from these plants was evaluated against medically significant bacteria. *Enterococcus faecalis, Acinetobacter baumannii, Escherichia coli* and *Shigella sonnei* were Gram negative whereas *Staphylococcus aureus* was Gram positive bacteria. All 15 plants showed activity against at least one bacterium. The two orchid plants, *Coelogyne stricta* and *Otochilus albus*, exhibited broad-spectrum activity by showing a high zone of inhibition against all tested bacteria with zone of inhibition (20mm) and

(21mm) against *Acinobacter baumannii* respectively. Similarly, The MIC values for the tested orchid extracts range from 1.25 mg/ml to 2.5 mg/ml, while the MBC values range from 0.3125 mg/ml to 0.625 mg/ml.

So *Otochilus albus* exhibits strong antibacterial properties against *Acinobacter baumannii* species, and its corresponding MIC and MBC values provide further evidence of its antibacterial effectiveness. Therefore, it is worthy to conduct additional investigations into this specific species to reveal its potential benefits. Furthermore, there is scope for conducting additional laboratory experiments and research work to develop new pharmaceutical products.

Keywords: Orchids, Phytochemical compounds, Antibacterial activity, MBC, MIC

सारांश

ओर्किडेसी, मोनोकोटिलेडोनस बिरुवाहरूको एक अत्यधिक उन्नत र व्यापक रूपमा फैलिएको परिवार। अर्किडमा विभिन्न रोगहरू विरुद्ध महत्त्वपूर्ण चिकित्सीय गतिविधिहरूको साथ प्राकृतिक यौगिकहरूको समृद्ध स्रोत फेला परेको छ। त्यसैले यो अध्ययन मुख्यतया कृत्रिम औषधिहरू प्रतिस्थापन गर्न प्राकृतिक औषधिहरू उत्पन्न गर्न जीवाणुरोधी गतिविधिमा केन्द्रित थियो। नेपालका विभिन्न क्षेत्रबाट अर्किडका बिरुवासङ्कलन गरिएको र त्यसपछि अन्नपूर्ण अनुसन्धान केन्द्रमा गरिएको अर्क तयारी र प्रयोगशालाको काम सम्पन्न भएको थियो ।

यस अध्ययनको उद्देश्यको लागि कुल पन्ध्र अर्किड प्रजातिहरू चयन गरिएको थियो। आर्किडका विभिन्न भागहरू परकोलेसन र इन्टरमिटेन्ट सोनिकेशन विधिहरूको संयोजनमार्फत मेथनोल प्रयोग गरेर निकासीको लागि प्रयोग गरिएको थियो। त्यसपछि विट्रो जीवाणुरोधी मूल्यांकन मा विभिन्न एकाग्रता संग अगर राम्रो प्रसार प्रविधि प्रयोग गरेर आयोजित गरिएको थियो। १५ प्रजातिहरू मध्ये, उच्चतम उपज १५% हाबेनेरिया मार्जिनाटाबाट प्राप्त भएको थियो, र सबैभन्दा कम सिलोजिन स्ट्रिक्टा 1% बाट प्राप्त भएको थियो। गुणात्मक विश्लेषणमा, विभिन्न अभिकर्मकहरू ट्यानिन, फ्लेभोनोइड्स, ग्लाइकोसाइड्स, फिनोल्स र अल्कलोइडहरू मापन गर्न प्रयोग गरिएको थियो। एक गुणात्मक फाइटोकेमिकल स्क्रिनिंगले संकेत गर्दछ कि अल्कलोइडर र फ्लेवोनोइड्स परीक्षण गरिएका सबै आर्किड प्रजातिहरूको अर्कहरूमा उपस्थित थिए। फिनोल र कार्बोहाइड्रेट दुवै सिलोजिन स्ट्रिक्टा र एरिया ग्रामिनिफोलियामा अनुपस्थित थिए। यी बिरुवाहरूबाट मेथनोल अर्कको जीवाणुरोधी गतिविधि चिकित्सकीय रूपमा महत्त्वपूर्ण ब्याक्टेरियाको विरुद्ध मूल्यांकन गरिएको थियो। एंटरोकोकस फेकलिस, एसिनेटोबैक्टर बौमन्नी, एस्चेरिचिया कोली र सिजेल्ला सोनेई ग्राम नकारात्मक थिए जबकि स्टैफिलोकोकस ऑरियस ग्राम पॉजिटिव जीवाणु थिए । सबै १५ वटा बोटबिरुवामा कम्तीमा एउटा ब्याक्टेरियाविरुद्ध सक्रियता देखिएको थियो । दुई आर्किड बिरुवाहरू, सिलोजिन स्ट्रिक्टा र ओटोचिलस अल्बसले क्रमशः एसिनोब्याक्टर बौमानीको बिरूद्ध निषेध (20 मिमी) र (21 मिमी) को क्षेत्रको साथ सबै परीक्षण गरिएको ब्याक्टेरियाको विरुद्ध निषेधको उच्च क्षेत्र देखाउँदै व्यापक स्पेक्ट्रम गतिविधि प्रदर्शन गरे। त्यस्तै, परीक्षण गरिएको आर्किड अर्कको लागि एमआईसी मानहरू 1.25 मिलीग्राम / एमएल देखि 2.5 मिलीग्राम / एमएल सम्म छन्, जबकि एमबीसी मानहरू 0.3125 मिलीग्राम / एमएल देखि 0.625 मिलीग्राम / एमएल सम्म छन्।

त्यसैले ओटोचिलस अल्बसले एसिनोब्याक्टर बौमानी प्रजातिहरूको बिरूद्ध बलियो जीवाणुरोधी गुणहरू प्रदर्शन गर्दछ, र यसको सम्बन्धित एमआईसी र एमबीसी मानहरूले यसको जीवाणुरोधी प्रभावकारिताको थप प्रमाण प्रदान गर्दछ। यसैले, यसको सम्भावित लाभहरू प्रकट गर्न यो विशिष्ट प्रजातिमा थप अनुसन्धान गर्न योग्य छ। यसबाहेक, त्यहाँ नयाँ फार्मास्युटिकल उत्पादनहरू विकास गर्न थप प्रयोगशाला प्रयोगहरू र अनुसन्धान कार्य सञ्चालन गर्न स्कोप छ।

कुञ्जीशब्द: आर्किड, फाइटोकेमिकल यौगिक, जीवाणुरोधी गतिविधि, एमबीसी, एमआईसी

CHAPTER: 1 INTRODUCTION

1.1 Background of the Study

Orchidaceae, a highly advanced and widely spread family of monocotyledonous plants, encompasses approximately 30,000 accepted species (Govaerts et al., 2023). In Nepal, 517 species under 105 genera have been reported (Vaidya, 2019). Orchids are extensively used in traditional medicine for treating severe illnesses (Pant and Raskoti, 2013). Orchid species are traditionally used as tonics in Nepal to improve digestion, stimulate body fluid production, and reduce fever (Ng *et al.,* 2012; Xu *et al.,* 2013). Orchids have been employed worldwide in traditional healing and treatment of various diseases. Bridging indigenous knowledge and ethno-pharmacological studies with modern research can lead to a more effective approach in drug discovery (Bulpitt *et al.,* 2007). The potential of orchids as a source for novel drugs with medicinal properties can provide to the demand for life-saving medications (Joshi *et al.,* 2023; Yang *et al.,* 2006).

Medicinal plants hold immense value as they serve various purposes, including traditional medicine, modern medicine, nutraceuticals, food supplements, folk remedies, and as a source of chemical compounds for synthetic drugs (Ncube *et al.,* 2008). Nepal's rich tradition of medicinal plants dates back to the Vedic period, and the Nepal Himalayas are recognized as a sacred sanctuary harboring potent medicinal and aromatic plants (Baral and Kurmi, 2006). Orchids have been found to contain a rich source of natural compounds with significant therapeutic activities against various diseases. These compounds exhibit antioxidant, anti-rheumatic, anti-inflammatory, antiviral, anti-carcinogenic, anticonvulsive, diuretic, neuroprotective, relaxing, anti-aging, wound healing, hypoglycemic, antitumor, anticancer, antimicrobial, and antiviral properties (Joshi *et al.,* 2023; Shimura *et al.,* 2007; Kumar *et al.,* 2000; Devkota *et al.,* 2022; Pant *et al.,* 2022; Chand *et al.,* 2016).

Bacterial diseases are a worldwide problem due to development of resistance towards different sorts of anti-toxins (Paudel *et al.,* 2018). Due to the increase of multiple drug resistant (MDR) pathogens nosocomial and community- acquired deadly infections has been increased which causes extensive utilization of antibiotics (Köck *et al.,* 2010; Mongalo *et al.,* 2013). Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi and Acinetobacter baumannii* have

inherent resistant to most available antibiotics (Cazer *et al.*, 2023; Kim *et al.*, 2005; Patzer and Dzierzanowska, 2007).

Due to its traditional importance as a medicinal plant, there has been a growing interest in understanding the components found in different types of orchids and their potential medicinal properties. Furthermore, researchers have devised techniques for successfully cultivating these plants (Xu *et al.*, 2013; Teixeira *et al.*, 2017). So present study will be focused in the evaluation of the antibacterial activity of selected orchids. The plenitude and variety of orchids are diminishing all through the world, starting with genetic disintegration also, finishing with nearby and worldwide species misfortune (Subedi *et al.*, 2013).

Phytochemicals are bioactive chemicals found in plants, are naturally produced in different parts of the plant, such as the bark, leaves, stems, roots, flowers, fruits, and seeds (Jayanthi and Lalita, 2012). These plant-derived compounds, including alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids, and terpenoids, are crucial bioactive constituents with significant therapeutic benefits (Dhandapani and Sabna, 2008). Several studies, including those by (Chand *et al*., 2016; Paudel *et al*., 2017), have demonstrated the diverse medicinal properties exhibited by these metabolites. Plants produce secondary metabolites like terpenoids, phenolics, and flavonoids to protect themselves, and these compounds also hold medicinal properties that are highly valued by humans. Terpenoids are structurally diverse natural products formed by the sequential fusion of branched five-carbon units. Plant phenolics, aromatic compounds with acidic hydroxyl groups attached to the phenyl ring, are derived from the phenylpropanoid and phenylpropanoid acetate pathways. Flavonoids, a diverse collection of over 4500 compounds, possess various beneficial properties, including antioxidative, antiviral, antimicrobial, antiplatelet, and antitoxic effects.

Certain orchid species, like *Dendrobium* and *Bulbophyllum*, are recognized for their antibacterial activity due to the presence of phytochemical compounds in different parts of the plant. Flavonoids and polyphenols are particularly important bioactive compounds known for their ability to absorb and neutralize free radicals, quench singlet and triplet oxygen, decompose peroxidases, induce anti-carcinogenic enzymes, or inhibit cancer-promoting enzymes. Orchids containing alkaloids, flavonoids, phenols, and polyphenols have been used in Ayurveda and Traditional Chinese Medicine (TCM) for their anticancer properties. In contrast to modern medications, traditional medicines are known for their reduced toxicity and similar clinical efficacy (Aawad *et al.*, 2011). Various plants have been found to exhibit

antibacterial properties, emphasizing the pressing requirement for the creation of new therapeutic antibacterial agents to address the increasing challenge of multi-drug resistant bacterial infections (Poudel *et al.*, 2018).

1.2 Rationale of Study

Numerous ethnic groups have traditionally utilized orchid plants to treat various bacterial illnesses. However, the effectiveness of these plants in fighting bacteria needs scientific confirmation. The global concern over antibiotic resistance is growing, as more disease-causing microorganisms are becoming resistant to multiple antibiotics. Scientists worldwide are investigating the impact of orchids on bacteria and there has been limited research on the antimicrobial properties of specific orchids in Nepal. Dealing with bacterial diseases has become a significant challenge due to the emergence of many multi drug-resistant bacteria, leading to heavy reliance on synthetic antibiotics. Herbal medicine could serve as a potential substitute for synthetic antibiotics, and studying the phytochemistry of orchid species may aid in developing herbal remedies. Although the orchids of Nepal have not been extensively studied, previous literature suggests that they hold promise as a source of diverse bioactive compounds. So based on these bioactive compounds the study of antibacterial analysis of orchids may fulfill the research gap.

1.3 Objective

General objective

The main objective of this research is to analyze phytochemical screening and antibacterial activity of selected orchid species.

Specific objective

- To screen the secondary metabolites in the methanol extract of different parts of orchid species.
- To evaluate antibacterial properties of Methanolic extract of orchid species.

CHAPTER: 2 LITERATURE REVIEW

2.1 Orchid Diversity and their medicinal value

Orchidaceae, one of the most extensive plant families among angiosperms, is a highly advanced group found in a variety of terrestrial ecosystems worldwide, excluding Polar Regions and deserts (Zhang *et al.,* 2022). Numerous orchids possess important aesthetic (Su *et al.,* 2020) and therapeutic importance (Tang *et al.,* 2016) leading to the frequent extraction of their natural plant resources, which diminishes both species diversity and population numbers. Orchids are found in various locations around the world, ranging from tropical regions to high-altitude areas. They are known for their extravagance in nature (White and Sharma, 2000). Meanwhile, viewed orchids as significant not only for their aesthetic beauty but also for their potential pharmaceutical uses and their role as environmental indicators (Joshi *et al*., 2009).

Habenaria dentata can be found across various regions in China, yet individual populations in its natural habitat are limited in size and dispersed. Moreover, due to its significant medicinal and aesthetic importance, the number of wild populations is rapidly decreasing (Yang *et al.,* 2023). Various species of *Anoectochilus*, including *Anoectochilus formosanus, Anoectochilus koshunensis* and *Anoectochilus roxburghii* are utilized in Chinese traditional medicine. *Anoectochilus roxburghii*, commonly known as the "King Medicine" in China, is found in southern China, Japan, Sri Lanka, India, and Nepal (Li and Zou, 1995; Tseng *et al.*, 2006).

Cultivating orchids has gained popularity in the commercial sector due to their exotic nature and high market value so these plants are currently trending because they showcase a captivating array of color variations, possess excellent longevity, and are reputed for their medicinal properties (Choudhary *et al.,* 2023). The leaves of orchid species have diverse applications in treating different conditions. For instance, the leaf paste of *Acampe praemorsa* is employed for treatment of rheumatism and pain, while the leaf juice of *Luisia zeylanica* is utilized in the treatment of wounds, boils, and burns, among other uses. (Chakraborty *et al.,* 2022).

2.2 Phytochemical Screening in Orchids

The qualitative analysis where all five types of phytochemicals, including alkaloids, flavonoids, saponins, steroids and terpenoids, as well as tannins were present in 13 samples of orchids tested (Chand *et al.*, 2016).

Alkaloids are collections of nitrogen-containing compounds with simple or intricate structures, typically characterized by their basic properties. These substances are commonly present as secondary metabolites in plants, and to a lesser extent, they can also be found in microorganisms and animals (Bribi, 2018). Flavonoids are diverse phenolic compounds occurring naturally in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine, are being actively sought after for their health benefits and have become essential in numerous applications, including nutraceuticals, pharmaceuticals, medicine, and cosmetics (Maridass *et al.*, 2008). Phenol belongs to a class of organic compounds characterized by a hydroxyl (-OH) group attached to a carbon atom within an aromatic ring and exhibits higher water solubility compared to alcohols (Zetola *et al.,* 2005).Tannins are diverse compounds with varying affinities for proteins and other biological macromolecules in aqueous solutions, leading to the formation of insoluble precipitates, and some tannin structures exhibit notably weak interactions (Kou *et al.,* 2022).Carbohydrates, the largest category of organic molecules in living beings, serve as a primary metabolic energy source for both plants and herbivorous animals (Keerthiga and Anand, 2014).

Several types of *Dendrobium* plants contain alkaloids, aromatic compounds, sesquiterpenoids, and polysaccharides as their primary constituents. These components make these *Dendrobium* species valuable sources for various medicinal purposes, such as tonics with astringent, analgesic, anti-pyretic, antioxidant, antimicrobial, ant diabetic, anticancer, anti-inflammatory, anti-metastasis, and anti-angiogenesis properties (Poudel *et al.*, 2022).

Dendrobium panduratum contains steroid, terpenoid, alkaloids, tannins, phenols, and flavonoids (Johnson and Janakiramam, 2013). *Geodorum densiflorum* leaves possess various chemical compounds including alkaloids, glycosides, steroids, saponins, carbohydrates, tannins, and flavonoids (Theng and korpenwar, 2014). The analysis conducted by Bhattacharjee *et al.* (2015) focused on examining the alkaloid, terpenoid, flavonoid, phenol, tannin, steroid, and glycoside components of *Vanda tessellata*, an orchid.

Bulbophyllum neilgherrense was analyzed to detect the existence of alkaloids, tannin, phenol, and reducing sugar in the plant (Harshitha *et al.,* 2013). An extract from the *Satyrium nepalense* plant underwent a preliminary qualitative chemical test indicating the existence of carbohydrates/glycosides, alkaloids, flavonoids, and unsaturated sterols/triterpenes (Mishra and Saklani, 2012). The analysis detected the presence of flavonoids in a total of 27 different genera and 61 species. Among these, 37 species of orchids tested positive for reducing sugar, while 21 species showed negative results for reducing sugar (Maridass *et al*., 2008).

Dendrobium nobile was subjected to qualitative analysis, which showed the presence of various compounds such as flavonoids, tannins, glycosides, alkaloids, and steroids (Meitei *et al.,* 2019). The presence of flavonoid and phenolic compounds was found in *Spiranthes sinensis* (Kou *et al.,* 2022). A preliminary phytochemical screening of *Geodorum densiflorum* identified the presence of various compounds such as alkaloids, flavonoids, steroids, terpenoids, tannins, and reducing sugars. The screening revealed the existence of these chemical constituents in the plant (Keerthiga and Anand, 2014).

A phytochemical screening on *Dendrobium superhum* indicated the presence of saponins, alkaloids, glycosides, and tannins (Benez, 1995). The ethanol extracts from both the stem and leaf of *Dendrobium lasianthera* were found to contain tannin and alkaloid compounds, while no saponins, flavonoids, triterpenoids, or quinones were identified in the screening (Agustini *et al.,* 2022). Research involving chloroform and methanol extracts from *Dendrobium lasianthera* organs revealed that the bioactive components consisted of terpenoids and phenolics (Laurentius *et al.,* 2016). A study on *Dendrobium crumenatum* phytochemistry revealed the presence of saponins, terpenoids, and alkaloids in its composition (Sandrasagaran *et al.,* 2014).

2.3 Antibacterial Screening

The term 'antibacterial activity' describes a material's capacity to eliminate or inhibit the growth of bacteria. This aspect holds great importance in medications designed for addressing or averting bacterial infections. Antibacterial properties can be present in various substances, covering antibiotics and bioactive compounds. Antibiotics, hailed as the medical wonders of the 20th century, play a vital role in managing bacterial diseases, yet their irrational and inappropriate usage has contributed to the emergence of resistant microbial strains (Gupta and Birdi, 2017).

The development of antibiotic-resistant bacteria is a biological adaptation process that occurs when an antimicrobial agent is applied, suppressing susceptible organisms and favoring the survival of resistant ones (Sibanda and Okoh, 2007). Antibiotic-resistant bacteria also known as "Superbugs" are on the rise, leading to a growing prevalence of infections that cannot be effectively treated with current medications. This results in the emergence of illnesses resistant to multiple drugs. The issue of bacterial resistance is rising, forming uncertainty on the future utility of antibiotics. Therefore, it is important to continue exploring new and effective approaches to battle these diseases. To address this challenge, measures should be put in place to minimize the problem and reduce the excessive use of antimicrobial drugs. Additionally, research efforts should focus on explaining the genetic factors behind resistance and undertaking investigations for the development of novel antibiotics, whether they are synthetically created or naturally derived.

According to several studies, *Staphylococcus aureus* is increasingly becoming a major challenge in the field of antimicrobial chemotherapy (Tacconelli, 2011; Zetola *et al.,* 2005). However, among various strains tested, only *Staphylococcus aureus* was vulnerable to all of the orchid root extracts (Mongalo *et al.,* 2013).Out of 53 endophytic growths, the one called *Epicoccum nigrum,* which was taken from the base of *Dendrobium thyrsiflorum*, had the strongest inhibitory effect on *Staphylococcus aureus* (Young mei xing *et al.*, 2011). The growth of *Fusarium tricinctum* obtained from *Dendrobium devonianum* had the ability to inhibit the growth of both bacterial and parasitic microbes. On the other hand, the same type of growth obtained from *Dendrobium thyrsiflorum* exhibited antagonistic effects specifically against *Escherichia coli* (Young mei xing *et al.,* 2011).

The plant species *Dendrobium nobile* exhibited large zone of inhibition specifically against *Staphylococcus aureus,* but was relatively less against other types of microorganisms. In contrast, all of the plant extracts tested demonstrated strong inhibition against *Staphylococcus aureus. Pholidota imbricata* and *Coelogyne cristata* displayed the highest levels of resistance against *Vibrio cholera* and *Staphylococcus aureus* (Zotela *et al.,* 2005). Several Vanda species, including *Vanda coerulea,* have shown significant antibacterial and antifungal properties, with effectiveness against a range of bacteria such as *Bacillus cereus, Streptococcus faecalis, Streptococcus pneumonia, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Proteus vulgaris,* and *Enterobacter aerogenes* (Priya *et al.*, 2011).

Although root extracts of *Otochilus albus* generally exhibit higher activity compared to leaf extracts, numerous studies have shown that the methanol extract of *Otochilus albus* of the leaf demonstrates activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia faecalis*, *E. coli*, and *Pseudomonas aeruginosa (*Darabpour *et al.,* 2011). On the other hand, the methanol extract of the root of *Otochilus albus* is inactive against *Bacillus cereus* and *Escherichia faecalis*, suggesting that the leaf could serve as a substitute for the root as a defense mechanism (Mongalo *et al.,* 2013). *Coelogyne stricta* (leaf) and *Dendrobium amoeneum* demonstrated effective activity against *Klebsiella pneumonae*. On the other hand, *Pholidota articulata* and *Pholiodota imbricata* were found to have acceptable action against *Escherichia coli*. However, *Eria spicata, Bulbophyllum affine, Vanda cristata*, and *Rynchostylis retusa* exhibited weak action against all microbes in the study (Marasini and Joshi, 2013).

A certain concentrations of *Dendrobium amoenum, Dendrobium crepidatum, Dendrobium moniliforme, and Dendrobium longicornu* were able to uniquely inhibit the growth of *Staphylococcus aureus, Escherichia coli, Klebseilla pneumoniae, Salmonella typhi*, and *Acinetobacter baumannii*. The antibacterial compounds extracted from these *Dendrobium* species may have a different mechanism of action compared to currently used antibiotics and could be useful as antibacterial agents against bacteria strains that have developed resistance to multiple drugs (Paudel *et al.,* 2018). A study conducted to evaluate the antibacterial activities of 55 indigenous medicinal plants using the disc diffusion method on dried extracts of petroleum ether, 95% alcohol, and sterile water. The test organisms used were *Staphylococcus aureus, Agrobacterium tumefaciens, Bacillus subtilis, Salmonella typhi, Escherichia coli, Shigella dysenteriae, Candida albicans, Saccharomyces cervisiae,* and *Candida neoformans*. The results showed that some of the plant extracts exhibited weak to moderate activities against both bacteria and fungi (Shakya, 1982).

A study conducted in Nepal to examine the antimicrobial properties of certain orchid plants using in vitro screening. UV-A radiation was used in duplicate assays to enhance the activity of the extracts. All 21 plants tested showed activity against at least two fungi. Six extracts were only active when exposed to UV-A radiation, and 14 extracts showed increased antibiotic or antifungal effects when exposed to light (Taylor *et al.*, 1995). Ten different species of orchids were tested against various bacterial strains, including *Vibrio cholera, Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, and *Klebsiella pneumonia*. Among

the plants tested, *Coelogyne stricta* and *Dendrobium amoeneum* showed good activity against *Klebsiella pneumonia*, while *Pholidota articulata* and *Pholidota imbricata* exhibited good activity against *Escherichia coli*. *Dendrobium nobile* also demonstrated good inhibition against *Staphylococcus aureus*. However, *Eria spicata, Bulbophyllum affine, Vanda cristata*, and *Rynchostylis retusa* showed weak activity against all bacterial strains (Marasini and Joshi, 2012). *Dendrobium amoenum* has antibacterial properties against five different microorganisms. However, the plant did not show any zone of inhibition against *Salmonella paratyphi* and *Escherichia coli*, while exhibiting equal zone of inhibition against the other three organisms: *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Kleibsella pneumonia (*Shrestha *et al.,* 2015).The extracts from *Dendrobium amoenum, Dendrobium crepidatum, Dendrobium moniliforme* and *Dendrobium longicornu* were able to inhibit the growth of various bacteria including *Staphylococcus aureus, Escherichia coli, Klebseilla pneumoniae, Salmonella typhi,* and *Acinetobacter baumannii (*Paudel *et al.,* 2018). Some fungi extracted from *Dendrobium moniliforme* and *Dendrobium transparens* have potent antibacterial properties against Gram-positive bacteria and are particularly effective against *Escherichia coli*, except for *Hypoxylon fragiforme*. Although many fungi can inhibit the growth of *Pseodomonas aeruginosa,* endophytes *,Hypoxylon fragiforme* and *Fusarium equiseti* did not show this ability (Roshni *et al.*, 2018).

The antibacterial effects of *Coelogyne brachyptera* leaves were examined against Gram-positive *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*, as well as Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. The findings showed that the *Coelogyne brachyptera* leaf extracts exhibited potent activity against the Gram-positive bacterial strains, with an inhibition zone diameter of 20 mm for *Staphylococcus aureus* and 26 mm for methicillin-resistant *Staphylococcus aureus*. However, they only showed weak activity against the Gram-negative bacteria, with an inhibition zone diameter of 14 mm for E. coli and 11 mm for *Pseudomonas aeruginosa* (Buyun *et al.*, 2020). The leaf extracts of a *Coelogyne* species have moderate effects on both *Bacillus cereus* (14.3 ± 1.4 mm) and *Staphylococcus aureus* (13.6 ± 1.2 mm) (Wati *et al.,* 2021). In an experiment conducted that exhibited inhibitory effects against six bacterial strains including *Streptococcus Pyogenes, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Chromobacterium violaceum,* and *Escherichia coli*. The largest zone of inhibition was observed against *Streptococcus Pyogenes* (15.4 ± 0.22 mm) followed by *Bacillus subtilis*

(15.3 ± 0.22 mm), while the least zone of inhibition (11.3 ± 0.23 mm) was observed in the case of *Pseudomonas aeruginosa* (Singh *et al.*, 2020).

CHAPTER: 3 MATERIALS AND METHODS

3.1 Sample material preparation

3.1.1 Selection and collection of Orchid plants

We obtained authorization for plant material collection from the Department of Forest, located in Babarmahal, Kathmandu, prior to conducting the collection of plant specimens. Fifteen orchids were selected and collected from various locations, including Kathmandu and Syangja, during the months of August and September 2018 based on their indigenous use. The collection process included gathering plant parts from additional locations such as Makawanpur, Dolakha, Kaski, and Syangja during the months of August to October. Fresh plants were washed with tap water and left to air dry for a few days. For hard parts such as stems, roots, and barks, small pieces were created. The plant material was dried completely by being spread under shade on blotting paper, and direct sunlight exposure was avoided to preserve the active compounds (Yam *et al.,* 2019).

3.1.3 Plant identification

The plants were identified and authenticated by Dr. Mukti Ram Paudel and confirmed using literatures.

3.1.4 Packing and storage

Waterproof bags were utilized to package thoroughly dried samples, while cotton bags were employed to store incompletely dried samples. The purpose was to enhance air circulation and prevent rotting during storage. These packaged samples were stored at room temperature to avoid exposure to direct sunlight (Gul *et al.,* 2017).

3.1.5 Grinding

The electric grinder was used to grind the completely dried samples, resulting in fine powders. These powders were then stored in a polythene pack to prevent moisture and direct sunlight exposure. They would be used at a later time when needed (Gaya *et al.,* 2013).

3.2 Laboratory Analysis

The test organisms utilized in the experiments include *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Acinetobacter baumannii* and *Shiegella sonnie*. These

organisms were obtained from the National Public Health Laboratory (NPHL), Teku, Kathmandu, Nepal.

Gram Negative :	**Gram Positive :**
Escherichia coli: ATCC 25922	*Staphylococcus aureus*: ATCC 25923
Enterococcus faecalis: ATCC 29212	
Acinetobacter baumannii: ATCC 19606	
Shigella sonnei: ATCC 25931	

3.2.1 Extraction

The extraction technique used was Percolation with intermittent Sonication. To begin, 20 g of the dried plant materials were placed separately in glass bottles with lids. Methanol was then poured into the glass bottles in a ratio of 1:10, based on the weight of the plant material and the volume of the solvent in ml. The solution underwent Sonication for one and a half hours per day for three consecutive days. Afterward, it was left to settle for one day. The next day, the solution was filtered, and the remaining residue was collected for the second round of Sonication.

The methanolic extract was concentrated under reduced pressure using a rotary vacuum evaporator, and the resulting solvent was collected and used for a second round of extraction with the residue. The concentrated extract was then transferred to clean and dry petri plates, which were left to dry in an incubator at 37°C for three days. On the fourth day, the extract was scraped with a clean blade and stored in cryovials.

To calculate the percentage yield of the extract, the following formula was used

$$\text{Percentage yield (\%)} = \left[\frac{Dry\ wt.of\ extract}{Dry\ wt.of\ plant\ material}\right] \times 100$$

The dry extracts obtained were then sealed and kept at a temperature of 40°C until they were needed (Gaya *et al.,* 2013; Gul *et al.,* 2017; Pochapski *et al.,* 2011;).

3.2.2 Extract Dilution

A weight of 25 mg of the crude extract from each sample was measured and dissolved in 1 ml of methanol. The resulting stock solution, prepared at a concentration of 25 mg/ml, was utilized for the qualitative phytochemical screening.

To perform antimicrobial screening, 100 mg of the crude extract from each sample was weighed and then dissolved in 1 ml of Dimethyl sulfoxide (DMSO). The resulting solution was considered the stock solution and was kept at 4°C until needed.

3.2.3 Phytochemical analysis

3.2.3.1 Qualitative phytochemical analysis

The plant sample's crude methanolic extract underwent an initial phytochemical screening to identify their primary phytochemical constituents, as per the protocols outlined.

3.2.3.1.1 Test for Carbohydrate

For the carbohydrate test, mix 2 mL concentrated HCl with a small amount of phloroglucinol and an equal amount of the aqueous extract solution. Heat the mixture over a flame, and a positive result is indicated by the appearance of a red color (Shaikh and Patil, 2020).

3.2.3.1.2 Test for Tannins

In the tannin test, a plant extract is dissolved in a solution of 5 mL distilled water, 1% gelatin, and 10% NaCl. A positive result is indicated by the formation of a white precipitate, revealing the presence of tannins in the extract (Shaikh and Patil, 2020).

3.2.3.1.3 Test for Flavonoid

The crude extract was mixed with 2 ml of a 2% NaOH solution, which instantly produced a yellow color. Adding a few drops of diluted acid to the solution resulted in it becoming colorless, indicating the presence of flavonoids (Horborne, 1973; Trease and Evans, 1989).

3.2.3.1.4 Test for Alkaloids

The crude extract was combined with 2 ml of 1% HCL and heated gently. A few drops of Mayer's and Wagner reagent were then added to the mixture. The presence of alkaloids is indicated by the turbidity of the resulting precipitate (Horborne, 1973; Trease and Evans, 1989).

3.2.3.1.5 Test for Phenol

The crude extract was dissolved in 2 ml of a 2% solution of $FeCl_3$. The presence of phenol is indicated by a bluish black or dark green coloration (Horborne, 1973; Trease and Evans, 1989).

3.2.4 Antimicrobial activity

3.2.4.1 Preparation of Culture media

Nutrient Agar (NA)

Approximately 28 gm. of the prepared medium (Hi Media Laboratories Pvt. Ltd., Mumbai, India) were accurately measured and placed into a conical flask containing distilled water. The contents were completely dissolved in the water, and the final volume was adjusted to 1000 ml. The mixture was then boiled to ensure uniform mixing. To sterilize the media, it was subjected to 15 lbs of pressure in an autoclave at a temperature of 121° C for 15 minutes. Autoclave tape was used as an indicator to confirm the completion of sterilization. Subsequently, the media was removed from the autoclave and allowed to cool to approximately 45-50°C before being poured onto sterilized and appropriately labeled Petri dishes. Around 20 ml of the media was poured onto each Petri dish with a diameter of 9 cm. The plates were then left to solidify, and the pouring process was conducted within a sterile cabinet (Rehman *et al.,* 2011)

Nutrient Broth (NB)

Approximately 13 grams of readymade medium of NB powder (HiMedia Laboratories Pvt. Ltd., Mumbai, India) were accurately measured and placed into a conical flask. The powder was then dissolved in distilled water, and the volume was adjusted to reach a final volume of 1000 ml. This liquid was subsequently transferred to culture bottles and sterilized by autoclaving at a pressure of 15 lbs and a temperature of 121°C for 15 minutes. Autoclave tape was utilized to verify the completion of the sterilization process. Finally, the media was cooled using a laminar air flow system and used for the suspension-type bacterial culture (Rauf *et al.,* 2011).

Muller Hinton Agar (MHA)

A quantity of 38 grams of MHA powder (obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India) was measured and then mixed with distilled water. The mixture was then adjusted to reach a final volume of 1000 ml. The resulting content was heated until it reached boiling point to ensure complete dissolution of the medium. To sterilize the medium, it was autoclaved at a pressure of 15 lbs and a temperature of 121°C for 15 minutes. After sterilization, the medium was allowed to cool down to a temperature range of 40-50°C and was carefully mixed before being poured onto sterile Petri dishes under aseptic conditions for further use (Kiran *et al.,* 2017).

3.2.4.2 Preparation of the standard culture inoculums

To obtain pure cultures of bacteria including *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Acinetobacter baumannii* and *Shiegella sonnie* each bacterium was streaked onto separate nutrient agar plates. The plates were then incubated at 37°C for approximately 24 hours, resulting in pure and isolated colonies. Using a sterilized inoculating loop, each distinct colony was aseptically transferred to Nutrient Broth for suspension culture. The inoculated bottles were placed in an incubator at 37°C overnight. The turbidity of the bacterial suspension was adjusted to the 0.5 McFarland standard for the antibacterial test. These inoculums were used to swap plates and test the antibacterial effects of plant extracts (Juvatkar *et al.,* 2015).

3.2.4.3 Transfer of the bacteria on the Petri dishes

To prepare for the transfer of bacteria, the agar plates were accurately labeled with the date, name of the bacteria, and name code of the extracts. A sterile cotton swab was used to transfer the inoculum of bacteria into a petri dish that contained solid Mueller Hinton Agar. The swab was first immersed in a well-mixed saline test culture, and excess inoculum was removed by pressing the saturated swab against the inner wall of the culture tube. The bacteria were then spread on the media using the swab, and the petri plates were rotated 90 degrees to ensure even spreading. Separate swabs were used for each species of bacteria, and the culture plates were left to dry for a few minutes (Juvatkar *et al.,* 2015).

3.2.4.4 Making Dilution of extracts

The plant extracts were diluted using Dimethyl Sulphoxide DMSO to create four different solutions. Four tubes were used and labeled as A1, A2, A3, and A4. The first tube, A1, was filled with the undiluted extract, which is 100% concentrated. In the A2 tube, 50 ml of the extract was mixed with 50 ml of DMSO, resulting in a 50% concentration. Then, 50 ml of the mixture from A2 was transferred to A3 and mixed with 50 ml of DMSO, resulting in a 25% concentration. Finally, 50 ml of the mixture from A3 was transferred to A4 and mixed with 50 ml of DMSO, resulting in a 12.5% concentration (Weber *et al.,* 1992).

3.2.4.5 Placing plant extracts

Holes with a diameter of 6mm were created using a cork borer. A total of five holes were made at a consistent distance from each other along the circular edges of the plates. These extracts were prepared at different concentrations by diluting them with DMSO. Each extract was diluted to four different concentrations. DMSO was used as the negative control. Therefore, all five holes were filled with four concentrations of the extract and the negative control. The antibiotic disc was used as the positive control and was placed in the center of the plate (Weber *et al.,* 1992).

3.2.4.6 Observation of results

The results were observed after incubating the plates at 37°C for 24 hours. We recorded the presence or absence of inhibition zones. We observed and recorded the zones of inhibition where they were present. We measured the diameter of the inhibition zone using a millimeter ruler. The presence of an inhibitory zone around the hole indicated the absence of bacterial growth, which was recorded as a positive result, while the absence of a zone was recorded as a negative result. The tests were performed in triplicates to ensure the consistency of the results.

3.2.5 Determination of Minimum Inhibition Concentration and Minimum Bactericidal Concentration

The minimum inhibition concentration and minimum bactericidal concentration (MBC) was determined using the two-fold serial dilution approach described by (Baron *et al.*, 1994) using the crude extract from various fractions of Orchid plants that had antibacterial activity. The steps are described as follows.

The experiment included positive and negative controls along with tubes numbered from 1 to 12. Initially, 1 ml of Nutrient Broth (NB) was added to the positive control and tubes 1 to 10. Tube number 1 was filled with 1 ml of a crude extract from a medicinal plant, serving as the negative growth control. The contents of each tube were mixed and diluted in a two-fold manner, resulting in a steadily decreasing concentration from tube 2 to tube 10. The positive control remained empty of plant extract.

Next, 50 µl of inoculum with a turbidity similar to a McFarland standard of 0.5 was added to all tubes, except the negative control. The tubes were then incubated at 37 ℃ for 24 hours. After incubation, their turbidity was compared to the positive and negative controls.

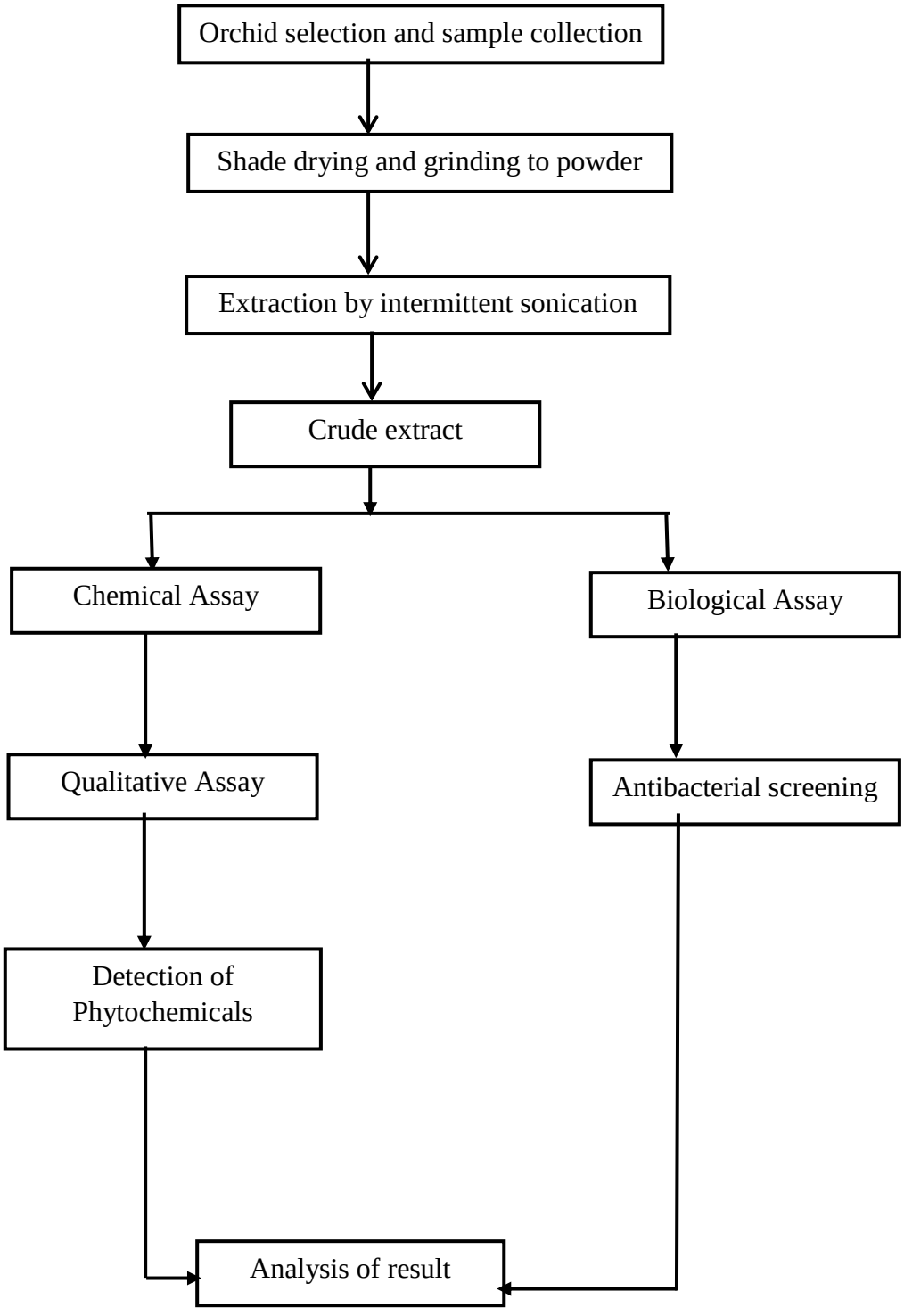
Incubation at 37 ℃ for 24 hours allowed researchers to examine the turbidity levels and draw conclusions about the plant extract's impact on microbial growth. Based on the fact that growth occurs in the positive control and any other tube where the extract concentration is insufficient to prevent growth, the findings were examined. The lowest concentration of a drug needed to prevent apparent turbidity, which shows that the growth of organisms is being inhibited, is known as the minimum inhibitory concentration (MIC). It was sometimes difficult to tell whether the turbidity was brought on by bacterial growth or by the turbidity of the plant extract.

In order to determine the lowest concentration of antimicrobial agent required to eradicate the bacteria and produce a sterile culture, a minimum bactericidal concentration (MBC) test was carried out. An NA plate was obtained and labeled with a marker from 1 to 10, along with positive and negative controls, to determine the minimum bactericidal concentration (MBC). Subcultures of the samples from the tubes were then grown on nutrient agar plates, one for each labeled number. The plates were then incubated for 24 hours at 37 ℃. They were then checked for bacterial growth. MBC refers to the labeled tube number in which there has been no growth of the organism after 24 hours of incubation.

3.3 Statistical Analysis

All the experiments were performed in triplicate for each sample and values were reported as mean ± SD using Duncan multiple range test in SPSS.

Flow chart of Method



CHAPTER: 4 RESULTS

4.1 Yield of Extracts

After the extraction process, the extract's percentage yield ranged from 1% to 15%. The highest yield was obtained from *Habenaria marginata*, and the lowest was from *Coelogyne stricta*. In the extraction process, *Rhynchostylis retusa* and *Bulbophyllum affine* yielded high quantities, while *Eria graminifolia* and *Liparis deflexa* produced comparatively lower amounts. Similarly, the texture and consistency of the extract also varied. The majority of them were sticky, while a few were powdery. Percentage yield (%) = $\left[\frac{\text{Dry wt.of extract}}{\text{Dry wt.of plant material}}\right] \times 100$

Table: 1 Percentage yield of the crude methanolic extracts

Plants Name	**Plant's part used**	**Dry weight of plant in gm**	**Weight of extract in gm**	**Percentage yield (%)**
Aerides odorata	Whole plant	80	4.8	6
Bulbophyllum affine	Whole plant	70	6.51	9.3
Bulbophylum leopordium	Whole plant	75	4.5	6
Coelogyne stricta	Pseudobulb	10	0.1	1
Eria graminifolia	Whole plant	65	2.6	4
Habenaria dentata	Tuber	70	5.25	7.5
Habenaria marginata	Tuber (Leaf)	80	12	15
Habenaria marginata	Tuber (Root)	80	11.2	14
Liparis deflexa	Tuber	70	2.59	3.7
Otochilus albus	Pseudobulb	70	6.3	9
Panisea uniflora	Pseudobulb	60	3	5
Pholidota articulata	Pseudobulb	85	7.225	8.5
Rhynchostylis retusa	Whole plant	60	6	10
Satyrium nepalense	Leaf	70	4.55	6.5
Satyrium nepalense	Root	80	5.04	6.3

4.2 Phytochemical Screening

The table below presents the results of a qualitative phytochemical screening conducted on the methanolic extract of specific Orchid species. The findings indicate that alkaloids and flavonoids were present in the extracts of all the Orchid species that were tested. Both Phenol and Carbohydrate were absence in *Coelogyne stricta* and *Eria graminifolia*.

Table: 2 Qualitative Phytochemical screening of selected species of Orchid

S.N.	Plant Name	Alkaloids	Flavonoids	Phenol	Carbohydrate	Tannins
1	*Aerides odorata*	+	+++	-	+++	+++
2	*Bulbophyllum affine*	+	+	+	-	+
3	*Bulbophylum leopordium*	+	+	+	-	+
4	*Coelogyne stricta*	+	+	-	-	+
5	*Eria graminifolia*	+	+	-	-	+
6	*Habenaria dentata*	+	+	+	+	+
7	*Habenaria marginata* (L)	+	+	-	+	+
8	*Habenaria marginata* (R)	+	+	-	+	+
9	*Liparis deflexa*	+	+	+	+	-
10	*Otochilus albus*	+	+	+	-	+
11	*Panisea uniflora*	+	+	+	-	+
12	*Pholidota articulata*	+	+	+	-	+
13	*Rhynchostylis retusa*	+	+++	++	+	++
14	*Satyrium nepalense* (L)	+	+	+	+	-
15	*Satyrium nepalense* (R)	+	+	+	+	-

Legend: (+) indicate presence and (–) indicates absence

4.3 Antibacterial Screening

The methanol extract obtained from various plant parts was used to test for antibacterial activity. The antibacterial activity was tested using the disc diffusion method. A positive sign indicates the production of an inhibition zone, and a negative sign denotes the absence of an inhibition zone, indicating the growth of microorganisms. The results of the table indicate that the orchid plants tested were effective against the bacteria used. This study involved screening 15 Orchid plants for their antibacterial properties against five strains of bacteria that can cause stomach disorders such as stomachaches, diarrhea, dysentery, and indigestion.

Table: 3 Bacterial strains with respective standard antibiotic drugs

Gram Negative :	**ATCC No.**	**Antibiotics**
Escherichia coli:	ATCC 25922	Ciprofloxacin
Enterococcus faecalis:	ATCC 29212	Gentamicin
Acinetobacter baumannii:	ATCC 19606	Meropenem
Shigella sonnei:	ATCC 25931	Methicillin/ Ciprofloxacin
Gram Positive:	**ATCC No.**	**Antibiotics**
Staphylococcus aureus:	ATCC 25923	Methicillin/ Ciprofloxacin

Different antibiotic drugs were taken as positive control for respective bacterial strains and DMSO (the solvent used for the dilution of different orchid extract) was taken as negative control for respective bacterial strains. Different concentration of plant extract (10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml) were tested against five bacterial strain and compare with their respective standard antibiotic. The zone of inhibition was measured for all tested bacteria and the result were expressed on mm including 6mm diameter of well.

The table below summarizes the results obtained from screening the methanolic extracts of different orchid plants against pathogenic bacteria. After screening 15 different species, it was observed that the zone of inhibition varied depending on the species and the concentration of the extracts used. The size of the zone where bacteria growth was inhibited increased with higher concentrations of the plant extracts

Table: 4 Antibacterial properties of methanolic extracts of different orchid genera against tested bacteria strains.

S.N.	Plants Name	*Escherichia coli*	*Enterococcus faecalis*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Shigella sonnei*
1	*Aerides odorata*	+	-	+	+	-
2	*Bulbophyllum affine*	-	-	+	+	+
3	*Bulbophylum leopordium*	+	+	+	+	+
4	*Coelogyne stricta*	+	+	+	+	+
5	*Eria graminifolia*	+	-	+	-	-
6	*Habenaria dentata*	+	+	+	-	-
7	*Habenaria marginata* (L)	-	+	+	+	-
8	*Habenaria marginata* (R)	+	+	-	+	-
9	*Liparis deflexa*	-	+	+	-	-
10	*Otochilus albus*	-	+	+	+	+
11	*Panisea uniflora*	-	+	+	+	-
12	*Pholidota articulata*	+	+	-	-	-
13	*Rhynchostylis retusa*	+	-	+	+	+
14	*Satyrium nepalense* (L)	-	-	+	-	+
15	*Satyrium nepalense* (R)	-	-	+	-	-

(Note: All the plants show positive result for positive and negative result for negative control)

Fifteen distinct orchid genera were subjected to testing against one variety of gram-positive bacteria and four varieties of Gram-negative bacteria. The results revealed that every orchid

genus exhibited antimicrobial properties against the bacteria under examination. Notably, *Coelogyne stricta* and *Otochilus albus* displayed inhibitory effects against nearly all of the tested bacterial strains. Moreover, *Rhynchostylis retusa* and *Bulbophyllum leopardinum* were capable of inhibiting the growth of 80% of the bacteria, while *Panisea uniflora*, *Aerides odorata*, *Habenaria dentata* (L), *Habenaria marginata* (L), *Habenaria marginata* (R), and *Bulbophyllum affine* demonstrated effectiveness against 60% of the tested bacterial strains. Additionally, *Pholidota articulata, Eria graminifolia, Liparis deflexa*, and *Satyrium nepalense* (L) exhibited antibacterial activity against 40% of the tested bacteria. Among the five bacterial strains scrutinized, *Shigella sonnei* displayed the highest level of resistance, with only five out of the 15 orchid plants utilized in the study demonstrating inhibitory effects on its growth. In contrast, *Acinetobacter baumannii* was the most susceptible bacterium, as its growth was hindered by 13 out of the 15 orchid plants that were examined.

4.4 Evaluation of Antibacterial Activity of Medicinal Plants

Table- 5: Number of microorganisms inhibited by tested orchid genera

S.N.	Plant Name	No. of bacteria inhibited	% of bacteria inhibited
1	*Aerides odorata*	3	60
2	*Bulbophyllum affine*	3	60
3	*Bulbophylum leopordium*	4	80
4	*Coelogyne stricta*	5	100
5	*Eria graminifolia*	2	40
6	*Habenaria dentata*	3	60
7	*Habenaria marginata* (L)	3	60
8	*Habenaria marginata* (R)	3	60
9	*Liparis deflexa*	2	40
10	*Otochilus albus*	5	100
11	*Panisea uniflora*	3	60
12	*Pholidota articulata*	2	40
13	*Rhynchostylis retusa*	4	80
14	*Satyrium nepalense* (L)	2	40
15	*Satyrium nepalense* (R)	1	20

Table-6: zone of inhibition (ZOI) shown by different medicinal plants against tested bacteria (Continue....)

Name of plants	*Conc. (mg/ml)*	Zone of inhibition against different bacterial strains (Diameter in mm)				
		Escherichia coli	***Enterococccus faecalis***	***Acinetoobacter baumannii***	***Staphylococcus aureus***	***Shiegella sonnii***
Aerides odorata	10	18.5 ± 1.80^{g}	-	18.83 ± 0.76^{j}	-	12 ± 1^{e}
	5	16.4 ± 1.44^{f}	-	16.5 ± 1.5^{i}	-	10.5 ± 0.5^{c}
	2.5	14 ± 1^{e}	-	14 ± 1.32^{g}	-	8.33 ± 0.57^{b}
	1.25	12.33 ± 2.08^{d}	-	12.4 ± 1.63^{e}	-	-
Bulbophyllum affine	10	-	-	10.5 ± 0.5^{c}	10.5 ± 0.5^{c}	12.33 ± 0.57^{e}
	5	-	-	8 ± 0^{b}	8.16 ± 0.28^{b}	10.5 ± 0.5^{d}
	2.5	-	-	-	-	8 ± 0^{b}
	1.25	-	-	-	-	-
Bulbophylum leopordium	10	-	14.5 ± 0.5^{f}	15.5 ± 0.5^{h}	10.5 ± 0.5^{c}	11 ± 0^{d}
	5	-	12.5 ± 1.15^{d}	13.5 ± 1.32^{g}	-	-
	2.5	-	10.33 ± 0^{c}	11 ± 0^{e}	-	-
	1.25	-	8 ± 0.5^{b}	-	-	-
Coelogyne stricta	10	10.16 ± 0.76^{c}	14.5 ± 0.57^{f}	20 ± 0^{k}	15.33 ± 0.57^{e}	15.16 ± 0.28^{f}
	5	8.5 ± 0.5^{b}	12.33 ± 0^{d}	18.5 ± 0.5^{j}	12.16 ± 1.03^{d}	12.5 ± 0.5^{e}
	2.5	-	10 ± 0^{c}	12 ± 0^{e}	10.83 ± 0.76^{c}	10 ± 0^{c}
	1.25	-	8 ± 0^{b}	10 ± 0^{c}	8.33 ± 0.28^{b}	-
Gentamicin		22.5 ± 1.5^{h}	23.16 ± 0^{j}	22.66 ± 0.28^{l}	19.66 ± 1.15^{i}	-
Methicillin		-	-	-	-	20.83 ± 2.36^{i}
Meropenem		19.48 ± 1.77^{g}	20.66 ± 0.28^{i}	23.16 ± 1.04^{l}	19.66 ± 1.15^{i}	-
Ciprofloxacin		26.6 ± 1.96^{i}	25.33 ± 0^{k}	27.33 ± 0.28^{m}	25.33 ± 0.28^{j}	26.83 ± 0.28^{j}

Name of plants	***Conc.* *(mg/ml)***	**Zone of inhibition against different bacterial strains (Diameter in mm)**				
		Escherichia coli	***Enterococccus faecalis***	***Acinetobacter baumannii***	***Staphylococcus aureus***	***Shiegella sonnii***
Eria graminifolia	10	14.5 ± 0.5^{e}	-	12.5 ± 0.5^{f}	-	-
	5	12 ± 0^{d}	-	10.5 ± 0.5^{c}	-	-
	2.5	10.33 ± 0.28^{c}	-	8 ± 0^{b}	-	-
	1.25	8.16 ± 0.28^{b}	-	-	-	-
Habenaria dentata	10	12.5 ± 0.5^{d}	14.5 ± 0^{f}	10 ± 0^{c}	-	-
	5	10 ± 0^{c}	12 ± 0.5^{d}	8.5 ± 0.5^{b}	-	-
	2.5	8.5 ± 0.5^{b}	10.5 ± 0.28^{c}	-	-	-
	1.25	7.33 ± 0.28^{b}	8.33 ± 0.5^{b}	-	-	-
***Habenaria marginata* (L)**	10	-	13.5 ± 0.5^{e}	16 ± 0^{i}	-	12 ± 0^{e}
	5	-	12.5 ± 0^{d}	14.5 ± 0.5^{h}	-	10.5 ± 0.86^{d}
	2.5	-	10 ± 0.5^{c}	12.33 ± 0.76^{e}	-	8.66 ± 0.57^{b}
	1.25	-	8.5 ± 0.5^{b}	10.5 ± 0.5^{c}	-	-
***Habenaria marginata* (R)**	10	-	10.5 ± 0.5^{c}	14.5 ± 0.5^{h}	-	-
	5	-	8.5 ± 0^{b}	12.33 ± 1.25^{e}	-	-
	2.5	-	-	10.5 ± 0.5^{c}	-	-
	1.25	-	-	8 ± 0^{b}	-	-
Gentamicin		22.5 ± 1.5^{h}	23.16 ± 0^{j}	22.66 ± 0.28^{l}	19.66 ± 1.15^{i}	-
Methicillin		-	-	-	-	20.83 ± 2.36^{i}
Meropenem		19.48 ± 1.77^{g}	20.66 ± 0.28^{i}	23.16 ± 1.04^{l}	19.66 ± 1.15^{i}	-
Ciprofloxacin		26.6 ± 1.96977^{i}	25.33 ± 0^{k}	27.33 ± 0.28^{m}	25.33 ± 0.28^{j}	26.83 ± 0.28^{j}

Name of plants	***Conc. (mg/ml)***	**Zone of inhibition against different bacterial strains (Diameter in mm)**				
		Escherichia coli	***Enterococccus faecalis***	***Acinobacter baumannii***	***Staphylococcus aureus***	***Shiegella sonnii***
Liparis deflexa	10	14.16 ± 0.28^{e}	16.5 ± 0.5^{g}	21 ± 0^{k}	19.66 ± 0.57^{f}	16.5 ± 0.5^{h}
	5	12.33 ± 0.28^{d}	14.5 ± 0.28^{f}	18.16 ± 0.76^{j}	17.33 ± 1.04^{h}	14.5 ± 0.86^{g}
	2.5	10 ± 0^{c}	10.16 ± 0^{c}	16.33 ± 0.57^{i}	16.5 ± 0.5^{g}	12.16 ± 0.57^{f}
	1.25	8 ± 0^{b}	8 ± 0.5^{b}	14 ± 0^{h}	14 ± 0^{f}	10.16 ± 0.28^{e}
Otochilus albus	10	-	9.5 ± 0.5^{c}	12.5 ± 0.5^{g}	-	12.5 ± 0.5^{f}
	5	-	7.5 ± 0^{b}	10 ± 0^{e}	-	10 ± 0^{e}
	2.5	-	-	-	-	8.5 ± 0.5^{d}
	1.25	-	0 ± 0.57735^{a}	-	-	7.5 ± 0.5^{c}
Panisea uniflora	10	10.16 ± 0.28^{c}	12.66 ± 0.5^{f}	-	-	-
	5	8.5 ± 0.5^{b}	10.5 ± 0.57^{e}	-	-	-
	2.5	7.33 ± 0.28^{b}	8.33 ± 0^{c}	-	-	-
	1.25	-	-	-	-	-
Pholidota articulata	10	9 ± 0.5^{b}	-	10 ± 0^{e}	9.16 ± 0.76^{e}	8.5 ± 0.86^{d}
	5	8.16 ± 0.28^{b}	-	9.33 ± 0.28^{d}	6.83 ± 0.57^{c}	7.16 ± 0.28^{c}
	2.5	7 ± 0^{b}	-	7.16 ± 0.28^{b}	6.16 ± 0.28^{b}	6.5 ± 0^{b}
	1.25	-	-	-	-	-
Gentamicin		22.5 ± 1.5^{h}	23.16 ± 0^{j}	22.66 ± 0.28^{l}	19.66 ± 1.15^{i}	-
Methicillin		-	0 ± 0.28^{a}	-	-	20.83 ± 2.36^{i}
Meropenem		19.4833 ± 1.77506^{g}	20.66 ± 0.28^{i}	23.16 ± 1.04^{l}	19.66 ± 1.15^{i}	-
Ciprofloxacin		26.6 ± 1.96977^{i}	25.33 ± 0^{k}	27.33 ± 0.28^{m}	25.33 ± 0.28^{j}	26.83 ± 0.28^{j}

Name of plants	***Conc.* *(mg/ml)***	**Zone of inhibition against different bacterial strains (Diameter in mm)**				
		Escherichia coli	***Enterococccus faecalis***	***Acinetoobacter baumannii***	***Staphylococcus aureus***	***Shiegella sonnii***
Rhynchostylis retusa	10	-	-	10.16 ± 0.28^{f}	8 ± 0^{d}	-
	5	-	-	8.33 ± 0.28^{c}	-	-
	2.5	-	-	-	-	-
	1.25	-	-	-	-	-
***Satyrium nepalense* (L)**	10	-	-	14.5 ± 1.32^{h}	-	-
	5	-	-	12.33 ± 0.76^{g}	-	-
	2.5	-	-	10.83 ± 0.28^{f}	-	-
	1.25	-	-	8 ± 0^{c}	-	-
***Satyrium nepalense* (R)**	10	15.5 ± 0.5^{e}	16.33 ± 0.28^{h}	-	-	12.5 ± 0.86^{f}
	5	13.33 ± 1.52^{d}	14.66 ± 0.76^{g}	-	-	10.5 ± 0.5^{e}
	2.5	-	12.33 ± 0^{f}	-	-	8 ± 0^{c}
	1.25	-	8 ± 1.04^{c}	-	-	-
Gentamicin		22.5 ± 1.5^{h}	23.16 ± 0^{j}	22.66 ± 0.28^{l}	19.66 ± 1.15^{i}	-
Methicillin		-	-	-	-	20.83 ± 2.36^{i}
Meropenem		19.48 ± 1.77^{g}	20.66 ± 0.28^{i}	23.16 ± 1.04^{l}	19.67 ± 1.15^{i}	-
Ciprofloxacin		26.6 ± 1.96^{i}	25.33 ± 0^{k}	27.33 ± 0.28^{m}	25.33 ± 0.28^{j}	26.83 ± 0.28^{j}

Note: (-) indicates no ZOI observed in orchid species.

Values in a column of tested bacteria with different alphabets are significantly different at $p \leq 0.05$.

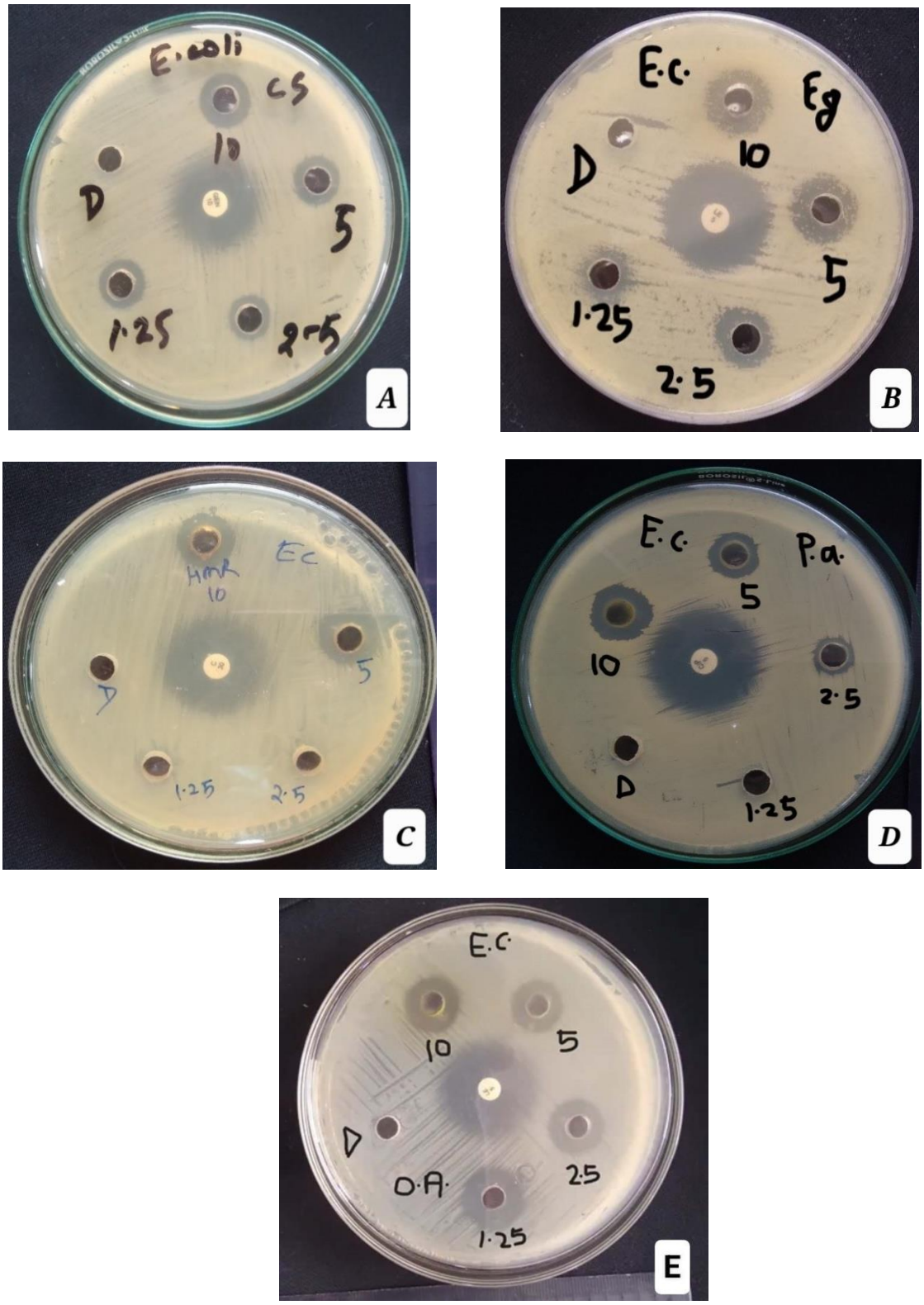


Fig 1: Antibacterial activity of different orchid extracts against *E. coli*. *(A) Coelogyne stricta* (B) *Eria graminifolia* (C) *Habenaria marginata* (D) *Pholidota articulata (*E) *Otochilus albus*

(Culture condition: MHA medium, 37 ℃ 18 hrs.)

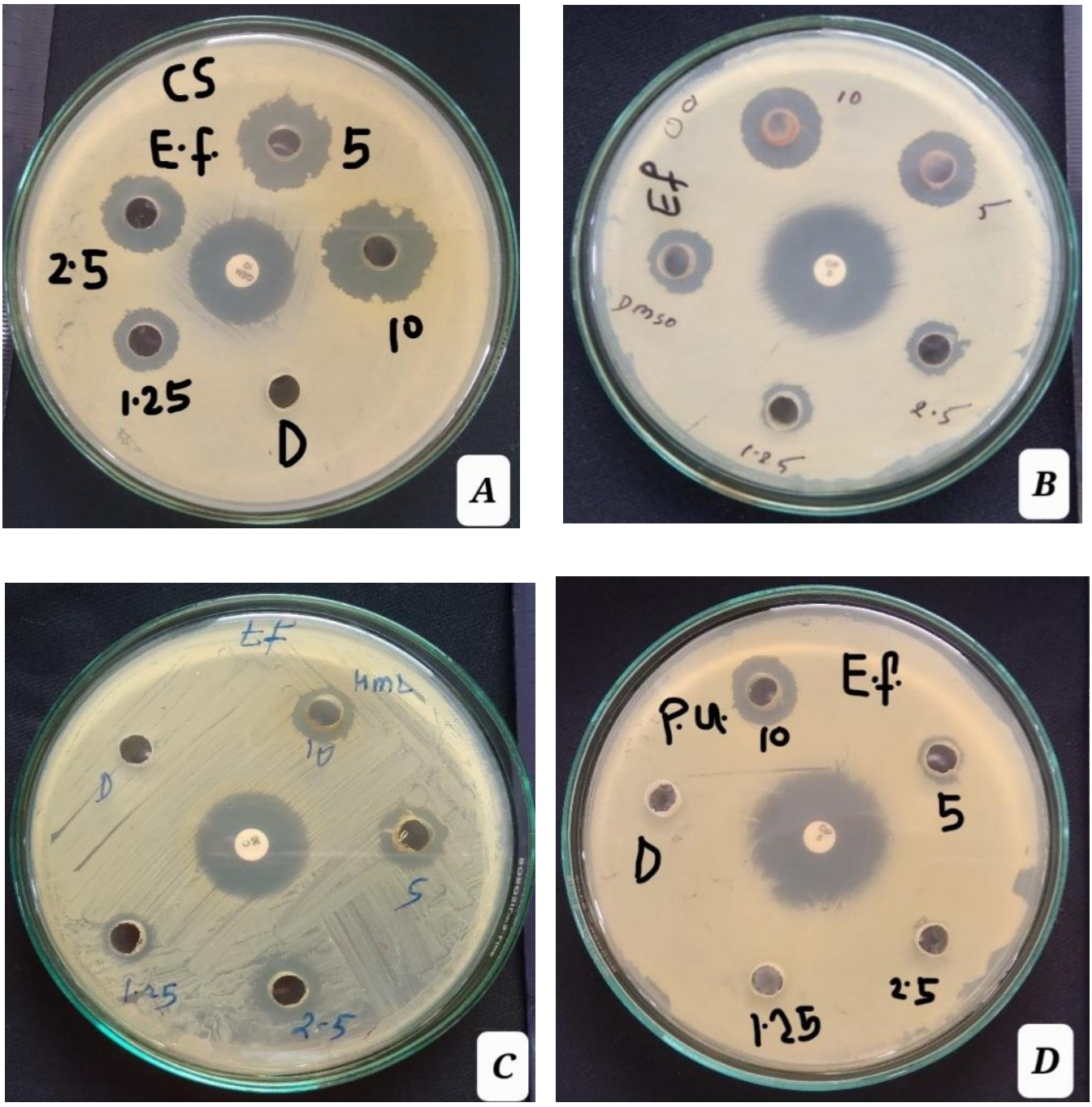


Fig 2: Antibacterial activity of different orchid extracts against *E. faecalis* (A) *Coelogyne stricta* (B) *Otochilus albus* (C) *Habenaria marginata* (D) *Panisea uniflora*

(Culture condition: MHA medium, 37 ℃ ***18 hrs.)***

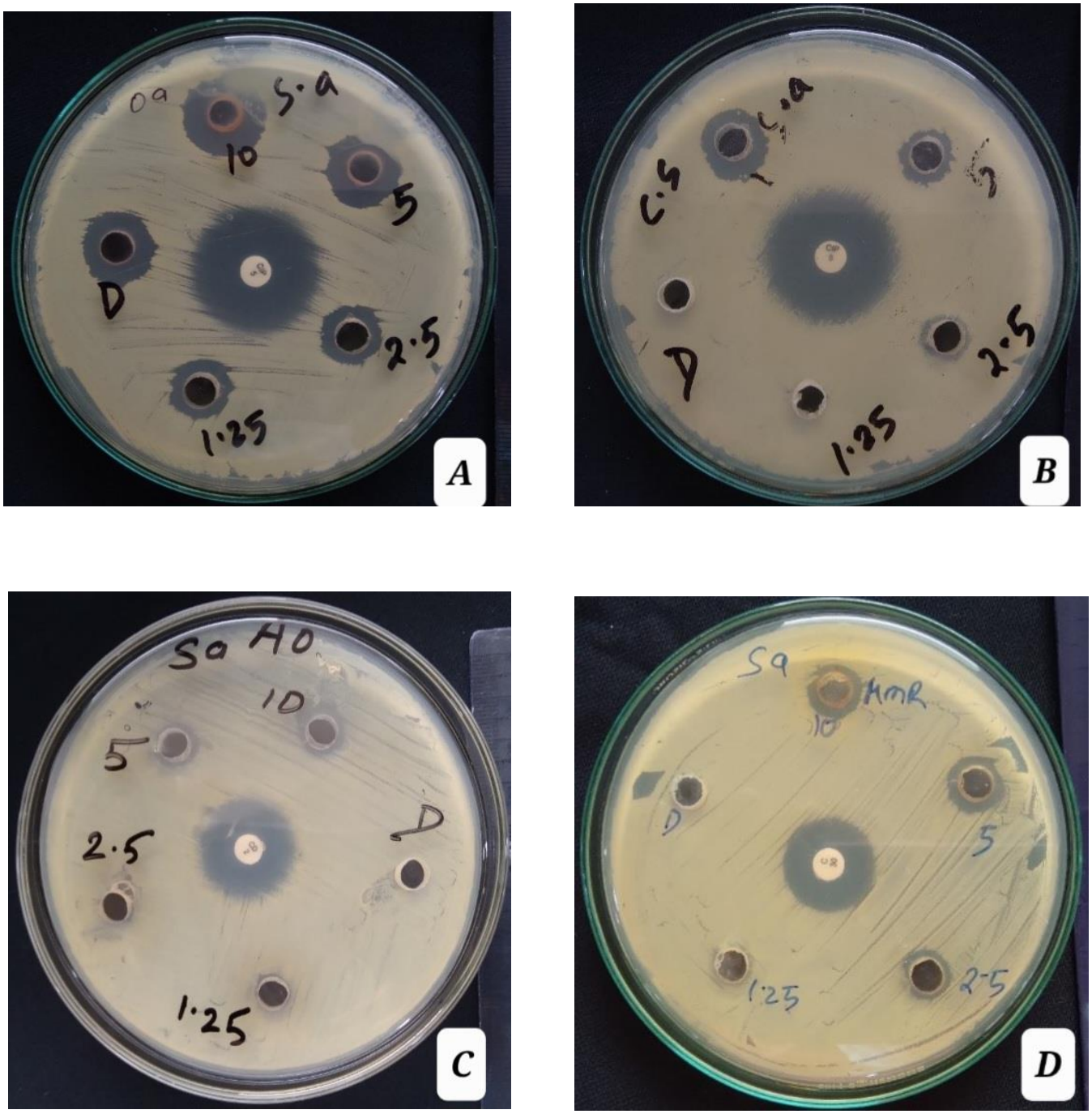


Fig 3: Antibacterial activity of different orchid extracts against *S. aureus* *(A) Otochilus albus* **(**B) *Coelogyne stricta* (C) *Aerides odorata* (D) *Habenaria marginata*

(Culture condition: MHA medium, 37 ℃ ***18 hrs.)***

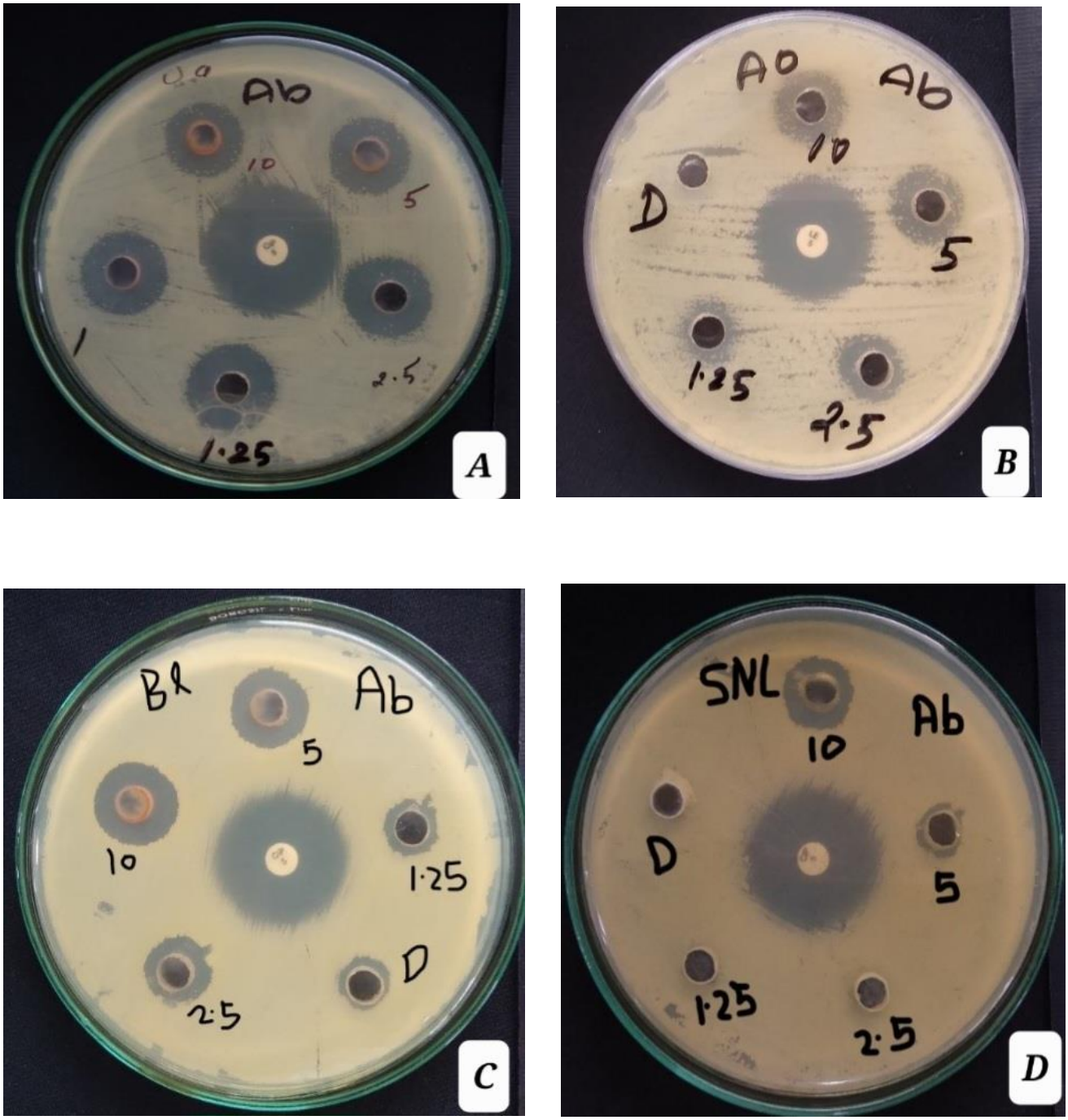


Fig 4: Antibacterial activity of different orchid extracts against *A. baumannii*

(A) Otochilus albus (B) *Aerides odorata* (C) *Bulbophyllum leopardinum* (D) *Satyrium nepalense*

(Culture condition: MHA medium, 37°C18 hrs)

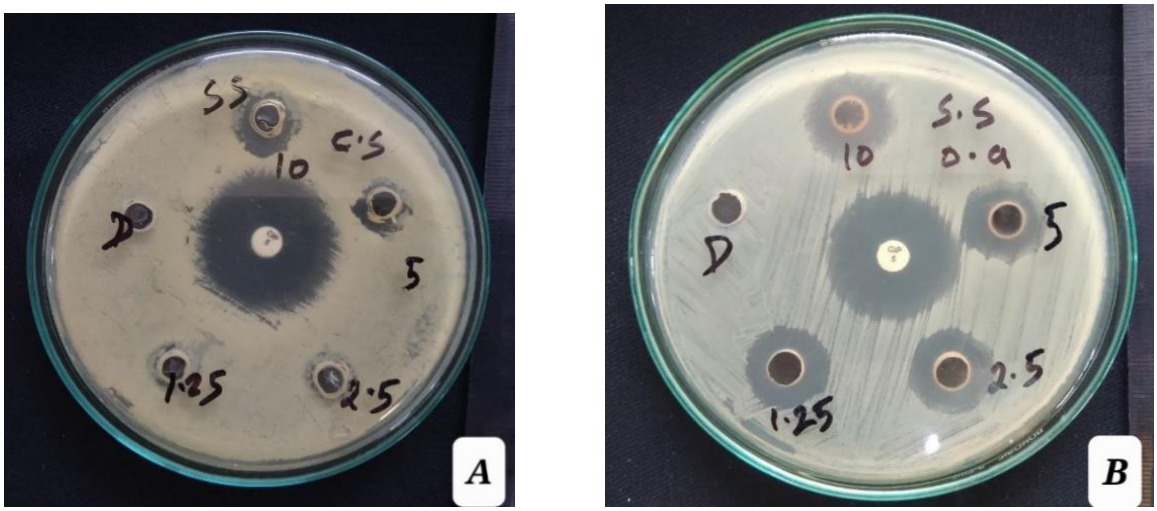


Fig 5: Antibacterial activity of different orchid extracts against *S. sonnii* *(A) Coelogyne stricta* (B) *Otochilus albus*

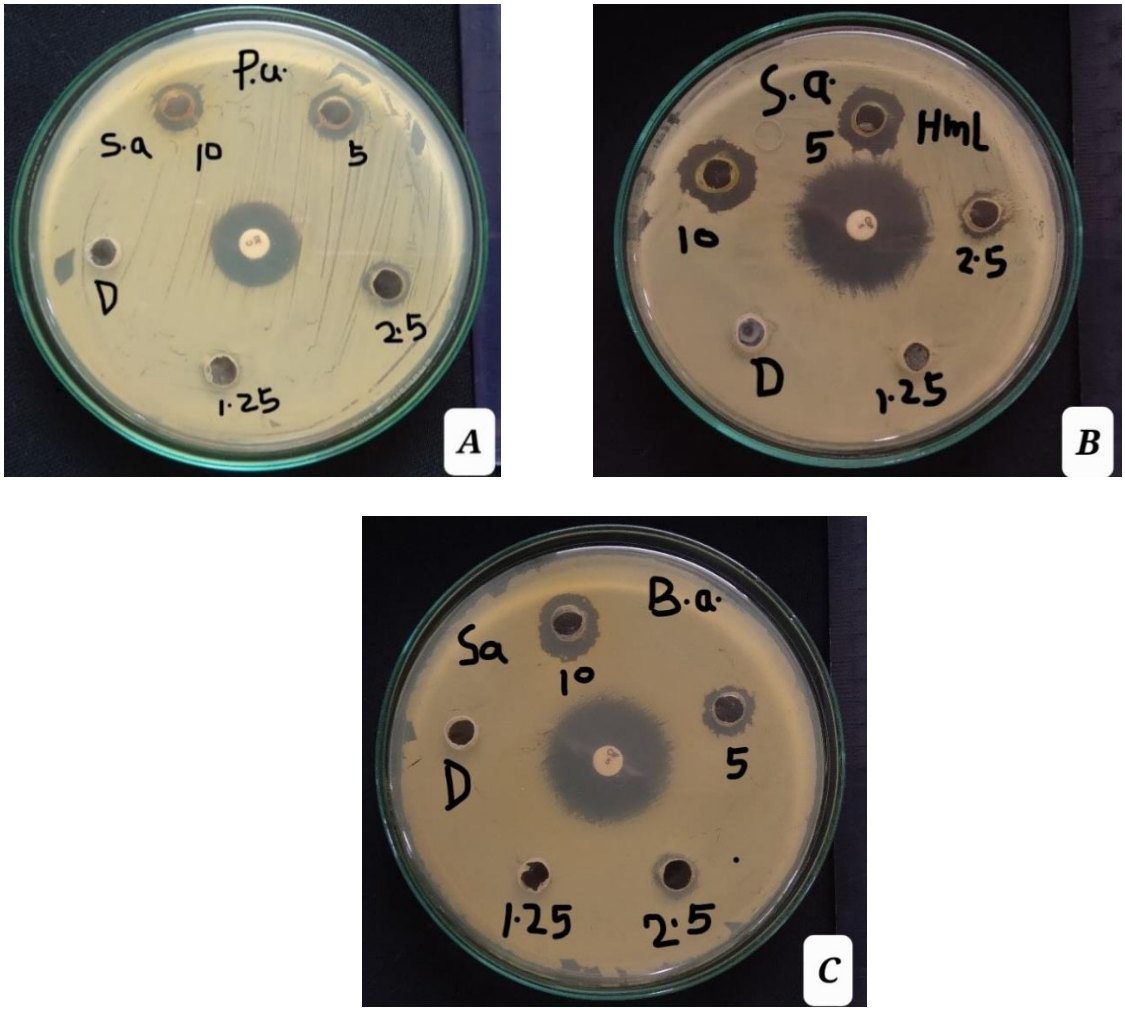


Fig 6: Antibacterial activity of different orchid extracts against *S. aureus* *(A) Panissea uniflora* **(**B) *Habenaria marginata* Leaf (C) *Bulbophyllum affinie*

(Culture condition: MHA medium, 37 ℃ ***18 hrs)***

4.5 Determination of MIC and MBC Value:

The MIC and MBC test was performed for all plant extracts that were judge as active (ZOI>6mm) against the tested microorganisms by agar well diffusion method.

Table :7 MIC and MBC value of Orchid extracts against tested bacteria

	Plant extracts	**Test Organisms**	**MBC (*m*g/ml)**	**MIC (*m*g/ml)**
1		*Escherichia coli*	2.5	0.625
		Enterococccus faecalis	-	-
		Acinobacter baumannii	2.5	0.625
		Staphylococcus aureus	-	-
	Aerides odorata	*Shiegella sonnii*	-	-
2		*Escherichia coli*	-	-
		Enterococccus faecalis	-	-
		Acinobacter baumannii	1.25	0.3125
		Staphylococcus aureus	1.25	0.3125
	Bulbophyllum affine	*Shiegella sonnii*	1.25	0.3125
3		*Escherichia coli*	-	-
		Enterococccus faecalis	1.25	0.3125
		Acinobacter baumannii	2.5	0.625
		Staphylococcus aureus	1.25	0.3125
	Bulbophylum leopordium	*Shiegella sonnii*	2.5	0.625
4		*Escherichia coli*	2.5	0.625
		Enterococccus faecalis	1.25	0.3125
		Acinobacter baumannii	2.5	0.625
	Coelogyne stricta	*Staphylococcus aureus*	1.25	0.3125
		Shiegella sonnii	2.5	0.625
5		*Escherichia coli*	2.5	0.625
	Eria graminifolia	*Enterococccus faecalis*	-	-

		Acinobacter baumannii	1.25	0.3125
		Staphylococcus aureus	-	-
		Shiegella sonnii	-	-
6		*Escherichia coli*	2.5	0.625
		Enterococccus faecalis	1.25	0.3125
		Acinobacter baumannii	2.5	0.625
		Staphylococcus aureus	-	-
	Habenaria dentata	*Shiegella sonnii*	-	-
7		*Escherichia coli*	-	-
		Enterococccus faecalis	2.5	0.625
		Acinobacter baumannii	1.25	0.3125
		Staphylococcus aureus	2.5	0.625
	Habenaria marginata (L)	*Shiegella sonnii*	-	-
8		*Escherichia coli*	2.5	0.625
		Enterococccus faecalis	2.5	0.625
		Acinobacter baumannii	-	-
		Staphylococcus aureus	1.25	0.3125
	Habenaria marginata (R)	*Shiegella sonnii*	-	-
9		*Escherichia coli*	-	-
		Enterococccus faecalis	1.25	0.3125
		Acinobacter baumannii	1.25	0.3125
		Staphylococcus aureus	-	-
	Liparis deflexa	*Shiegella sonnii*	-	-
10		*Escherichia coli*	1.25	0.3125
		Enterococccus faecalis	2.5	0.625
		Acinobacter baumannii	2.5	0.625
	Otochilus albus	*Staphylococcus aureus*	2.5	0.625

		Shiegella sonnii	1.25	0.3125
11		*Escherichia coli*	-	-
		Enterococccus faecalis	2.5	0.625
		Acinobacter baumannii	1.25	0.3125
		Staphylococcus aureus	2.5	0.625
	Panisea uniflora	*Shiegella sonnii*	-	-
12		*Escherichia coli*	2.5	0.625
		Enterococccus faecalis	2.5	0.625
		Acinobacter baumannii	-	-
		Staphylococcus aureus	-	-
	Pholidota articulata	*Shiegella sonnii*	-	-
13		*Escherichia coli*	1.25	0.3125
		Enterococccus faecalis	2.5	0.625
		Acinobacter baumannii	2.5	0.625
		Staphylococcus aureus	1.25	0.3125
	Rhynchostylis retusa	*Shiegella sonnii*	2.5	0.625
14		*Escherichia coli*	-	-
		Enterococccus faecalis	-	-
		Acinobacter baumannii	1.25	0.3125
		Staphylococcus aureus	-	-
	Satyrium nepalense (L)	*Shiegella sonnii*	-	-
15		*Escherichia coli*	-	-
		Enterococccus faecalis	-	-
		Acinobacter baumannii	2.5	0.625
		Staphylococcus aureus	-	-
	Satyrium nepalense (R)	*Shiegella sonnii*	-	-

The table presents the results of an antibacterial activity study involving various plant extracts against different test organisms. The effectiveness of each plant extract is measured in terms of Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) values. The plant extracts were tested against several bacterial strains, including *Escherichia coli, Enterococcus faecalis, Acinetobacter baumannii, Staphylococcus aureus,* and *Shigella sonnei.*The MBC values for these extracts against the test organisms ranges from 1.25 to 2.5 mg/ml, and the MIC values range from 0.3125 to 0.625 mg/ml.

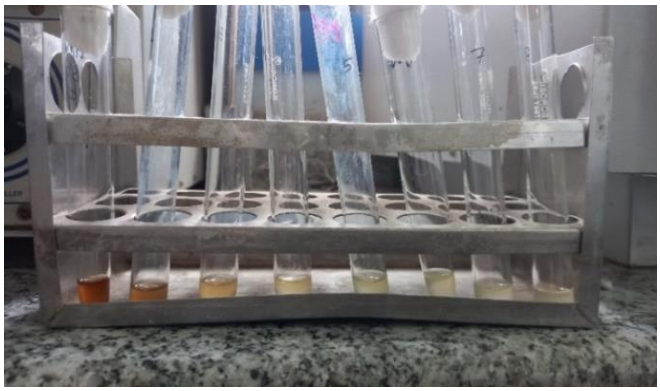


Fig 7: Determination of MIC value of *Otochilus albus* against *A.baumannii*

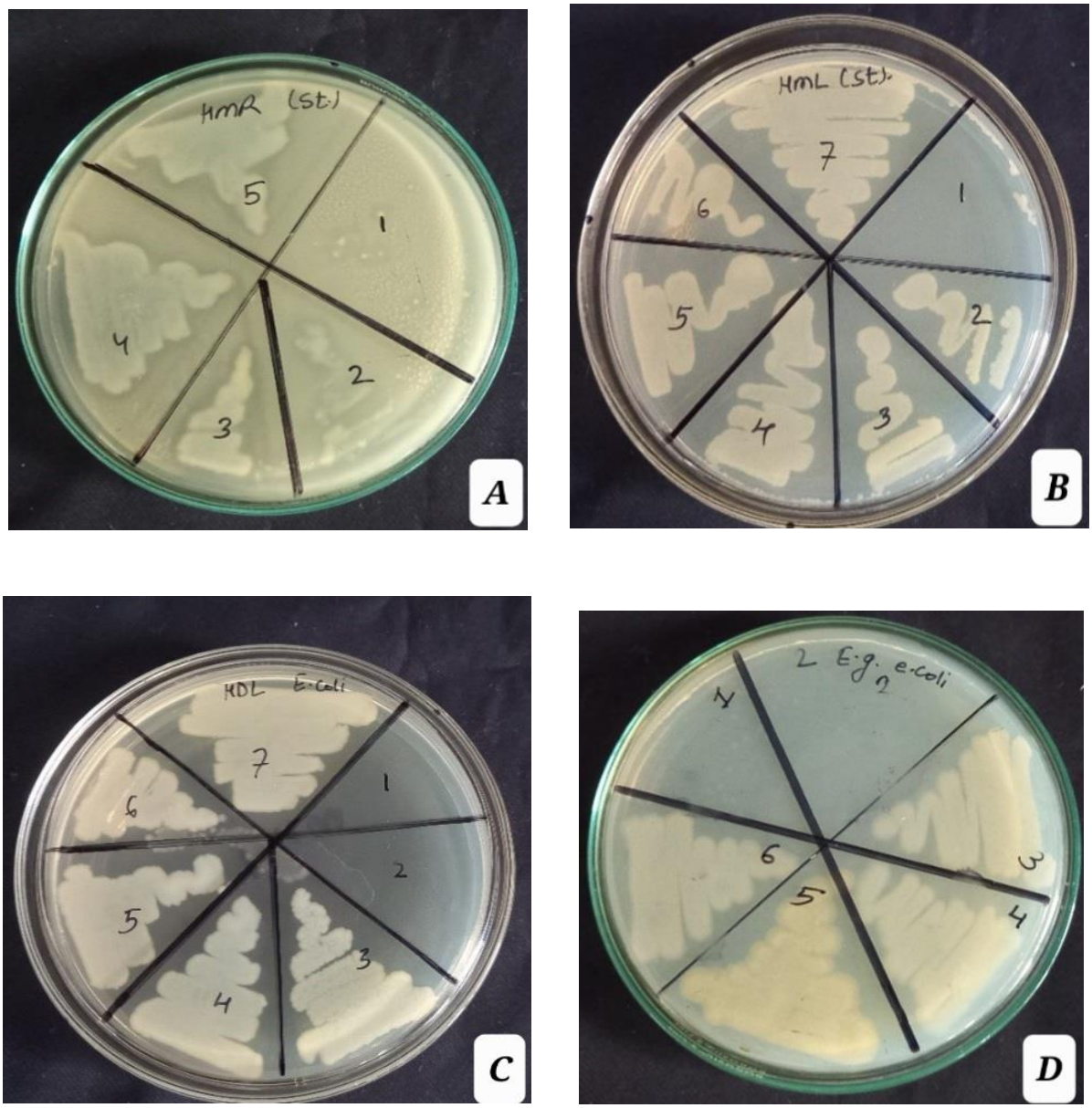


Fig 8: Determination of MBC value ; A. *Habenaria marginata* R against *S. aureus* **B.***Habenaria marginata* L against *S. aureus* **C**. *Habenaria dentata* against *E. coli;* **D.** *Eria graminii* against *E. coli*

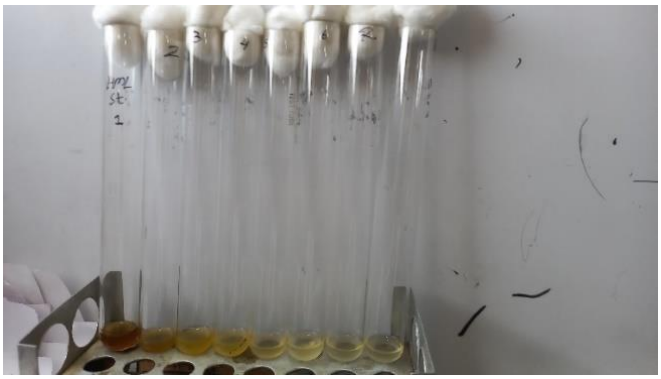


Fig 9: Determination of MIC value of *Coelogyne stricta* against *A. baumannii*

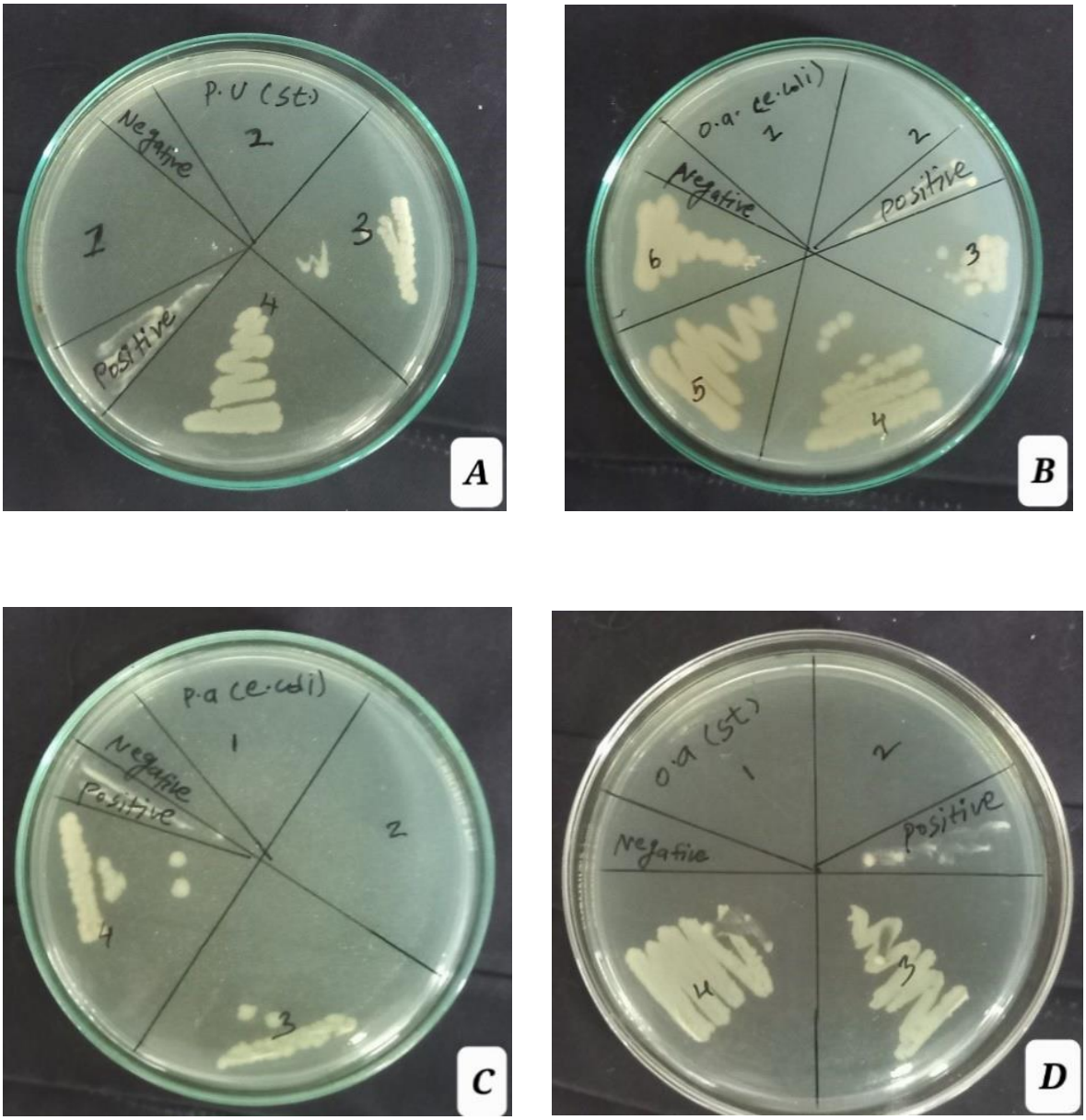


Fig: 10 Determination of MBC value **A**. *Panissia uniflora* aginst *S. aureus***B**. *Otochilus albus* against *E. coli* **C**. *Pholiodota articulata* against *E. coli* **D.** *Otochilus albus* against *S. aurues*

CHAPTER: 5 DISCUSSIONS

The findings may indicate a promising new resource for replacing the extensive use of antibiotics. With the widespread misuse of antibiotics, infections caused by multidrug-resistant strains are becoming increasingly resilient to antimicrobial drugs due to their natural mechanisms. Hence, there is a growing need to employ antimicrobial agents derived from plants for treating infections caused by multidrug-resistant strains. Considering the current trend among florists where orchid waste, harnessing these waste materials to develop high-value pharmaceutical or cosmetic products with antimicrobial properties could represent a viable solution within an economic framework.

5.1 Qualitative Phytochemical Assay

Plants synthesize phytochemicals via complex biochemical processes as a defense mechanism against predators, pathogens, and environmental challenges. These compounds function as restrictions, signaling agents, and contributors to diverse biological functions like pollination, communication between hosts and pathogens, as well as attraction of animals and insects (Das and Gezici, 2018).The current study conducted a qualitative phytochemical screening of methanol extracts from selected species of Orchid. The analysis indicated the existence of various secondary metabolites such as alkaloids, flavonoids, phenols, tannins, and carbohydrates. These compounds are likely to be present in high quantities in the plants themselves.

The results align with previous studies conducted on other species of Orchid, which have also indicated the existence of alkaloids, flavonoids, phenols, tannins, and carbohydrates (Chand *et al.*,2016; Maridass *et al.*, 2008; Marjoka *et al.*, 2016). Radhika and Murthy (2016) carried out an initial assessment of the phytochemical properties of *Rhynchostylis retusa* (L.) Blume. Based on the qualitative analysis, it was discovered that the plant contains several compounds including alkaloids, tannins, flavonoids, glycosides, saponins, and coumarins. Which resembles with our result. The analysis of phytochemicals in Orchid species has shown that they contain alkaloids, phenols, flavonoids, tannins and other compounds. Specifically, the presence of tannins and flavonoids has been linked to antimicrobial properties. It is possible that the antibacterial effects observed against certain strains of bacteria are due to the presence of these phytochemicals (Bhattacharya, 2015).

Mishra *et al.,* (2018) reported the absence of alkaloid in the methanolic extract from *Satyrium nepalense* but it was present in the present study. They also found the presence of carbohydrate, glycoside, flavonoid, phenolic compound, and tannins which supports with our results. Chand et al., (2016) also found to contain alkaloids, flavonoids, saponins, steroids and terpenoids, as well as tannins in ethanolic extract of 13 orchid samples. But a few individual samples were found to be missing certain phytochemical classes where some results matches with present results.

Joshi *et al.,* (2023) conducted a study on a selection of 6 orchid species, including *Eria graminifolia, Otochilus albus*, and *Pholiodata articulata*, which correlated with our own investigation. The findings revealed the presence of alkaloids, flavonoids, and tannins. However, saponins were absent in *Otochilus albus* and *Pholiodota articulata*. The positive result was obtained when conducting a qualitative test to detect the presence of alkaloids in *Aerides odoratum* and *Rhynchostylis retusa* (Akter *et al.,* 2018). The investigation of phytochemical analysis in *Ansellia Africana, Trydactylescottelli , Polystachyabella* and *Liparis bowkeri* through qualitative analysis unveiled differing levels of flavonoids, saponins, alkaloids, tannins, terpenoids, steroids, and glycosides (Chagona *et al.,* 2021). This result showed relatively similarity with present results.

(Joseph *et al.*, 2018) reported similar work of *Bulbophyllum mysorensis* that revealed the presence of alkaloid, flavonoid and absence of carbohydrate, phenol and tannins that supports the present study. (Keerthiga and Anand, 2014) carried out Preliminary phytochemical screening of *Geodorum densiflorum* that showed presence of alkaloids, steroids, carbohydrates, flavonoids, tannin and saponins. This report supports the present investigation. *Rhyncostylis retusa* confirmed higher levels of flavonoids, terpenoids, phlobatannins, and tannins, while glycosides, saponins, and steroids were found in lower amounts. Additionally, the species showcased a relatively high presence of seven secondary metabolites: tannins, flavonoids, steroids, phlobatannins, saponins, glycosides, and terpenoids (Marjoka *et al.*, 2016). This report closely resembles the present result. Bhatnagar et al., (2017) also found to contain alkaloids, flavonoids, Phenol but absence of tannins in ethanolic extract of *Rhynchostylis retusa*. And presence of alkaloid, flavonoids, carbohydrate, tannins but absence of phenol in *Satyrium nepalense* such type of results matches with present results. The findings of this study have focused specifically on the potential medicinal properties of this plant and have promoted ongoing research on medicinal orchids in Nepal.

5.2 Antibacterial Assay

The plant extracts were tested for their antimicrobial properties using the agar well diffusion method, which involved measuring the zone of inhibition against the test microorganism. The antibacterial compounds within the plant material diffused into the agar media, leading to the inhibition or killing of bacteria and resulting in a clear zone around the disc on the agar surface. The measurement of this zone of inhibition allows us to assess the effectiveness of the antimicrobial substances present in the plant extract.

The size of the zone of inhibition formed is influenced by a combination of external and internal factors. External factors like the pH of the medium, the duration and temperature of incubation, the volume of the well, the concentration of plant extracts, and the size of the inoculum can be controlled and standardized during the experiment, thereby minimizing errors caused by these factors. On the other hand, intrinsic factors, such as the characteristics of the medicinal plants used (including their components), their solubility, and diffusing properties, are predetermined and cannot be easily adjusted. This may lead to variations in the infusibility of the antibacterial agents, potentially resulting in high-potency antibiotics not exhibiting a proportional zone of inhibition corresponding to their efficacy (Prasai *et al.,* 2004).

In recent years, there has been a significant increase in infections, and antibiotic resistance has become a persistent problem. It is possible that natural products found in higher plants could offer a new source of antimicrobial agents (Ahmad and Aquil, 2007). Antimicrobial agents can destroy pathogens through various mechanisms. The primary method of action involves disrupting cell wall synthesis, inhibiting protein synthesis, interfering with nucleic acid synthesis, and inhibiting metabolic pathways (Neu, 1992).

Total five pathogenic bacteria *Escherichia coli, Enterococcus faecalis, Acinetobacter baumannii, Staphylococcus aureus,* and *Shigella sonnei* were used to screen the plant extracts. Such bacteria can cause different kind of diseases such as skin diseases, respiratory diseases, diarrhea and dysentery, meningitis, pneumonia, gastrointestinal diseases, staph skin infection, urinary tract infection, septic shock, blood infection, high fever, conjunctivitis, keratitis etc. *Escherichia coli* can cause very rare respiratory Pnumonie and urinary diseases. *Staphylococcus aureus* can affect the boil cuts and wounds.

Previous research conducted on various orchid genera has shown comparable findings. Multiple studies have indicated that different Orchid genera possess antibacterial activity. Marasini and Joshi (2012) reported that *Bulbophyllum affine* exhibited a 10mm zone of inhibition against *Staphylococcus aureus* but had no effect on *Escherichia coli. Rhynchostylis retusa*, on the other hand, showed an 8mm zone of inhibition against *Staphylococcus aureus* and no effect on *Escherichia coli*. In addition, *Pholiodota articulata* displayed a 12mm zone of inhibition against *Staphylococcus aureus* and an 8mm zone of inhibition against *Escherichia coli*. Similarly, *Coelogyne stricta* showed a 14mm zone of inhibition against *Staphylococcus aureus* and an 8mm zone of inhibition against *Escherichia coli.*

The extract solutions were divided into four concentrations, with the variations in concentration made based on DMSO. The most effective plant, *Bulbophylum leopordium,* inhibits bacterial development by around 80%, followed by *Panisea uniflora, Aerides odorata, Habenaria dentata L, Habenaria marginata (*L), *Habenaria marginata* (R), *and Bulbophyllum affine*, which is only 60% effective. Six different bacterial species were investigated, and *Shigella sonnei* emerged as the most resilient. The growth of *Acinetobacter baumannii*, the most sensitive bacteria, can be inhibited by 12 out of 15 orchid genera.

Rashmi *et al.,* (2015) reported antibacterial activity of selected orchids of Karnataka, India viz *Leucas zeylanica, Dendrobium nutantiflorum, Prosopis pallida, Carex breviscapa.* All the selected orchids showed good activity against tested bacteria. This report supports the present investigation and more or less similar extent in ZOI. Devi *et al.,* (2013) reported that ethanolic extract of *Coelogyne nervosa* shows the maximum Zone of Inhibition against the bacteria *Psedomonas aeruginosa* (15mm), *Shiegella sonnii* (12mm) and *Corynebacteria* spp (9mm). In the present study out of three only *Shiegella sonnii* was employed and it shows more or less similar zone of inhibition i.e. 12-16mm. This slight difference may be due to difference in plant genera, harvesting period, solvent etc.

Jagtap *et al.*, (2014) reported antimicrobial activity of *Habenaria longicorniculata*, J. Graham against five strains of microorganism. Plant extract inhibit the growth of all tested microorganisms with ZOI ranges from 6 -10 mm. Bhattacharjee *et al.,* (2014) reported similar work of *Rhynechostylis retusa* and *Vanda tessellate* against *Staphulococcus aureus, Bacillus subtilis, Vibrio cholera, Escherichia coli, Klebsiella pneumoniaee*. The ZOI ranges from 5-15mm. These findings support the present study with slight difference in ZOI. This difference may be due to difference in genus, solvent and bacterial strain.

The Methanolic extract of *Satyrium nepalense*, at a concentration of 8mg/100µl, exhibited potent antibacterial effects against various food pathogenic bacteria, including *Streptococcus mutans, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebseilla pneumonia* (Mishra and Saklani, 2012).so this result also resembles with our experiment. The inhibitory zones were observed after applying the undiluted alcoholic extract of *Rhynchostylis retusa*. The diameters of these zones measured 4.13 mm, 6.48 mm, 5.86 mm, and 4.40 mm against *Bacillus subtilis, Micrococcus lutea,* and *Staphylococcus aureus,* respectively (Kaushik, 2019). When comparing these results with ours, we found significantly lower values than our own findings.

The MBC aimed to determine the antimicrobials exact effectiveness using the two-fold serial dilution method, reducing the concentration by two times. The size of the Zone of Inhibition (ZOI) could be maximal when the MBC value is low, indicating high diffusibility but low bactericidal activity. On the other hand, a substance might have a higher MBC value with a smaller ZOI, indicating strong bactericidal properties but limited diffusibility. *Coelogyne stricta* showed significant antibacterial potential against all test organisms, with MBC values ranging from 1.25 to 2.5 mg/ml and MIC values ranging from 0.3125 to 0.625 mg/ml. However, *Pholidota articulata* exhibited least activity, as indicated by the small data for MBC and MIC values. *Otochilus albus* demonstrated potent antibacterial effects against most test organisms, with MBC values between 1.25 to 2.5 mg/ml and MIC values ranging from 0.3125 to 0.625 mg/ml.

On the other hand, *Bulbophyllum leopordium, Eria graminifolia, Aerides odorata, Panisea uniflora, Liparis deflexa,* and *Habenaria marginata* (L) showed no least activity against any of the tested organisms, as indicated by the small data for MBC and MIC values for most cases. *Habenaria marginata* (R) displayed significant antibacterial activity against *Escherichia coli* and *Enterococcus faecalis*, with MBC and MIC values of 2.5 mg/ml and 0.625 mg/ml, respectively, but had no activity against other test organisms. *Bulbophyllum affine* showed moderate antibacterial potential, with MBC values of 1.25 mg/ml against *Acinetobacter baumannii* and MIC values of 1.25 mg/ml against *Staphylococcus aureus* and *Shigella sonnei*. *Satyrium nepalense* (R) demonstrated some activity against *Acinetobacter baumannii* with an MIC of 2.5 mg/ml, while *Habenaria dentata* exhibited activity against *Escherichia coli* and *Enterococcus faecalis,* with MBC values of 2.5 mg/ml and 1.25 mg/ml, respectively. However, both plants showed no activity against other test organisms. Several

researchers have demonstrated that plant extracts have a higher impact on gram positive bacteria's susceptibility compared to Gram negative bacteria (Lin *et al.*, 1999, Parekh and Chanda, 2007).

Bhatnagar *et al.*, (2017) examined that a portion of *Rhynchostylis retusa* revealed significant activity against *Acinetobacter* species, exhibiting a minimum inhibitory concentration (MIC) of 0.104 mg/mL. Similarly, *Satyrium nepalense* showed remarkable antibacterial efficacy against both *Staphylococcus aureus* and *Escherichia coli*, with MIC values of 0.625 mg/mL and 0.125 mg/mL, respectively, which corresponds to our findings. Marasaini and Joshi, (2012) found the MIC and MBC value of the extracts of *Pholidota imbricata* and *Coelogyne cristata* were found to be 0.625 mg/ml, 0.3125 mg/ml and 0.125 mg/ml, 0.250 mg/ml that aligns with our result.

Overall, this study indicates that some of the tested plant extracts, such as *Coelogyne stricta*, *Otochilus albus*, and *Habenaria marginata* (R), have potential as sources of antibacterial agents, while others, like *Pholidota articulata* and *Bulbophyllum leopordium*, may not possess significant antibacterial properties against the tested organisms. The results suggest that different plant extracts possess varying degrees of antibacterial activity against the tested organisms, and further investigation is warranted to explore their potential as natural antibacterial agents..

CHAPTER: 6 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

15 different orchid genera were tested against 5 bacterial strains. The highest zone of inhibition value was found to be 21mm was shown by *Otochilus albus* and 20mm by *Coelogyne stricta* against bacteria *Acinobacter baumannii*. While Ciprofloxacin, serving as the positive control, exhibits a diameter of 27 mm with *Coelogyne stricta* and Meropenem display 23 mm each, in comparison, alongside *Otochilus albus*.

It is necessary to introduce new, biologically safe, active drugs, ecofriendly in nature and effective as antimicrobial agents. Hence, identification and purification of pure compounds from *Coelogyne stricta,* and *Otochilus albus* hold a promising sector in modern pharmaceutical industry to provide the effective feasible, safety and cheaper medication that addresses neither to unmeet therapeutic needs. In conclusion, of the present investigation Orchid could potential source for antibacterial activity, that can further studies for novel phytochemicals.

6.2 Recommendation

This is just a preliminary study of antibacterial screening of some orchid species of Nepal. Because of time constrains limited work has been done in this research. Certain recommendation have been suggested from this research which are:

- We can use other solvent (e.g. hexane, chloroform, petroleum ether, ethyl acetate) instead of methanol during extraction by which other active compound of plant could be extracted.
- *Otochilus albus* showed the broad spectrum antibacterial activity which can be further used to isolation of the therapeutic antibacterial compounds and carry out further Pharmacological evaluation.
- The extracts can be concentrated and used for test of other antibacterial activity
- Samples from other different places should be collected and used for different scientific test considering its medicinal value.

REFERENCES

Abu-Shanab, B., Adwan, G. M., Abu-Safiya, D., Jarrar, N. and Adwan, K. (2004). Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. *Turkish Journal of Biology*, 28(2): 99-102.

Abu-Shanab, B., Adwan G. M., Jarrar, N., Abu-Hijleh, A. and Adwan, K. (2006). Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against methicillin-resistant *Staphylococcus aureus*. *Turkish Journal of Biology*, 30(4): 195-198.

Acharya, K. P. and Rokaya, M. B. (2010). Medicinal orchids of Nepal: are they well protected?. *Our Nature*, 8(1): 82-91.

Adwan, G. and Mhanna, M. (2009). Synergistic Effects of Plant Extracts and Antibiotics on *Staphylococcus aureus* Strains Isolated from Clinical Specimens. *Asian Pacific Journal of Tropical Medicine*, 2: 46-51.

Akter, M., Huda, M. K. and Hoque, M. M. (2018). Investigation of secondary metabolites of nine medicinally important orchids of Bangladesh. *Journal of Pharmacognosy and Phytochemistry*, 7(5): 602-606.

Allero, A.A. and Afolagan, A.J. 2006. Antibacterial activity of *Solanum tomentosum*. *African Journal of Biotechnology* 5: 369-372.

Altammar Khadijah, A. Y. (2010). *Antibactertial property and toxcity study of medicinal plant Tinospora Crispa l./Altammar Khadijah Ahmed Y* (Doctoral dissertation, University of Malaya).

Agustini, V., Simaremare, E., Gunawan, E., Awom, J. and Wopi, S. (2020). Phytochemical Screening, Antibacterial and Cytotoxic Activity of *Dendobium lasianthera* from Papua.

Awaad, A. S., El-Meligy, R. M., Qenawy, S. A., Atta, A. H. and Soliman, G. A. (2011). Anti-inflammatory, antinociceptive and antipyretic effects of some desert plants. *Journal of Saudi Chemical Society*, 15(4):367-373.

Ayoola, G. A., Sofidiya, T., Odukoya, O. and Coker, H. A. B. (2006). Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. *J. Pharm. Sci.* and *Pharm. Pract*, 8: 133-136.

Bharathi, B.V., Rao, D.K., Chiranjeevi, D., Venkateswarlu, V. and Rao, V.M.U. (2013). Phytochemical analysis and biological evaluation of *Pholidota articulata* Lindl., *International Journal of Pharmacy and Pharmaceutical Sciences*, 5 (2): 501-505.

Bañez, S. E. S. (1995). Phytochemical Screening and Pharmacological Testing of" Sanggumay" Orchid (*Dendrobium superhum* Reichb.). *The Vector: International Journal of Emerging Science, Technology and Management (IJESTM)*, 4(1):451-454

Bao, J., Cai, Y., Sun, M., Wang, G., and Corke, H. (2005). Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. *Journal of Agricultural and Food Chemistry*, 53(6): 2327-2332.

Baral, S. R. and Kurmi, P. P. (2006). A compendium of medicinal plants in Nepal.

Bauer, A. W. (1966). Antibiotic susceptibility testing by a standardized single diffusion method. *Am. J. Clin. Pathol.*, 45: 493-496.

Bhandari, P., Shrestha, K. and Subedi, C. K. (2020). Orchids of Panchase Forest, Central Nepal: A Checklist. *Journal of Plant resources*, 18(1): 143.

Bharal, A., Kashyap, M., Sohpal, V. K. and Sembi, J. K. (2014). Evaluation of antimicrobial properties of terrestrial orchids (collected from northern Himalayas) against certain human pathogens. *Int J Bioassays*, 3: 3036-3039.

Bhatnagar, M., Sarkar, N., Gandharv, N., Apang, O., Singh, S. and Ghosal, S. (2017). Evaluation of antimycobacterial, leishmanicidal and antibacterial activity of three medicinal orchids of Arunachal Pradesh, India. *BMC Complementary and Alternative Medicine*, 17(1):1-10.

Bhattacharjee, B., Islam, T., Rahman, Z. and Islam, S. M. S. (2015). Antimicrobial activity and phytochemical screening of whole plant extracts of *Vanda tessellata* (Roxb). *Journal of Pharmaceutical Science*, 4:72-83.

Bhattacharya, S. (2014). Phytochemical and biological studies on *Rhynchostylis retusa* (L.) Blume, an important medicinal orchid of India. *Journal of Pharmacognosy and Phytochemistry*, 2(4), 95-102.

Bribi, N. (2018). Pharmacological activity of alkaloids: a review. *Asian journal of botany*, 1(1), 1-6.

Bulpitt, C. J., Li, Y., Bulpitt, P. F. and Wang, J. (2007). The use of orchids in Chinese medicine. *Journal of the royal society of medicine*, 100(12): 558-563.

Cardoso, J. C., Zanello, C. A. and Chen, J. T. (2020). An overview of orchid protocorm-like bodies: Mass propagation, biotechnology, molecular aspects, and breeding. *International Journal of Molecular Sciences*, 21(3): 985.

Chagona, P., Kwamboka, N., Gaya, H., Makonde, H., Adem, A., Osano, K. and Kawaka, F. (2021). Phytochemical analysis and antibacterial activity of the Kenyan wild orchids. *Micro Environer*, 1(02):93-100.

Chakraborty, A., Pal, N., Thanvi, H. A., Dhal, A., Debi, C., Kumar, S. and Mishra, S. (2022). Medicinally important common orchids of India. Medico-Bio wealth of India, 5: 25-29.

Chand, M. B., Paudel, M. R. and Pant, B. (2016). The antioxidant activity of selected wild orchids of Nepal. *Journal of Coastal Life Medicine*, 4(9): 731-736.

Chase, M.W. (2001). The origin and biogeography of Orchidaceae. In Pridgeon, A.M., Cribb, P.J., Chase M.W. and Rasmussen, F. eds. Genera orchidacearum, Vol. II: Orchidoideae (part I). pp. 1-5. Oxford University Press, Oxford.

Chen, C. C., Wu, L. G., Ko, F. N. and Teng, C. M. (1994). Antiplatelet aggregation principles of *Dendrobium loddigesii*. *Journal of natural products*, 57(9): 1271-1274.

Chitme, H. R., Chandra, R. and Kaushik, S. (2004). Studies on anti-diarrhoeal activity of *Calotropis gigantea* R. Br. in experimental animals. *J Pharm Pharm Sci*, 7(1): 70-5.

Choudhary, D., Mashkey, V. K., Goutam, E., Shrivastava, M., Rawat, M., Kumari, A. and Tripathi, V. (2023). Medicinal orchids: Traditional uses and recent advances. *Annals of Phytomedicine*, 12(1): 1-9.

Chugh, S., Guha, S. and Rao, I. U. (2009). Micropropagation of orchids: a review on the potential of different explants. *Scientia Horticulturae*, 122(4): 507-520.

Collee J.G., Fraser. A.G., Marmion B.P. and Simmon. A. (1999). *Mackie and McCartney Practical Medical Microbiology*. Churchill Living Stone.

Cribb, P. J., Kell, S. P., Dixon, K. W. and Barrett, R. L. (2003). Orchid conservation: a global perspective. *Orchid conservation*, 124.

Cazar, M. E., Abad, D. H., Idrovo, A. M. and Barrera, D. A. (2023). Assessment of antioxidant activities of *Epidendrum secundum* Jacq, a terrestrial orchid from southern Ecuadorian highlands. *South African Journal of Botany*, *154*:380-386.

Darabpour, E., Bavi, A. P., Motamedi, H. and Nejad, S. M. S. (2011). Antibacterial activity of different parts of *Peganum harmala* L. growing in Iran against multi-drug resistant bacteria. *EXCLI journal*, 10: 252.

Das, K. and Gezici, S. (2018). Secondary plant metabolites, their separation and identification, and role in human disease prevention. *Ann. Phytomedicine*, *7*:13-24.

Denis, O., Jans, B., Deplano, A., Nonhoff, C., De Ryck, R., Suetens, C. and Struelens, M. J. (2009). Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA)

among residents of nursing homes in Belgium. *Journal of Antimicrobial Chemotherapy*, 64(6):1299-1306.

Devkota, H. P., Logesh, R., Adhikari-Devkota, A. and Paudel, M. R. (2022). Orchids of Genus Bletilla: Traditional Uses, Phytochemistry, Bioactivities, and Commercial Importance. In *Orchids Phytochemistry, Biology and Horticulture: Fundamentals and Applications* 573-590 Cham: Springer International Publishing.

Dhandapani, R. and Sabna, B. (2008). Phytochemical constituents of some Indian medicinal plants. *Ancient science of life*, 27(4): 1.

Dressler, R. L. (1968). Pollination by euglossine bees. *Evolution*, 202-210.

Fabricant, D. S. (2001). Approaches to drug discovery using higher plants. *Environmental Health Perspectives*, 109(1): 69-75.

Gaya C., Kawaka J., Muchugi A. and Ngeranwa J. (2013). Variation of alkaloids in the Kenyan *Zanthoxylum gilletii* (De Wild Waterman). African Journal of Plant Science, 7(9): 438-444.

Govaerts, P., Bernet, K., Kratochvil, G., Gerlach, G., Carr, P., Alrich, A.M., Pridgeon, J., Pfahl, M.A., Campacci, D.H., Baptista, H., Tigges, J., Shaw, P., Cribb, A., George, K., Kreuz, J. (2023). Plants of the World Online: Orchidaceae. Royal Botanical Gardens, Kew

Gupta, P. D. and Birdi, T. J. (2017). Development of botanicals to combat antibiotic resistance. Journal of Ayurveda and integrative medicine, 8(4): 266-275.

Ghimire, R. (2022). Distribution of orchid species in Nepal: A comprehensive overview. *International Journal of Multidisciplinary Research and Growth Evaluation*.

Goldhirsch, A., Winer, E. P., Coates, A. S., Gelber, R. D., Piccart-Gebhart, M., Thürlimann, B. and Wood, W. C. (2013). Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Annals of oncology*, 24(9): 2206-2223.

Gregersen, T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *European journal of applied microbiology and biotechnology*, 5: 123-127.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S. and Jahan, N. (2017). Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. The Scientific World Journal, 2017: 5873648. 10.1155/2017/5873648

Harborne, J. B. (1973). Methods of plant analysis. *Phytochemical methods: a guide to modern techniques of plant analysis*, 1-32.

Haroun, M. F. and Al-Kayali, R. S. (2016). Synergistic effect of *Thymbra spicata* L. extracts with antibiotics against multidrug-resistant *Staphylococcus aureus* and *Klebsiella pneumoniae* strains. *Iranian Journal of Basic Medical Sciences*, 19(11): 1193.

Harshitha, K., Nishteswar, K. and Harisha, C. R. (2013). Pharmacognostical and preliminary phytochemical investigations on different parts of *Bulbophyllum neilgherrense* Wight.-An orchid used in folk medicine. *Global Journal of Research on Medicinal Plants* and *Indigenous Medicine*, 2(4): 259.

Hossain, M. M., Sharma, M. and Pathak, P. (2013). In vitro propagation of *Dendrobium aphyllum* (Orchidaceae)—seed germination to flowering. *Journal of Plant Biochemistry and Biotechnology*, 22: 157-167.

Hsiao, Y. Y., Pan, Z. J., Hsu, C. C., Yang, Y. P., Hsu, Y. C., Chuang, Y. C. and Chen, H. H. (2011). Research on orchid biology and biotechnology. *Plant and Cell Physiology*, 52(9): 1467-1486.

Jayanthi, P. and Lalitha, P. (2012). Biological activities of few nitrogen heterocyles using pass and lipophilicity using adme boxes. *Pharma Science Monitor*, 3(4).

Johnson, M. and Janakiraman, N. (2013). Phytochemical and TLC studies on stem and leaves of the orchid *Dendrobium panduratum* subsp. villosum Gopalan and AN Henry.

Joshi, G. C., Tewari, L. M., Lohani, N., Upreti, K., Jalal, J. S. and Tewari, G. (2009). Diversity of orchids in Uttarakhand and their conservation strategy with special reference to their medicinal importance. *Rep Opin*, 1(3): 47-52.

Joshi, P. R., Joshi, S., Paudel, M. R. and Pant, B. (2023). Screening of In vitro α-amylase Inhibitory Activity of Wild Orchids of Nepal. *Journal of Nepal Biotechnology Association*, 4(1): 1-7.

Juvatkar, P., Waghulde, S., Naik, P. and Gorde, N. (2015). Antimicrobial activity of bark of Moringa oleifera L.

Kaushik, P. (2019). Antibacterial potential of the Himalayan orchids. *J Orchid Soc India*, 33:11-22.

Keerthiga, M. and Anand, S. P. (2014). Physicochemical, preliminary phytochemical analysis and antibacterial activity against clinical pathogens of medicinally important orchid *Geodorum densiflorum* (Lam) Schltr. *Int J Pharm Pharm Sci*, 6(8): 558-61.

Kim, H. W., Knowles, J. C. and Kim, H. E. (2005). Hydroxyapatite porous scaffold engineered with biological polymer hybrid coating for antibiotic Vancomycin release. *Journal of materials science: materials in medicine*, 16: 189-195.

Kiran, V., Vivek, T., Deepak, K. and Khemchand, S. (2017). Comparative antimicrobial potential of *Tribhuvana-Mishrana* and its ingredients against clinical bacteria. *International Journal of Ayurvedic Medicine*, 8(1): 8-11.

Köck, R., Becker, K., Cookson, B., van Gemert-Pijnen, J. E., Harbarth, S., Kluytmans, J. A. J. W. and Friedrich, A. W. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. *Eurosurveillance*, 15(41).

Koju, L., Shrestha, N., Raskoti, B. B., Ale, R., Ghimire, N. P. and Shrestha, S. (2023). Spatial patterns, underlying drivers and conservation priorities of orchids in the central Himalaya. *Biological Conservation*, 283: 110121.

Kong, J. M., Goh, N. K., Chia, L. S. and Chia, T. F. (2003). Recent advances in traditional plant drugs and orchids. *Acta Pharmacologica Sinica*, 24(1):7-21.

Kumal, K., Pant, D. R., Tripathi, G. R. and Joshi, G. P. (2020). Phytochemical screening and antibacterial activity of selected mistletoes of Kathmandu Valley. Botanica Orientalis- Journal of Plant Science.14: 21-28.

Kumar, P. S., Subramoniam, A. and Pushpangadan, P. (2000). Aphrodisiac activity of *Vanda tessellata* (Roxb.) Hook. ex Don extract in male mice. *Indian Journal of Pharmacology*, 32(5): 300-304.

Kuo, Y. J., Pei, J. K. and Chao, W. W. (2022). Pharmacological and Chemical Potential of *Spiranthes sinensis* (Orchidaceae): A Narrative Review. *Plants*, 11(13): 1692.

Li, L., Weinberg, C. R., Darden, T. A. and Pedersen, L. G. (2001). Gene selection for sample classification based on gene expression data: study of sensitivity to choice of parameters of the GA/KNN method. *Bioinformatics*, 17(12): 1131-1142.

Li, M. and Zou, D. (1995). Study on pharmacology activity of *Anoectochilus roburghii* from three source. *Information Chin Pharmacol Soc*, 3: 26-28.

Lin, J., Opoku A.R, Gheeb, K. M, Hutchings A.D, Terblanche, S.E., Jager A.K. and Van, S.J. (1999). Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and antimicrobial activities. *Journal of Ethnopharmacology* 68: 267-274.

Liu, M., Li, X. Q., Weber, C., Lee, C. Y., Brown, J. and Liu, R. H. (2002). Antioxidant and antiproliferative activities of raspberries. *Journal of agricultural and food chemistry*, 50(10): 2926-2930.

Mabberley, D. J. (1997). *The plant-book: a portable dictionary of the vascular plants*. Cambridge university press.

Mabberley, D.J. (1997). *The plant-book*. 2nd edn. Cambridge University Press, Cambridge

Mangeot, S., Armstrong, K., Colvin, A. N., Yeates, K. O. and Taylor, H. G. (2002). Long-term executive function deficits in children with traumatic brain injuries: Assessment using the Behavior Rating Inventory of Executive Function (BRIEF). *Child Neuropsychology*, 8(4): 271-284.

Marasini, R. and Joshi, S. (2012). Antibacterial and antifungal activity of medicinal orchids growing in Nepal. *Journal of Nepal Chemical Society*, 29: 104-109.

Maridass, M., Hussain, M. I. and Raju, G. (2008). Phytochemical survey of orchids in the Tirunelveli hills of South India. *Ethnobotanical leaflets*, 2008(1): 95.

Marjoka, A., Alam, O. and Huda, M. K. (2016). Phytochemical screening of three medicinally important epiphytic orchids of Bangladesh. *Jahangirnagar University Journal of Biological Sciences*, 5(1): 95-99.

Matsen, J. M. (1979). Bacterial susceptibility testing and assays. *Clinical Diagnosis and Laboratory Methods. WB Saunders, Philadelphia*, 1900-193..

McFarland, J. (1907). The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of the American Medical Association*, 49(14): 1176-1178.

Medhi, R. P. and Chakrabarti, S. (2009). Traditional knowledge of NE people on conservation of wild orchids.

Meitei, A. L., Pamarthi, R. K., Kumar, R., Bhutia, N. T., Rai, D., Babu, P. K. and Singh, D. R. (2019). *Dendrobium nobile* orchid in traditional medicine-A phytochemical analysis. *Indian Journal of Horticulture*, 76(3):557-560.

Mishra, A. P., Saklani, S., Salehi, B., Parcha, V., Sharifi-Rad, M., Milella, L. and Srivastava, M. (2018). *Satyrium nepalense*, a high altitude medicinal orchid of Indian Himalayan region: chemical profile and biological activities of tuber extracts. *Cellular and Molecular Biology*, 64(8): 35-43.

Mishra, A. P. and Saklani, S. (2012). *Satyrium nepalense*: a rare medicinal orchid of western Himalaya (India); phytochemical screening, antimicrobial evaluation and conservation studies. *Indonesian Journal of Pharmacy*, 23(3): 162-170.

Moin, S., Sahaya, B. S., Servin, P. W. and Chitra, B. D. (2012). Bioactive potential of *Coelogyne stricta* (D. Don) Schltr: An ornamental and medicinally important orchid. *Journal of Pharmacological Research*, 5: 2191-2196.

Mongalo, N. I., Opoku, A. R. and Zobolo, A. M. (2013). Antibacterial activity of root and leaf extracts of *Jatropha zeyheri* Sond (Euphorbiaceae). *African Journal of Biotechnology*, 12(5).

Ncube, N. S., Afolayan, A. J. and Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African journal of biotechnology*, 7(12).

Ng, T. B., Liu, J., Wong, J. H., Ye, X., Wing Sze, S. C., Tong, Y. and Zhang, K. Y. (2012). Review of research on *Dendrobium*, a prized folk medicine. *Applied microbiology and biotechnology*, 93: 1795-1803.

Pant, B. (2013). Medicinal orchids and their uses: Tissue culture a potential alternative for conservation. *African Journal of plant science*, 7(10): 448-467.

Pant, B. and Raskoti, B. B. (2013). *Medicinal orchids of Nepal*. Himalayan map house.

Pant, B., Chand, K., Paudel, M. R., Joshi, P. R., Thapa, B. B., Park, S. Y. and Rajbhandari, P. (2022). Micropropagation, antioxidant and anticancer activity of pineapple orchid: *Dendrobium densiflorum* Lindl. *Journal of Plant Biochemistry and Biotechnology*, 1-11.

Pant, B. (2013). Medicinal orchids and their uses: Tissue culture a potential alternative for conservation. *African Journal of plant science*, 7(10): 448-467.

Parekh, J. and Chanda, S. 2007. In vitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. *African Journal of Biomedical Research* 1(6): 92-9

Parvekar, P., Palaskar, J., Metgud, S., Maria, R. and Dutta, S. (2020). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against *Staphylococcus aureus*. *Biomaterial investigations in dentistry*, 7(1): 105-109.

Patzer, J. A. and Dzierżanowska, D. (2007). Increase of imipenem resistance among *Pseudomonas aeruginosa* isolates from a Polish paediatric hospital (1993–2002). *International journal of antimicrobial agents*, 29(2): 153-158.

Paudel, M. R., Bhattarai, H. D. and Pant, B. (2022). Traditionally used medicinal *Dendrobium*: a promising source of active anticancer constituents. In Orchids

Phytochemistry, Biology and Horticulture: Fundamentals and Applications (pp. 389-414). Cham: Springer International Publishing.

Paudel, M. R., Chand, M. B., Karki, N. and Pant, B. (2015). Antioxidant activity and total phenolic and flavonoid contents of *Dendrobium amoenum* Wall. ex Lindl. *Botanica Orientalis: Journal of Plant Science*, 9: 20-26.

Paudel, M. R., Chand, M. B., Pant, B. and Pant, B. (2018). Antioxidant and cytotoxic activities of *Dendrobium moniliforme* extracts and the detection of related compounds by GC-MS. *BMC complementary and alternative medicine*, 18:1-9.

Paudel, M.R., Rajbanshi, N., Shah, A.K., Acharya, S. and Pant, B. (2018) Antibacterial activity of selected *Dendrobium* species against clinically isolated multiple drug resistant bacteria. *African Journal of Microbiology Research*, 12(18): 426-432

Paul, P., Chowdhury, A., Nath, D. and Bhattacharjee, M. K. (2013). Antimicrobial efficacy of orchid extracts as potential inhibitors of antibiotic resistant strains of *Escherichia coli*. *Asian J Pharm Clin Res*, 6(3): 108-111.

Pearson, R. D., Steigbigel, R. T., Davis, H. T. and Chapman, S. W. (1980). Method of reliable determination of minimal lethal antibiotic concentrations. *Antimicrobial Agents and Chemotherapy*, 18(5): 699-708.

Pessoa, J., Tyteca, D., Pereira, A., Pereira, C., Borges, L., Caperta, A. D. and Monteiro, J. (2018). The orchid flora of Portugal–Addendum N. 7–Gymnadenia borealis new for Portugal and Galicia?–New localities of *Spiranthes aestivalis* and *Neottia nidus-avis*. *Journal Europaischer Orchideen*, 50: 235-246.

Peterson, L. R. and Shanholtzer, C. J. (1992). Tests for bactericidal effects of antimicrobial agents: technical performance and clinical relevance. *Clinical microbiology reviews*, 5(4): 420-432.

Pochapski, M. T., Fosquiera, E. C., Esmerino, L. A., Dos Santos E. B., Farago, P. V., Santos, F. A. and Groppo, F. C. (2011). Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from *Ipomoea batatas* (L.) Lam. Pharmacognosy magazine, 7(26): 165-170. 10.4103/0973-1296.80682

Prasai, T., Lekhak, B. and Baral, M.B. (2004). Antibacterial property of Medicinal plants against Gram negative bacteria isolated from water. *Proceeding of iv National Conference on Science and Technology*, 2: 410-415.

Priya, R. S., Kumar, S. R. and Britto, S. (2011). Antibacterial screening of the leaf extracts of *Vanda coerulea* Griff. ex Lindl. *International Journal of Research in Pharmaceutical Sciences*, 2(1): 60-62.

Radhika, B. and Murthy J. (2016) Preliminary phytochemical analysis and in vitro bioactivity against clinical pathogens of medicinally important orchid of *Rhynchostylis retusa* Blume. *American Journal of Pharm Tech Research*, 6 (4).

Rajbhandari, K. R. (2014). Orchids of Nepal: Status, threat and conservation. In *Proceedings of National Workshop on NTFP/MAPs Sector Action Plan Development: Orchid. Department of Plant Resources, Ministry of Forest and Soil Conservation and Central Department of Botany, Tribhuvan University, Kathmandu, Nepal* 1-40.

Rao, A. N. (2004). Medicinal orchid wealth of Arunachal Pradesh. *Indian Medicinal Plants of Conservation Concern (Newsletter of ENVIS Node, Foundation for Revitalisation of Local Health Traditions, Bangalore)*, 1(2): 1-5.

Raskoti, B. B. and Ale, R. (2019). Molecular phylogeny and morphology reveal a new epiphytic species of *Habenaria* (Orchidaceae; Orchideae; Orchidinae) from Nepal. *Plos one*, 14(10), e0223355.

Raskoti, B. B. and Ale, R. I. T. A. (2019). New species of orchids and notes on Orchidaceae of Nepal. *Phytotaxa*, 394(4): 257-266.

Rauf, A., Latif, A., Rehman, S. and Afaq, S. H. (2011). In-vitro antibacterial screening of extracts of *Usnea longissima* lichen. *Int J Appl Biol Pharm Technol*, 2:14-18.

Raven, P. H. and Axelrod, D. I. (1974). Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden*, 61(3): 539-673.

Rehman, S., Latif, A., Ahmad, S. and Khan, A. U. (2011). In vitro antibacterial screening of *Swertia chirayita* Linn. Against some gram negative pathogenic strains. *Int. J. Pharm. Res. Dev*, 4: 188-194.

Shaikh, J. R., and Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603-608.

Shrestha, R., Shah, S. and Pant, B. (2018). Identification of endophytic fungi from roots of two *Dendrobium* species and evaluation of their antibacterial property. *African Journal of Microbiology Research*, 12(29): 697-704.

Shimura, K., Okada, A., Okada, K., Jikumaru, Y., Ko, K. W., Toyomasu, T. and Yamane, H. (2007). Identification of a biosynthetic gene cluster in rice for mom lactones. *Journal of Biological Chemistry*, 282(47): 34013-34018.

Shrestha, P., Adhikari, S., Lamichhane, B. and Shrestha, B. G. (2015). Phytochemical screening of selected medicinal plants of the family Lythraceae. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 1(6):11–17. http://biosciencediscovery.com

Shrestha, P., Bista, M., Sharma, P., Shrestha, S., Lamichhane, B., Adhikari, S., Pandey, B. R. and Shrestha, B. G. (2015). Phytochemical screening, antimicrobial activity and cytotoxicity of Nepalese medicinal plants *Swertia chirayita* and *Dendrobium amoenum*. *Nepal Journal of Biotechnology*, 3(1): 48-54.

Sibanda, T. and Okoh, A. I. (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. African Journal of Biotechnology, 6(25).

Singh, H. K. (2012). Studies on the phytochemical constituents and in vitro antioxidant activities of *Rhynchostylis retusa* (Orchidaceae). *Journal of Pharmacy Research*, 5(5): 2905-2909.

Singh, L., Antil, R., Ashmita, P., and Dahiya, P. (2020). In-vitro biological activities of Vernonia cinerea (L.). *Plant Arch*, *20*(2), 4889-4900.

Sofowora. E.A. (1982) Medicinal Plants and Traditional Medicine in Af and a. *John Wiley and Sons, Chichester*, 256.

Subedi, A., Kunwar, B., Choi, Y., Dai, Y., van Andel, T., Chaudhary, R. P. and Gravendeel, B. (2013). Collection and trade of wild-harvested orchids in Nepal. *Journal of Ethnobiology and Ethnomedicine*, 9(1): 1-10.

Su, Z.H.; Li, Y.; Zhang, X.Y.; Chen, M.X.; Chen, L.J. and Li, Y.L. (2020). Ornamental Evaluation of Endemic Orchids from Guangdong Province. *Chin. J. Trop. Crops*, 8: 1560–1565.

Tacconelli, E. (2012). Burden of *Staphylococcus aureus* endocarditis: how real is the threat?. *Clinical Microbiology and Infection*, 18(2): 107-109.

Tang, H. (2016) *DNA Barcoding Identification and Ecological Suitability of Important Medicinal Plants of Orchidaceae*. Ph.D. Thesis, Sichuan Agricultural University, Yaan, China.

Teixeira da Silva, J. A. and Ng, T. B. (2017). The medicinal and pharmaceutical importance of *Dendrobium* species. *Applied Microbiology and Biotechnology*, 101: 2227-2239.

Theng, P. A. and Korpenwar, A. N. (2014). Studies on phytochemical, pharmacognostic and physicochemical investigations of an endangered orchid-*Geodorum densiflorum* (Lam.) Schltr. *Inte J of Bioassay*, 3(2): 1771-4.

Thitilertdecha, N., Teerawutgulrag, A. and Rakariyatham, N. (2008). Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts. *LWT-Food Science and Technology*, 41(10): 2029-2035.

Todar K. (2008). *Todar's Online Textbook of Bacteriology*. URL: http://textbookofbacteriology.net.

Trease, G. E., and Evans, W. C. (1983). *Pharmacognosy*.

Trease, G. E., and Evans, W. C. (1989). *Trease and Evans' pharmacognosy*. *(No Title)*.

Tseng, C. C., Shang, H. F., Wang, L. F., Su, B., Hsu, C. C., Kao, H. Y. and Cheng, K. T. (2006). Antitumor and immune stimulating effects of *Anoectochilus formosanus* Hayata. *Phytomedicine*, 13(5): 366-370.

Tyteca, D., Pessoa, J., Pereira, A., Pereira, C., Borges, L., Caperta, A. D. and Monteiro, J. (2018). The orchid flora of Portugal–Addendum N. 7–Gymnadenia borealis new for Portugal and Galicia?–New localities of *Spiranthes aestivalis* and *Neottia nidusavis*. *Journal Europaischer Orchideen*, 50: 235-246.

Vaidya, B., Shrestha, M. and Joshi, N. (2000). Report on Nepalese orchid species with medicinal properties. In *The Himalayan plants, can they save us? Proceeding of Nepal-Japan joint symposium on conservation and utilization of Himalayan medicinal resources* 146-152.

Wati, R. K., De Graaf, E. F., Bogarín, D., Heijungs, R., Van Vugt, R., Smets, E. F. and Gravendeel, B. (2021). Antimicrobial activity of necklace orchids is phylogenetically clustered and can be predicted with a biological response method. *Frontiers in Pharmacology*, 11: 586345.

Weber, N. D., Andersen, D. O., North, J. A., Murray, B. K., Lawson, L. D. and Hughes, B. G. (1992). In vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. Planta medica, 58(05): 417-423.

Wilson, M. F. (2007). Medicinal plant fact sheet: *Cypripedium*: Lady's slipper orchids. *Virginia: Arlington*.

Williams, G. C. (1979). The question of adaptive sex ratio in outcrossed vertebrates. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, 205(1161): 567-580.

Xing, Y. M., Chen, J., Cui, J. L., Chen, X. M. and Guo, S. X. (2011). Antimicrobial activity and biodiversity of endophytic fungi in *Dendrobium devonianum* and *Dendrobium thyrsiflorum* from Vietman. *Current microbiology*, 62: 1218-1224.

Xu, J., Han, Q. B., Li, S. L., Chen, X. J., Wang, X. N., Zhao, Z. Z. and Chen, H. B. (2013). Chemistry, bioactivity and quality control of *Dendrobium*, a commonly used tonic herb in traditional Chinese medicine. *Phytochemistry reviews*, 12: 341-367.

Vaidya, B. N. (2019). Nepal: a global hotspot for medicinal orchids. *Medicinal Plants: From Farm to Pharmacy*, 35-80.

Yam, E. L. Y., Hsu, L. Y., Yap, E. P. H., Yeo, T. W., Lee, V., Schlundt, J. and Wilder-Smith, A. (2019). *Antimicrobial resistance in the Asia Pacific region*: a meeting report

Yang, R. Y., Chang, L. C., Hsu, J. C., Weng, B. B., Palada, M. C., Chadha, M. L. and Levasseur, V. (2006). Nutritional and functional properties of Moringa leaves–From germplasm, to plant, to food, to health. *Moringa leaves: Strategies, standards and markets for a better impact on nutrition in Africa. Moringanews, CDE, CTA, GFU. Paris*, 1-9.

Yang, Y., Tang, J., Zou, R., Luo, Y., Deng, Z., Li, D. and Wei, X. (2023). The Genetic Diversity and Genetic Structure of the Germplasm Resources of the Medicinal Orchid Plant *Habenaria dentata*. Genes, 14(9):1749.

Zaidan, M. R., Noor Rain, A., Badrul, A. R., Adlin, A., Norazah, A. and Zakiah, I. (2005). In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop biomed*, 22(2): 165-170.

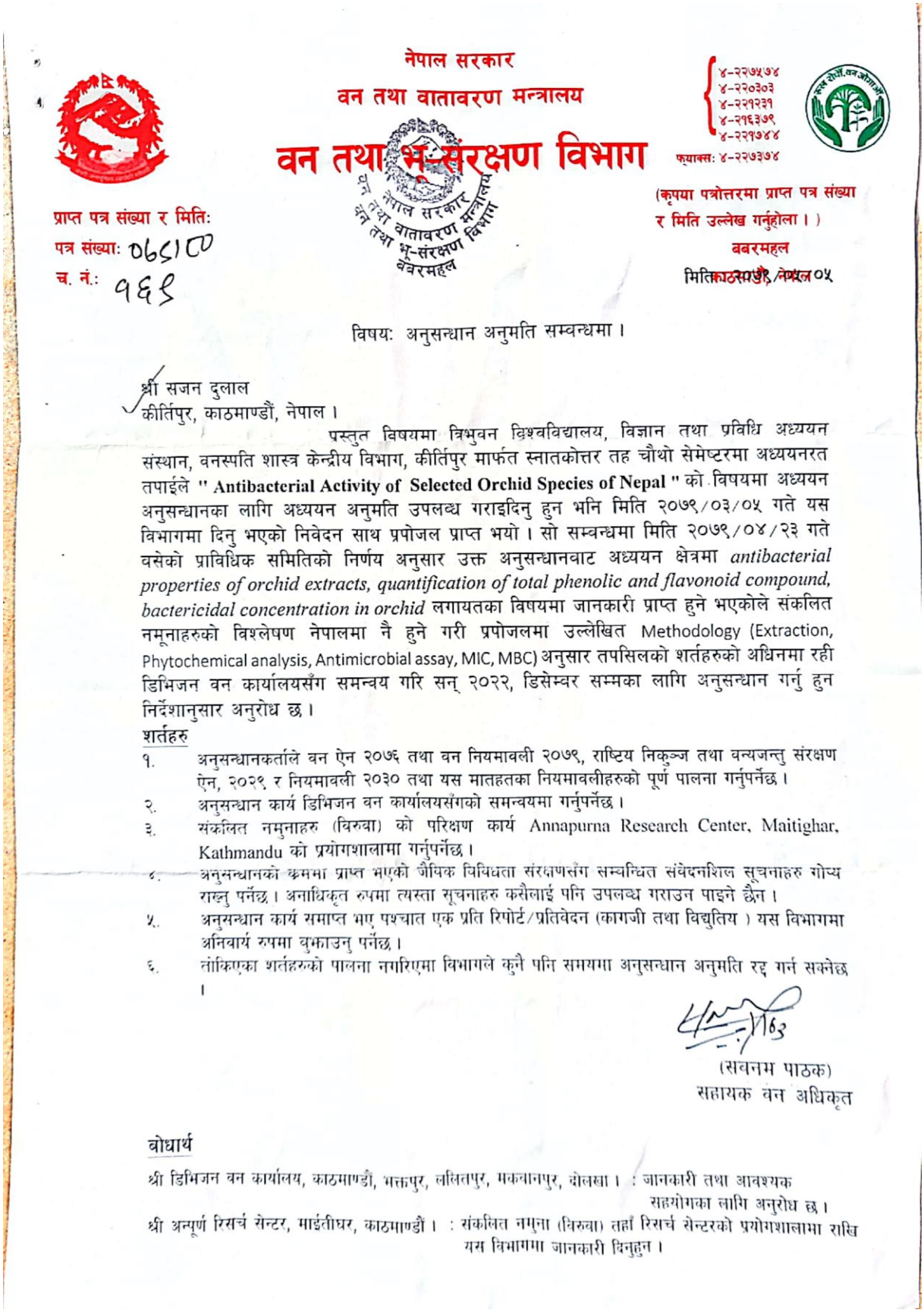
Zetola, N., Francis, J. S., Nuermberger, E. L. and Bishai, W. R. (2005). Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. *The Lancet infectious diseases*, 5(5): 275-286.

Zhang, Q., Wang, H. C., Cheng, Z., Wang, M. N., Li, L. Q. and Long, C. L. (2022). Current Status of Wild Orchid Resources in China, Focusing on Their Conservation and Utilization. China Biotechnology, 42(11): 59-72.

Zhao, L., Mitomo, H., Zhai, M., Yoshii, F., Nagasawa, N. and Kume, T. (2003). Synthesis of antibacterial PVA/CM-chitosan blend hydrogels with electron beam irradiation. *Carbohydrate Polymers*, 53(4): 439-446.

Annex

Ethical Review



नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वन तथा भू-संरक्षण विभाग
बबरमहल

४-२२७५७४
४-२२०३०३
४-२२१२३१
४-२१६३७९
४-२२१७४४
फ्याक्स: ४-२२७३७४

प्राप्त पत्र संख्या र मिति:
पत्र संख्या: ०६८।८०
च. नं.: ९६९

(कृपया पत्रोत्तरमा प्राप्त पत्र संख्या र मिति उल्लेख गर्नुहोला ।)

मिति: काठमाण्डौं, नेपाल ०५

विषय: अनुसन्धान अनुमति सम्वन्धमा ।

श्री सजन दुलाल
कीर्तिपुर, काठमाण्डौं, नेपाल ।

प्रस्तुत विषयमा त्रिभुवन विश्वविद्यालय, विज्ञान तथा प्रविधि अध्ययन संस्थान, वनस्पति शास्त्र केन्द्रीय विभाग, कीर्तिपुर मार्फत स्नातकोत्तर तह चौथो सेमेष्टरमा अध्ययनरत तपाईले " **Antibacterial Activity of Selected Orchid Species of Nepal** " को विषयमा अध्ययन अनुसन्धानका लागि अध्ययन अनुमति उपलब्ध गराइदिनु हुन भनि मिति २०७९/०३/०५ गते यस विभागमा दिनु भएको निवेदन साथ प्रपोजल प्राप्त भयो । सो सम्वन्धमा मिति २०७९/०४/२३ गते वसेको प्राविधिक समितिको निर्णय अनुसार उक्त अनुसन्धानवाट अध्ययन क्षेत्रमा *antibacterial properties of orchid extracts, quantification of total phenolic and flavonoid compound, bactericidal concentration in orchid* लगायतका विषयमा जानकारी प्राप्त हुने भएकोले संकलित नमूनाहरुको विश्लेषण नेपालमा नै हुने गरी प्रपोजलमा उल्लेखित Methodology (Extraction, Phytochemical analysis, Antimicrobial assay, MIC, MBC) अनुसार तपसिलको शर्तहरुको अधिनमा रही डिभिजन वन कार्यालयसँग समन्वय गरि सन् २०२२, डिसेम्वर सम्मका लागि अनुसन्धान गर्नु हुन निर्देशानुसार अनुरोध छ ।

शर्तहरु

१. अनुसन्धानकर्ताले वन ऐन २०७६ तथा वन नियमावली २०७९, राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण ऐन, २०२९ र नियमावली २०३० तथा यस मातहतका नियमावलीहरुको पूर्ण पालना गर्नुपर्नेछ ।
२. अनुसन्धान कार्य डिभिजन वन कार्यालयसँगको समन्वयमा गर्नुपर्नेछ ।
३. संकलित नमुनाहरु (विरुवा) को परिक्षण कार्य Annapurna Research Center, Maitighar, Kathmandu को प्रयोगशालामा गर्नुपर्नेछ ।
४. अनुसन्धानको क्रममा प्राप्त भएको जैविक विविधता संरक्षणसँग सम्वन्धित संवेदनशिल सूचनाहरु गोप्य राख्नु पर्नेछ । अनाधिकृत रुपमा त्यस्ता सूचनाहरु कसैलाई पनि उपलब्ध गराउन पाइने छैन ।
५. अनुसन्धान कार्य समाप्त भए पश्चात एक प्रति रिपोर्ट/प्रतिवेदन (कागजी तथा विद्युतिय) यस विभागमा अनिवार्य रुपमा बुझाउनु पर्नेछ ।
६. तोकिएका शर्तहरुको पालना नगरिएमा विभागले कुनै पनि समयमा अनुसन्धान अनुमति रद्द गर्न सक्नेछ ।

(सवनम पाठक)
सहायक वन अधिकृत

बोधार्थ

श्री डिभिजन वन कार्यालय, काठमाण्डौं, भक्तपुर, ललितपुर, मकवानपुर, दोलखा । : जानकारी तथा आवश्यक सहयोगका लागि अनुरोध छ ।
श्री अन्नपूर्ण रिसर्च सेन्टर, माईतीघर, काठमाण्डौं । : संकलित नमुना (विरुवा) तहाँ रिसर्च सेन्टरको प्रयोगशालामा राखि यस विभागमा जानकारी दिनुहुन ।