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The Potential Alternative Antibacterial Activity of Falcata (*Falcataria Falcata*) Leaf Methanolic Extract against *Staphylococcus Aureus* and *Escherichia Coli*

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Adventist Medical Center College Department of Pharmacy

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A Thesis Study Presented to
The Faculty of the Department of Pharmacy Adventist Medical Center College

In Partial Fulfillment
of the Requirements for the Degree Bachelor of Science in Pharmacy

by

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APPROVAL SHEET

This thesis entitled:

"The Potential Alternative Antibacterial Activity Of Falcata (*Falcataria falcata*) Leaf Methanolic Extract Against *Staphylococcus aureus* And *Escherichia coli*"

prepared and submitted by Laliefe B. Arnan, Katherine Joy S. Escueta, Nor Rashida P. Rashid, Elyca M. Tacbobo, and Queenie Rose M. Tinoy has been examined, and is recommended for approval and acceptance as partial fulfillment of the requirements for the degree Bachelor of Science in Pharmacy

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Approved and accepted in partial fulfillment for the Degree of Bachelor of Science in Pharmacy.

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CERTIFICATE OF ORIGINALITY

We hereby declare that this submission is our own work and that, to the best of our knowledge and belief, it contains no materials previously published or written by another person nor material to which to a substantial extent has been accepted for award of any other degree or diploma of a university or other institute of higher learning, except where due acknowledgement is made in the text.

We also declare that the intellectual content of this thesis is the product of our work, even though we have received assistance from others on style, presentation, and language expression.

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ABSTRACT

The Potential Alternative Antibacterial Activity Of Falcata (*Falcataria falcata*) Leaf Methanolic Extract Against *Staphylococcus aureus* And *Escherichia coli*

- **Laliefe B. Arnan; Katherine Joy S. Escueta; Nor Rashida P. Rashid; Elyca M. Tacbobo; Queenie Rose M. Tinoy**
- **Degree: Bachelor of Science in Pharmacy**
- **Thesis Adviser: Junnin Gay L. Garay, RPh, CPh, MS Pharm**

Falcata is a plant that can be found in the Philippines and is used for the production of wood veneer and plywood. While in Indonesia, it is used as traditional remedy for malaria (Budiarti et al. 2020). They belong to the Fabaceae family, a family known for having great antibacterial effects (Gamo et al. 2015). This study used a percolation extraction method and the percentage yield is calculated to determine the yield from the falcata extract. Disc diffusion method is used for susceptibility testing and determining the zone of inhibition for the different groups. The CLSI guidelines for *Staphylococcus aureus* and *Escherichia coli* will be used to determine the antibacterial effect of the extract, in terms of resistance, intermediate, and susceptible results.

From the results, the percentage yield of the methanolic crude leaf extract of *Falcataria falcata* is 2.67%. Leaves from the *Falcataria falcata* plant were extracted and tested against bacteria. The extracts showed promise in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria, with 75% concentration as being more effective. However, these bacteria showed some resistance to all extract concentrations: *S. aureus* (90% - resistant, 75% - intermediate, 50% - resistant, 25% - resistant); *E. coli* (90% - resistant, 75% - resistant, 50% - resistant, 25% - resistant). Further study is needed to determine the exact antibacterial properties of the plant.

Keywords:- *Falcataria Falcata, Antibacterial, Leaves, Methanolic Crude Leaf Extract, Disc Diffusion Method, Susceptibility Testing, Fabaceae Family, Staphylococcus Aureus, Escherichia Coli, Percentage Yield.*

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CHAPTER ONE
INTRODUCTION

This chapter presents the problem and its setting. It includes the background of the study, statement of the problem, research objectives, research hypothesis, theoretical framework, conceptual framework, significance of the study, scope and limitation, and the operational definition of terms used.

A. Background of the Study

One of the most widely used medications globally is antibiotics. Unfortunately, many individuals and healthcare providers disregard the potential for these drugs to lose effectiveness over time, assuming resistance only impacts future patients. This complacency, coupled with the widespread use of antibiotics, significantly accelerates the development of resistance, posing a serious threat to public health (Gandra et al. 2015). Data collected from 71 countries, including the most populated ones, reveals a disturbing trend. A 30% increase in global antibiotic consumption was observed between 2000 and 2010, rising from an estimated 50 billion to 70 billion standard units (Van Boeckel et al. 2014). This alarming rise underscores the urgent need to promote responsible antibiotic use and invest in research and development of new antibiotics to combat this growing public health challenge.

Medicinal plants, with their long history of use in traditional medicine, offer a promising avenue for exploration. A comprehensive review by Clark (2020) highlights the crucial role medicinal plants play in combating antibiotic-resistance pathogens. There are 459 compounds that are derived from plant materials and are known and utilized for significantly inhibiting antibacterial activity. In the case of Falcata methanolic leaf extract, its potential as an antibacterial agent is particularly intriguing. Falcata has a rich history of medicinal applications. Stuart (2022) documents its diverse uses, including treating tropical ulcers, inducing sleep, addressing venereal diseases, relieving chest congestion, and even serving as a traditional remedy for malaria in Papua Island, Indonesia.

Falcataria falcata is a member of the Fabaceae family, and plants belonging to this family, such as *Acacia rigidula,* possess antibacterial effects like inhibiting the microorganism growth of bacteria like *Providencia alcalifaciens, Yersinia enterocolitica*, *Enterococcus faecalis, Staphylococcus aureus,* and *Bacillus subtilis* (Cavazos 2021). Plants in the Fabaceae family are known to be rich in oestrogenic, antibacterial, antioxidant, anti-fungal, antifeedant and insecticidal activities (Gamo et al. 2015). *Cassia alata*, a family member of *Fabaceae,* shows significant antibacterial efficacy against *S. aureus* by its crude extracts of the root and stem (Toh et al. 2023). The leaves and pods of Carob (*Ceratonia siliqua* L.), under the same family, exhibited antibacterial activity against *Pectobacterium atrosepticum* (Saïda et al. 2015). These traditional uses provide valuable clues for further research, paving the way for scientific investigation into the potential of Falcata leaf extract as a natural antibacterial alternative.

Staphylococcus aureus is a gram-positive bacteria that can cause a variety of clinical diseases and infections that are common both in community and hospital settings. It is the causative agent of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections (Taylor and Unakal 2023).

Escherichia coli (*E. coli)* is a gram-negative bacillus, which is considered as the causative agent to many illnesses of diarrhea, such as traveler's diarrhea, dysentery, or bloody diarrhea. It is one of the most common bacteria that could cause uncomplicated cystitis, and also in other diseases, outside of the intestines; this includes pneumonia, bacteremia, and spontaneous bacterial peritonitis. (Mueller and Tainter 2023)

Trimethoprim-sulfamethoxazole (TMP-SMX) are two antimicrobial agents that work together to treat various bacterial infections. It includes a variety of aerobic gram-positive and gram-negative bacteria, fungi, and protozoa.TMP-SMX has been listed as one of the essential treatments required in a basic health system, according to the WHO recommendation. The medication is made as a fixed-ratio mix of trimethoprim and sulfamethoxazole (1:5) (Bakdach and Elajez 2020). Moreover, Trimethoprim-Sulfamethoxazole (TMP-SMX) has been used in genuine clinical settings to treat infections of the skin, soft tissues, bones, and joints. Hence, the spread of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and other aureus isolates has increased the demand for TMP-SMX (Sato et al. 2022).

In order to assess the antibacterial efficacy of *Falcataria falcata* methanolic crude leaf extract, researchers intend to examine the phytochemical components of the extract and measure the zone of inhibition, driven by the quest for organic and natural alternatives to treat bacterial infections.

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B. Statement of the Problem

The following inquiries are intended to be addressed by this research study. Initially, to determine whether concentrations of *Falcataria falcata* methanolic leaf crude extract (90%, 75%, 50%, and 25%) are more effective against *Staphylococcus aureus* and *Escherichia coli*,. The extract is first compared to a synthetic antibiotic, Trimethoprim-sulfamethoxazole based on the zone of inhibition. Additionally, the researchers wanted to identify the phytochemical compounds present in the methanolic crude leaf extract of *Falcataria falcata* and the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria.

C. Research Objectives

The purpose of this study is to determine how *Falcataria falcata* methanolic leaf crude extract works against *Staphylococcus aureus* and *Escherichia coli*. Specifically, it sought to ascertain the following goals:

- Determine the percent yield in the methanolic crude leaf extract of *Falcataria falcata.*
- Determine the phytochemical compounds present in the methanolic crude leaf extract of *Falcataria falcata.*
- Identify the significant differences in the zone of inhibition between the positive (Trimethoprim-sulfamethoxazole) and negative (distilled water) groups for the bacteria *Staphylococcus aureus* and *Escherichia coli*, as well as the experimental groups (90%, 75%, 50%, and 25% concentrations) of the methanolic crude leaf extract of *Falcataria falcata*.
- Determine whether the methanolic crude leaf extracts of *Falcataria falcata* exhibit intermediate, susceptible, or resistant antibacterial activity against the tested bacterias at each concentration.

D. Research Hypotheses

- H0: The *Falcataria falcata* plant has no antibacterial effect and had no significant differences between the four concentrations (90%, 75%, 50% and 25%) of methanolic leaf crude extract and the positive control group (Trimethoprim-sulfamethoxazole) against *Staphylococcus aureus* and *Escherichia coli* bacteria.
- H1: The *Falcataria falcata* plant has an antibacterial effect and had significant differences between the four concentrations (90%, 75%, 50% and 25%) of methanolic leaf crude extract and the positive control groups (Trimethoprim-sulfamethoxazole) against *Staphylococcus aureus* and *Escherichia coli* bacteria.

E. Theoretical Framework

The research by Davis and Stout (1971), as cited by Darwis et al. (2018) served as the foundation for the theoretical framework of this investigation. According to the Disc Plate Method of Microbiological Antibiotic Assay, the diameter immediately responds to the concentration of the antibiotic, and the identification and management of the numerous minute factors are what restrict accuracy. It was further emphasized that the disc plate method is still widely used and that microbiological test methodologies offer a reliable assessment of antibiotic activity with a low risk of interference from physiologically inactive components or degradation products. This investigation indicates that the chosen plant species' antibacterial activity and effectiveness are compatible with the research.

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F. Conceptual Framework

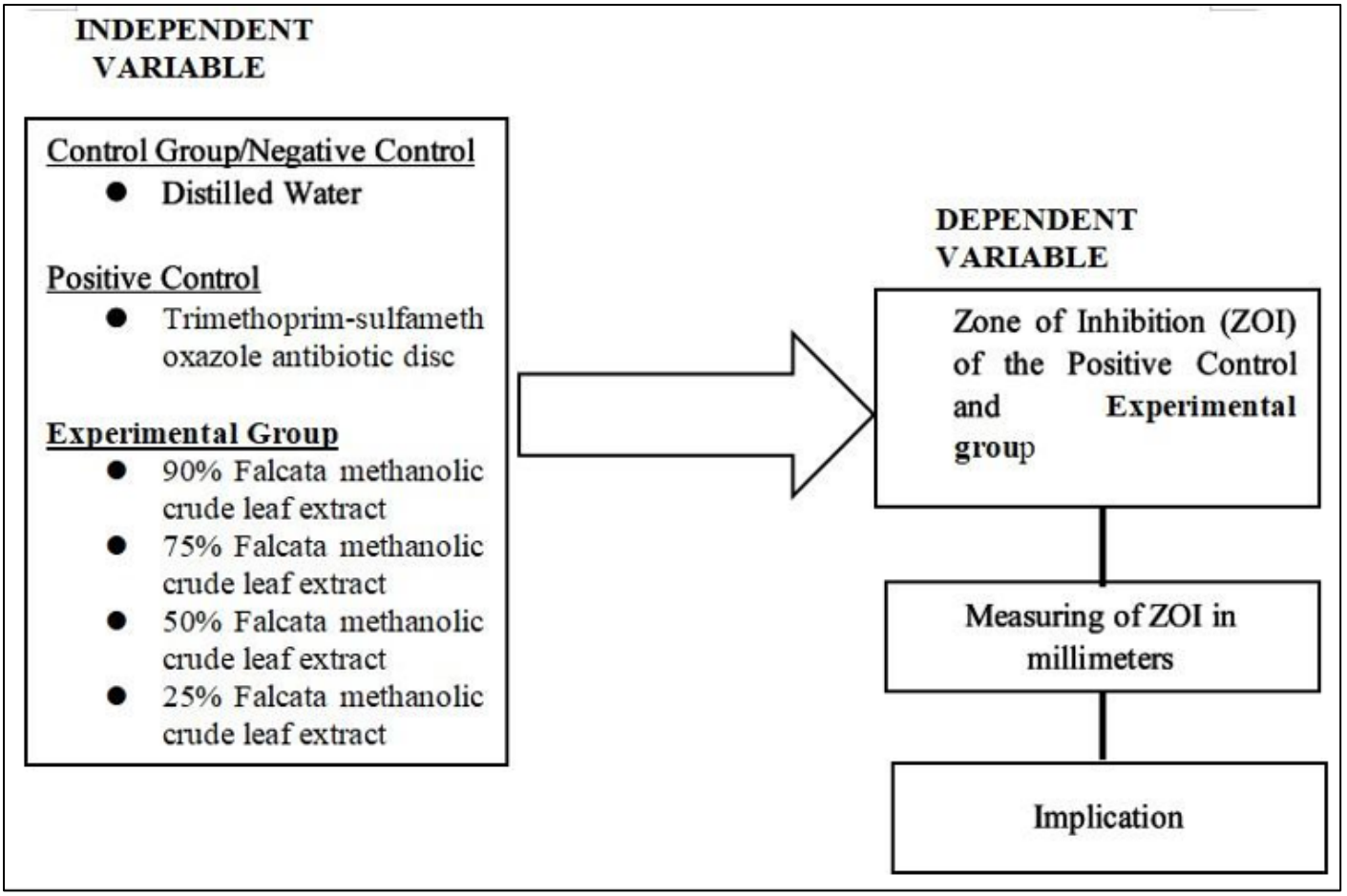

Fig 1: Dependent and Independent Variables (Ali et al. 2023).

G. Significance of the Study

Community, this research offers a promising path toward safer and more sustainable antibacterial solutions for individuals and communities. By exploring the potential of Falcata leaf extract, a readily available plant in many regions, the research empowers communities with locally accessible resources to combat bacterial infections. The community will be able to get recommendations and ideas from the researchers on the potential uses of this plant as an antibacterial.

Learners, this research serves as a valuable learning resource for students and researchers in various fields, including microbiology, pharmacology, and ethnomedicine. It provides insights into the scientific investigation of natural antibacterial properties of *Falcataria falcata* and encourages further research in this area.

Future Researchers, this research lays the foundation for further investigation into the antibacterial properties of Falcata leaf extract. Future studies can explore its efficacy against other bacterial strains, its mechanism of action, and the development of standardized formulations for safe and effective use.

H. Delimitations and Limitations of the Study

This study aimed to identify the plant's secondary metabolites and show that *Falcataria falcata*'s methanolic crude leaf extract is an effective natural antibacterial alternative for treating *Escherichia coli* and *Staphylococcus aureus*. The study has several important limitations as well as interesting options to consider.

This research compares the effectiveness of the various concentrations (90%, 75%, 50%, and 25%) of methanolic crude leaf extract of *Falcataria falcata* against Trimethoprim-sulfamethoxazole as a positive control and distilled water as a negative control only. This research did not investigate to confirm its efficacy and safety in rats but only in vitro on petri plates. Moreover, the limited scope of the study currently focuses only on Falcata methanolic crude leaf extract's antibacterial activity against gram positive bacteria (*S. aureus)* and gram negative bacteria (*E. coli)*. This would lead to an idea of which extract will be more susceptible to the bacteria.

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This preliminary study provides an indication of the potential of leaf extract from falcata (*Falcataria falcata*) as a natural antibacterial agent substitute. To overcome the obstacles and realize its full potential for enhancing public health, further research is necessary. Through the exploration and use of easily accessible natural resources such as falcata (*Falcataria falcata*), the research may contribute to the development of safer and more sustainable methods for fighting bacterial illnesses.

Furthermore, this study only used the disc diffusion method to evaluate the antibacterial activity of Falcata methanolic crude leaf extract. Khan et al. (2019) stated in their study that "disc diffusion method is the gold standard for confirming the susceptibility of bacteria." Moreover, this approach offers broad acceptance due to its provision of a straightforward and economical procedure for identifying numerous targets.

I. Operational Definition of Terms

➢ *The Following Terms are Defined for Better Understanding of the Study:*

- **Alkaloids.** It is mainly biosynthetically derived from amino acids, resulting in a variety of chemical structures, mostly isolated from plants. Therapeutically, alkaloids are particularly well known as anesthetics, cardioprotective agents, and anti-inflammatory agents. (Heinrich et al. 2021)

  It refers to one of the phytochemicals found in *Falcataria falcata* extract that has antibacterial properties.
- **Anthraquinones.** It is the most important quinone derivative of anthracene and the parent substance of a large class of dyes and pigments. (Britannica 2024)

  It refers to one of the phytochemicals and whether the methanolic leaf extract of *Falcataria falcata* has this kind of substance.
- **Antibacterial agent.** It is a group of materials that selectively destroy bacteria by interfering with bacterial growth or survival. (Li et al. 2020)

  It refers to the ability of *Falcataria falcata* methanolic leaf extract to inhibit 75% of the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria.
- **Cyanogenic glycosides.** It is a bioactive plant product derived from amino acids. (Gleadow and Møller 2014)

  It refers to one of the phytochemicals whether the methanolic leaf extract of *Falcataria falcata* has this kind of substance.
- **Experimental group.** It is the test subjects that receive the treatment or intervention under investigation. The performance of the experimental group is then compared against the well-established markers, the positive and negative controls. (Drew 2023)

  It refers to the different *Falcataria falcata* crude extract concentrations used for the antibacterial effect comparison between the negative control and positive control groups.
- **Flavonoids.** It belongs to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages. They have miscellaneous favorable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), and atherosclerosis (Panche et al. 2016).

  It refers to one of the phytochemicals found in *Falcataria falcata* extract that has antibacterial properties.
- **Intermediate.** The result indicates that there should be a combination of antibiotics to be fully effective against the bacteria (Hartline 2022).

  This refers to the compound having some effectiveness against the bacteria, but it may not be strong enough to completely eliminate them.
- **Leaf extract.** It is a process of separating active plant materials or secondary metabolites such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides from inert or inactive material using an appropriate solvent and standard extraction procedure. (Abubakar and Haque 2020)

  It refers to the dried powder obtained by the percolation method of the leaves of *Falcataria falcata*, then drying the extracts.
- **Negative control group.** refers to a group that does not receive the procedure or treatment and is expected not to yield a positive result. Its role is to ensure that a positive result in the experiment is due to the treatment or procedure. (Drew 2023)

  It refers to the distilled water used in the experiment for comparing between the experimental control and positive control groups.
- **Phytochemicals.** It is a plant-based bioactive compound produced by plants for their protection. These phytochemicals possess strong antioxidant activities and exhibit antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral activities. (Kumar et al. 2023)

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It refers to the chemical compounds identified in the study that have shown antibacterial properties in *Falcataria falcata* methanolic leaf extracts such as flavonoids, alkaloids, saponins, tannins, and steroids.

- **Positive control group.** It refers to a group in an experiment that receives a procedure or treatment known to produce a positive result. It serves the purpose of affirming the experiment's capability to produce a positive outcome (Drew 2023).

It refers to the Trimethoprim-sulfamethoxazole antibiotic disc used in the experiment, known to have susceptible effects on *Staphylococcus aureus* and *Escherichia coli.*

- **Resistant.** The result indicates that the antibiotic is not effective in killing against the bacteria (Hartline 2022).

This refers to the compound not having any significant effect on inhibiting or killing the bacteria.

- **Saponins.** It is a naturally occurring compound found in plants, possessing foaming and emulsifying properties and sometimes exhibiting antimicrobial effects. Investigate the potential contribution of saponins to the antimicrobial activity of the extract (Juang and Liang 2020).

It refers to one of the phytochemicals found in *Falcataria falcata* extract that has antibacterial properties.

- **Steroids.** It is any of a class of natural or synthetic organic compounds characterized by a molecular structure of 17 carbon atoms arranged in four rings. Therapeutic values include a large number of anti-inflammatory agents, anabolic (growth-stimulating) agents, and oral contraceptives (Kluger 2019).

It refers to one of the phytochemicals found in *Falcataria falcata* extract that has antibacterial properties.

- **Susceptible.** It indicates that the antibiotic is effective against killing the bacteria when it is exposed to it (Hartline 2022).

This refers to the compound being effective in inhibiting or killing the targeted bacteria.

- **Tannins.** It is a heterogeneous group of high - MW, water-soluble, polyphenolic compounds, naturally present in cereals, leguminous seeds, and, predominantly, in many fruits and vegetables, where they provide protection against a wide range of biotic and abiotic stressors. Tannins exert several pharmacological effects, including antioxidant and free radical scavenging activity as well as antimicrobial, anti-cancer, anti-nutritional, and cardio-protective properties (Smeriglio et al. 2016).

It refers to one of the phytochemicals found in *Falcataria falcata* extract that has antibacterial properties.

- **Zone of inhibition.** The clear area surrounding an antimicrobial agent-containing disc on an agar plate in a disc diffusion assay, indicates inhibition of microbial growth. (Giuliano et al. 2019)

It refers to the ability of the different methanolic leaf extracts of *Falcataria falcata* 90%, 75%, 50%, and 25% concentrations to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* using disc diffusion assay.

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CHAPTER TWO
REVIEW OF RELATED LITERATURE AND STUDIES

This chapter presents the related literature and studies, after in-depth research done by the researchers. The literature and studies adduced in this chapter address the different ideas, concepts, generalizations, conclusions, and also the different developments related to the study, starting from the past up to the present. This will serve as a guide for the researchers in developing the study. Moreover, the information included in this chapter helps in familiarizing details that are opposite and similar to the present study.

A. Taxonomy of Falcataria falcata

- Kingdom: Plantae
- Division: Magnoliophyta Class: Magnoliopsida
- Order: Fabales
- Family: Fabaceae
- Genus: Falcataria
- Species: *Falcataria falcate*

Fig 2: Falcataria Falcata Plant (wildlifeofhawaii.com)

Falcataria falcata is classified as belonging to the *Eukarya* domain, *Plantae* under kingdom, *Tracheophyta* under phylum, *Magnoliopsida* under class, *Fabales* under order, *Fabaceae* under family, *Falcataria* under genus, and *Falcataria falcata* species (Prematuri et al. 2020). Common species of *Falcataria falcata* include *Falcataria moluccana, Albizia fulva, Albizia moluccana, Albizia eymae, Albizia falcata, Adenanthera falcataria, Albizia falcataria, and Paraserianthes falcataria.* (NParks 2022).

According to Paquit and Rojo (2018), falcata wood is the main supplier of plywood and pulpwood. From the pulpwood and plywood industries, this contributes to improving the quality of life in the Philippines and the country's economy. Today, in the Philippines, plantation forestry is an effective approach to producing timber legally, ready to be marketed and sold. In fact, plant forestry decreases the exploitation of wild forests' resources by meeting the nation's demand for the production of wood (Murat 2020). The implementation of plantations creates regeneration in the degraded areas, which benefits future generations. *Gmelina spp., Swietenia spp., and Paraserianthes spp.* are the most common tree plantation species in the Philippines.

From the province of Caraga, the *Falcata moluccana* trees, with a minimum diameter of 25 cm, are loaded into trucks and, together with some stem logs, transported to the processing facility. The trees are utilized in the production of veneer and plywood inside the facility (Marasigan et al. 2022).

Falcata (*Falcataria falcata)* is a great multipurpose tree for the humid tropics, compared to the other species and it grows quickly. It is usually used for different wood products and may also be used for farming and in forest plantations, it can have intercropping (Alamsyah et al. 2018). The falcata trees have the capability to attain heights of 40 meters, with their initial branches commonly emerging at an elevation of up to 20 meters above the ground (Rojas-Sandoval 2022).

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Fig 3: A Fully Matured Falcata (*Falcataria falcata*) Tree (Stuart 2022)

B. Morphology of Falcataria falcata

According to Kawai et al. (2023), the leaves are composed of pinnately compound leaves, whose length varies from 23-30 cm. The leaves possess rusty pressed hairs and have a slender angled axis, having a gland located above the base. The leaflets are paired, with some each axis having 15-20 pairs. It is appeared to be small, oblong, and measuring 6-12mm on its length, and 3-5 mm on its width, with a short-pointed on its tip. The underside appears to be paler with fine hairs, while the topside has dull green color and is hairless. The bark shows many uses for the entire tree, including fire protection, mechanical support for the stem, and the translocation and storage of nutrients and carbon. As a result, bark morphology would change with size to correspond with ontogenetic changes in the composition and roles of trees. For instance, a thicker inner bark to achieve greater translocation and storage capacity may be linked to the increased photosynthesis in larger trees owing to their large canopy.

In order to anticipate changes in growth, carbon stocks, reactions to environments, and wood quality in F. falcata plantations during stand development, model parameterizations will be based on the size-related variations in features reported here (Kawai et al. 2023). However, due to the persistent growth in demand for wood, which rose by 216% between 2003 and 2014, it became necessary to ascertain the qualities of new wood sources, such as the branches of F. molucanna, and to utilize them (Marisagan et al. 2021). F. falcata is also referred to by a variety of species names, including Ademaanthera falcataria L., Albizia moluccana Miq., Paraserianthes falcataria, and Albizia falcataria (L.) Fosberg and Backer. (L.) Nielsen; according to Marasigan et al. (2022) all of which are regarded as synonyms for Falcataria moluccana.

F. falcata, also known as Falcataria moluccana, is most frequently found in mesic lowland regions, although it may grow up to 2300 meters above sea level. In addition to planted woods, disturbed regions, abandoned farms, montane forests, grassy plains, river flood terraces, and roadsides close to the sea, it is frequently found in secondary forests, primary deciduous forests, and mountain forests (Rojas-Sandoval 2022). F. moluccana trees are able to grow to enormous sizes when other trees cannot, according to tree community research. This shows that either resource monopolization or differentiation are occurring.

C. Phytochemical Analysis of Falcata Species

According to recent studies, the phytochemical components of *Eperua falcata's* wood wastes have phenolic compounds such as simple phenols, lignans, flavonoids, and tannins. Their chemical and biological attributes could include antioxidants, anti-radicals, anti-termite, antifungal, anticancer, inhibitors of type 1 HIV, antimutagens, and antibacterial qualities (Louis et al. 2023).

Furthermore, the bark of *Paraserianthes falcataria,* in the in-vitro anthelmintic study of (Baihaqi et al. 2020) shows that its bark waste in both aqueous and 70% methanol extracts contains compounds, such as tannins, flavonoids, alkaloids, saponins, and steroids in the phytochemical screening.

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In the crude extract of glucose lowering study by (Arini et al. 2019) of a bark waste of *Paraserianthes falcataria L.*, it has alkaloids, flavonoids, and triterpenoids. The in vivo study stated that triterpenoids can lower glucose levels.

From the antioxidant activity study of (Rumidatul et al. 2021), the findings of their phytochemical compounds of the twigs of *Falcataria moluccana* are flavonoids, phenolics, steroids, terpenoids, tannins, and saponins. Therefore, from these studies, most phytochemical results are obtained from bark and twigs, and they have phytochemical compounds of alkaloids, flavonoids, simple phenols, phenolic compounds, lignans, tannins, saponins, steroids, terpenoids, and triterpenoids.

- *Flavonoids*

Flavonoids are known to have antibacterial effects; they are known as polyphenolic compounds and are commonly known for their antibacterial activity because they inhibit the proliferation of microorganisms, including strains of bacteria that are resistant to multiple drugs (Shamsudin et al. 2022).

- *Tannins*

Tannins are a group of phenolic compounds found in various plant species, foods, and beverages (Smeriglio et al. 2016). They play an important role in terms of defense against different pathogens (Singh et al. 2021). Specifically, tannins are also found in various parts of plants, such as wood, bark, leaves, fruit skins, and seeds (Kennedy and Jones 2015).

Tannins are commonly found in plant cell vacuoles and are particularly abundant in the epidermal tissues. They are found in wood, bark, roots, leaves, fruits, and galas. Its effect protects the plants from fungi invasion, including insects, which can act as antiseptics. From human skin, they have drying effects, hasten wound healing, prevent bleeding, and inhibit microorganism growth (Prasad 2023).

- *Saponins*

Saponins are secondary metabolites that can be found in various plant species and are also amphiphatic molecules composed of a hydrophilic sugar moiety and a hydrophobic triterpene or a steroid aglycone (Mroczek 2015). These phenolic compounds have been extensively studied for their diverse biological activities, such as antimicrobial, anti-inflammatory, anti-cancer, and immunomodulatory properties (Mroczek 2015). All of the bioactive compounds that the saponins have, are valuable for nutraceutical and pharmaceutical applications (Kumar and Pansari 2016). According to (Wink 2015), saponins play an essential role in plant defense against different types of herbivores and pathogens. This has also been studied for their potential advantages for the benefits of human health, such as reducing cholesterol and modulating the gut microbiota (Mroczek 2015; Kumar and Pansari 2016).

- *Alkaloids*

Alkaloids are a wide range of secondary metabolites originating from plants, animals, fungi, and bacteria (Dey et al. 2020). According to Rahman et al. (2018) they have been extensively studied for their various biological activities, such as analgesic, antimicrobial, anti-inflammatory and psychoactive effects. Alkaloids also have been investigated for their potential pharmacological applications, including the treatment of neurological disorders and cancer (Rahman et al. 2018).

- *Steroids*

Steroids are chemically active hormone-like, either natural or synthetic. It belongs to a large group of chemical compounds that are distinguished by their certain carbon structure. Drugs that have steroids are used for relieving swelling and inflammation, like prednisone and cortisone, vitamin D, and sex hormones (Agidew 2022). Plant steroids exhibit many medicinal and pharmaceutical effects; this includes anti-tumor, immunosuppressive, protecting the liver, antibacterial, anthelmintic, cytotoxic, and enhancing heart function (Oklestkova 2021).

D. Bacteria

- *Staphylococcus Aureus (S.Aureus)*

Staphylococcus is a genus of gram-positive cocci bacteria from the family *Staphylococcaceae*. It is a major pathogen that is associated with high injection and mortality rates; it is one of the leading causes of hospital-acquired infections and post-surgical wound complications in the world. It can cause diseases such as infections of the respiratory tract, skin, and soft tissue, pleuro-pulmonaryskin; device-related infections; and infective endocarditis. *Staphylococcus aureus also* has an extraordinary ability to acquire resistance to any antibiotic (Karaman et al. 2020).

- *Escherichia coli (E.coli)*

Escherichia coli is the most common gram-negative, also known to be normal intestinal flora, but intestinal and extraintestinal illness could occur in humans. They are commonly found in the human gastrointestinal tract and their virulence is lacking.

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E. coli can be found outside the gastrointestinal tract; it can also cause various infections, such as urinary tract infections (UTI), pneumonia, bacteremia, and peritonitis. *E. coli* is one of the major nosocomial infections, such as catheter-associated UTIs and ventilator associated pneumonia (VAP). They are also found in soil, vegetables, meats that are undercooked, and in water (Mueller and Tainter 2023).

E. Disc Diffusion Assay

The disc diffusion method is the most flexible susceptibility testing method for testing antimicrobial agents. It consists of placing a paper disc that is saturated with antimicrobial agents on the swabbed bacteria in the agar medium, and a zone of inhibition is measured after the plate is incubated overnight to determine its presence or absence. This technique was conducted at the University of Washington in the mid-1960s. This technique is commonly called the "Kirby-Bauer method" and was published in 1966 by Bauer and his colleagues. The standardization of the method includes the disc size, inoculum size, time of incubation, and temperature. The results of this method are qualitatively measured as resistant, intermediate, and susceptible. The Clinical and Laboratory Standards Institute (CLSI) in the United States has been consistently expanding the disc diffusion method outlined by Bauer and his colleagues. There are several other international societies that use this similar technique, such as the European Union Committee for Antimicrobial Susceptibility Testing (EUCAST) and the British Society for Antimicrobial Chemotherapy (Tenover 2019).

From the Microbiology Manual of Rosanna Hartline (2022), the Kirby-Bauer Test result gives a prediction that is accurate, in which the antibiotics are effective or not against the pathogen. The Kirby-Bauer is considered simple to perform and does not cost much; this is considered to be used commonly in medical practice. In the results, the antibiotic is reported as either sensitive, susceptible, intermediate, or resistant.

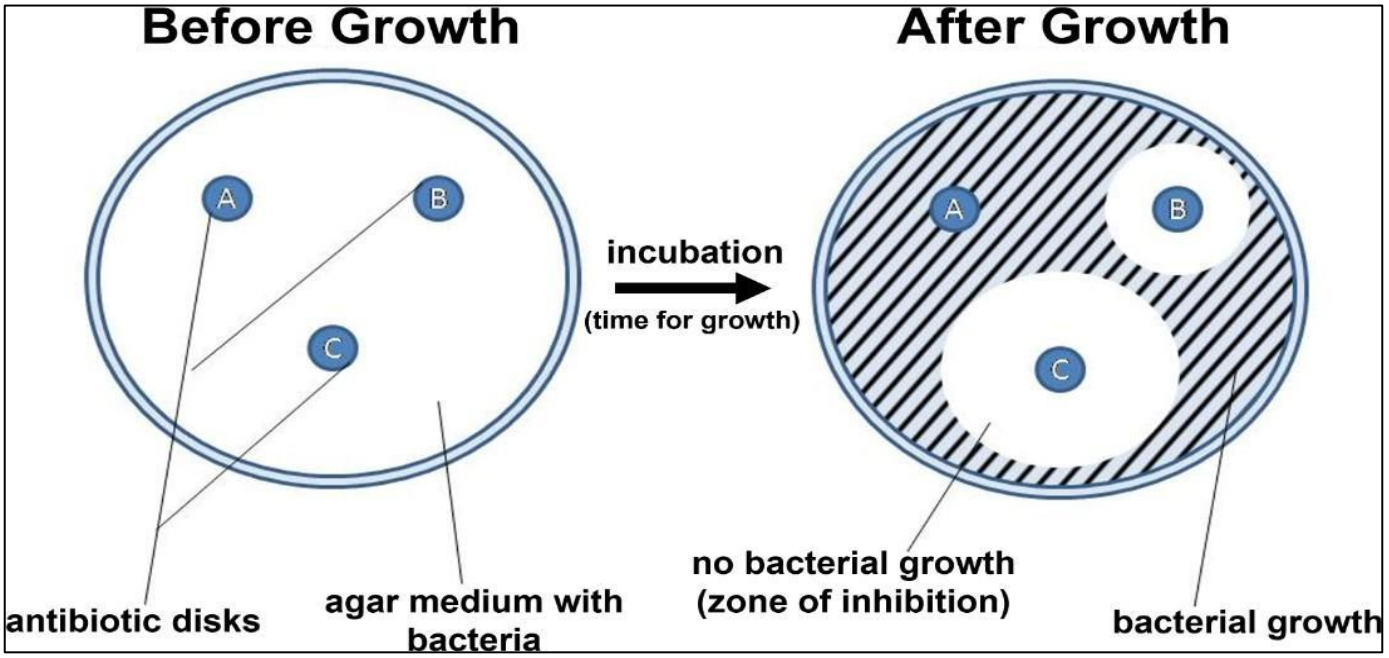

Fig 4: Kirby-Bauer Summary Diagram (Hartline 2022)

➢ *Positive Control*

In the experiment, it is the control group that is used for the testing that is recognized to produce results or to have understandable effects on the results (Spacey 2023).

- *Trimethoprim-Sulfamethoxazole (TMP-SMX Or Co-Trimoxazole)*

For this study, the researchers will be using Trimethoprim-Sulfamethoxazole, also known as co-trimoxazole as the positive control. It has abbreviations such as TMP-SMX, TMP-SMZ, TMP-SMX, or TMP-Sulfa. It is an antimicrobial agent for treating and preventing various infections caused by bacteria. It is affordable and useful to several illnesses, this agent is FDA-approved; its indication involves acute exacerbation of chronic bronchitis, otitis media for pediatric only, treatment for traveler's diarrhea, prophylaxis, Urinary Tract Infections, pneumocystis carinii pneumonia (one or both lungs are infected by a fungus), and toxoplasmosis. Non-FDA approved indications included prophylaxis in HIV-infected individuals, acne vulgaris, listeria, melioidosis, pertussis or whooping cough, *Staphylococcus aureus* infections, including methicillin-resistant *Staphylococcus aureus* (MRSA), etc. (Kemnic and Coleman 2022).

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According to the susceptibility test results of co-trimoxazole, 83% (24 of 29 bacteria) are found to be sensitive to cotrimoxazole, and 17% are resistant. The standard concentration of TMP-SMX antibiotic discs used in research laboratories is usually 1.25/23.75 µg (CLSI 2023).

Its mechanism of action involves sulfamethoxazole, which works directly on the synthesis of folate inside the microbial organisms by being antagonists of p-aminobenzoic acid (PABA) during the synthesis of dihydrofolate, the form that is converted to tetrahydrofolate, and trimethoprim as antagonists of the enzyme dihydrofolate reductase, which stops the production of tetrahydrofolate, the active form of folate and is responsible for synthesizing purines for DNA and protein production in bacteria. The combination of these antimicrobial agents results in a synergistic anti-folate effect (Kemnic and Coleman 2022).

The trimethoprim-sulfamethoxazole combination is effective against those bacteria that are gram-positive (including some strains of methicillin-resistant *S. aureus*) and a broad spectrum of gram-negative bacteria, protozoans such as the *Cystoisospora* and *Cyclospora* species and the fungus *Pneumocystis jirovecii* (Werth 2022). The combination TMP-SMX is also for the treatment of urinary tract infections caused by some bacteria, such as *E. coli, Klebsiella, Enterobacter, Morganella morganii, Proteus mirabilis,* or *P. vulgaris*. The drug is also being used for diseases that are caused by *Staphylococcus aureus,* including methicillin-resistant *S. aureus* [MRSA] (Sato et al. 2022).

- *Negative Control*

In an experiment, it is the control group that is used for the testing that is known to have no effect or predicted to have no effect on the results (Spacey 2023). This study will use distilled water as the negative control.

F. Nutrient Agar

Nutrient agar is a widely used microbiological growth medium; it contains important elements for the growth of bacteria, including carbohydrates, proteins, vitamins, and minerals, like *Escherichia coli*, that are commonly cultivated on nutrient agar for laboratory studies due to its capacity for adaptation and widely recognized properties. *Staphylococcus aureus* is also capable of growing on nutrient agar, according to Gandra et al. (2015). Although this medium may not be the most bacterially selective, it provides a rich supply of nutrients that support a variety of microorganisms, including *S. aureus*.

G. Antibacterial Primary Metabolites Against S. Aureus and E.coli

The third-largest family of higher plants is called *Fabaceae*. Fibers, vitamins (A, C, E, etc.), organic constituents (protein, oligosaccharides, carbohydrates, fats, oxalate, and phylococcus), and mineral constituents (calcium, magnesium, potassium, iron, zinc, and nitrogen) are all found in the Fabaceae family, according to the species. The primary components of the *Fabaceae* family are flavonoids. Several plant parts belonging to the *Fabaceae* family are responsible for treating a variety of illnesses in traditional medicine. Numerous characteristics of these plants have been clarified by in vitro and in vivo studies; there is also evidence of the medicinal effects of *Fabaceae* in various bodily systems. The oestrogenic, antibacterial, antioxidant, antifungal, antifeedant, and insecticidal properties of this plant family are present. Menorrhagia (during pregnancy), anemia, polymenorrhea, and ulcers are all treated using *fabaceae* (Gamo et al. 2015).

Senna alata (L.) belongs to the family of *Fabaceae.* The antimicrobial properties of *S. alata* leaves are demonstrated by the median zone of inhibition with 9.02mm (aqueous), 15.18mm (ethanol), and 9.02mm (pure extracts) against *Staphylococcus aureus*. Thus, it appears that *S. alata* leaves can be utilized in pharmaceuticals since they contain antibacterial properties with a minimum inhibitory concentration of 55% of the ethanolic extract (Avila 2022).

H. Synthesis

Falcata wood boosts the Philippine economy and raises the standard of living for its people by being a significant source of pulpwood and plywood (Alamsyah et al. 2018). Phytochemical analysis reveals compounds like tannins, flavonoids, alkaloids, saponins, and steroids, mostly present in bark, with potential health benefits, including glucose level reduction and antioxidant and anthelmintic effects (Rumidatul et al. 2021).

Trimethoprim-sulfamethoxazole (TMP-SMX or Co-trimoxazole) is being used for the treatment of infectious diseases that are caused by *Staphylococcus aureus,* including methicillin-resistant *S. aureus* [MRSA] (Sato et al. 2022).

Falcataria falcata is a member of *the Fabaceae* family, whose primary components are flavonoids, which are constituents having antibacterial properties against many kinds of infectious bacteria (Xie et al. 2015).

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CHAPTER THREE
MATERIALS AND METHODS

In this chapter, the research methodologies used in the study are presented, along with the details regarding the methods and procedures that are used in the preparation of the *Falcataria falcata* extract and the instruments used in obtaining the bacteria, and the proper procedures and guidelines to meet in order for the bacteria to be cultivated. Additionally, the statistical tools used to analyze the results of the data are also discussed.

A. Research Design

This study utilized an experimental research design to provide evidence concerning the antibacterial activity of the methanolic leaf extract of *Falcataria falcata*. The experiment used the methanolic extract of *F. falcata* plant material to test its effectiveness against *Staphylococcus aureus* and *Escherichia coli*. The extract is analyzed using the disc diffusion method, which involves placing a paper disc containing the extract in different concentrations on an agar plate inoculated with *S. aureus* and *E. coli* and measuring the zone of inhibition.

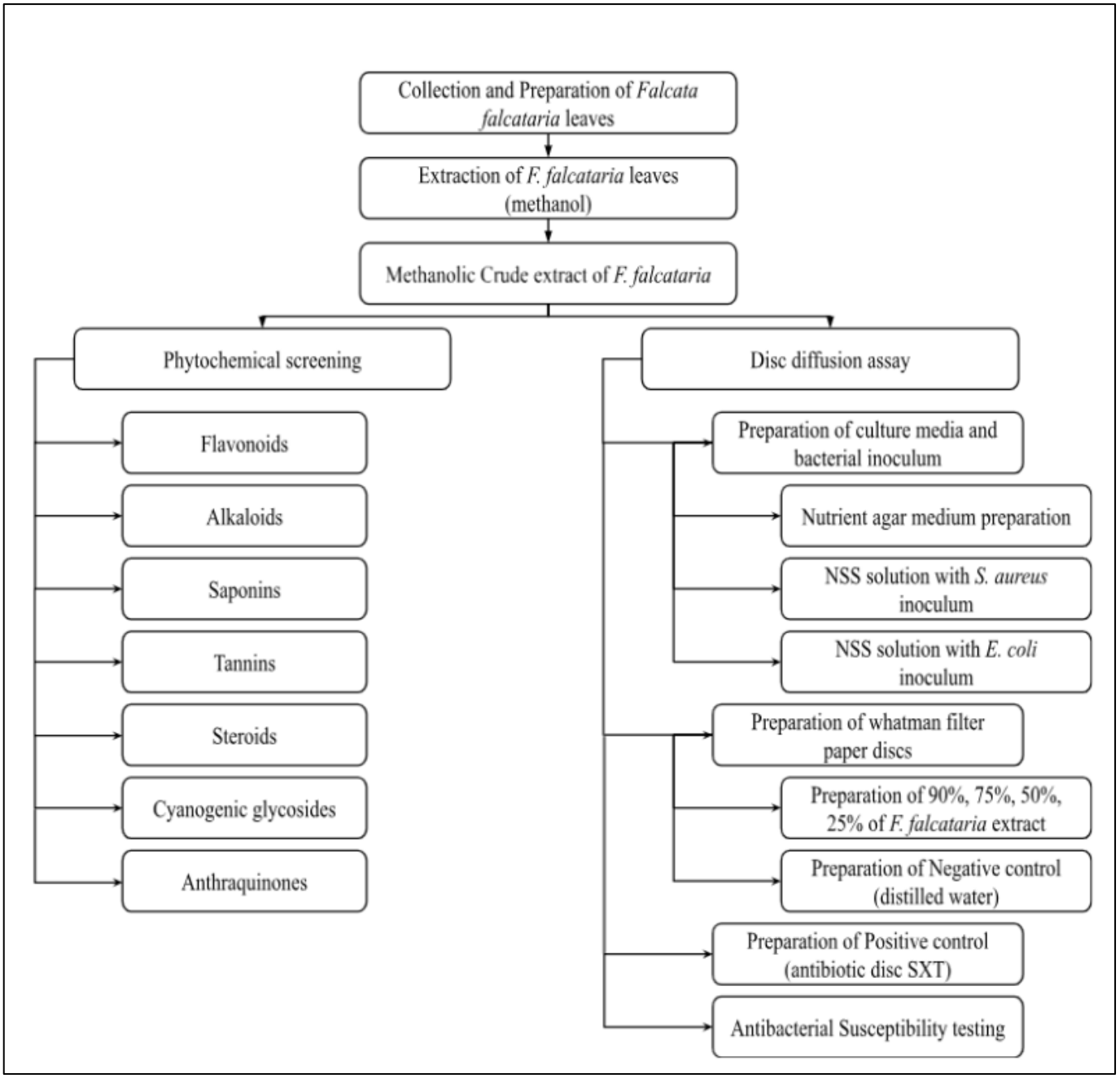

Fig 5: Flow of Research

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B. Research Setting

The plant material of *F. falcata* is harvested and gathered for further processing in Tingcob, Digkilaan, Iligan City, Lanao del Norte (8°16'25.5"N, 124°20'52.7"E). The plant samples are prepared and extracted in the Pharmacy Laboratory at Adventist Medical Center College (AMCC). The plant used in this study has undergone plant authentication, phytochemical analysis, and rotary evaporation processes at Biological Science Laboratory of Mindanao State University-Iligan Institute of Technology (MSU-IIT), Iligan City. The bacterial samples are collected at Central Mindanao University (CMU). To determine the antibacterial activity of the plant, a susceptibility test was conducted at the Medical Technology Laboratory located in AMCC. The researchers have requested the necessary chemicals for the tests and extraction from the school administrator.

C. Research Instruments

The instrument of the study employs multiple laboratory pieces of equipment, and some are also from the researcher's house. Firstly, the collection of plant material was done using a sack and chainsaws by the personnel hired by the researchers to collect the young leaves of F. falcata. The leaves were removed from their branches and placed in a net in a room away from direct sunlight at 22° C and a mat was placed beneath the net to prevent the leaves from dropping to the floor and to avoid contamination. For the preparation of the crude extract, a blender, sieve (No. 40 and No. 60), spatula, beakers, Hencik 2022 rotary evaporator, thermostatic water bath HH-2, and 3 laboratory percolators were used. For the culturing of the bacteria, the inoculating loop, alcohol lamp, sterile cotton applicator, whatman filter paper, nutrient agar (NA) powder, 9 cm glass petri plates, ruler, marker, micropipette tips 5-10 µL, Mini Incubator DSI-100D, Biobase autoclave BKM-Z18N, the Biobase Class II A2 biosafety cabinet, Asahi ES-801 single electric stove hot plate, Asuki TB-300 weighing scale, Wickerham card ZO8-76288-330, Top Pette FP-42096, rubber bands, and 4 Erlenmeyer flasks.

D. Data Gathering Procedure

- *Phase 1. Collection of the Plant Samples and Preparation of the Methanolic Crude Leaf Extract of Falcataria Falcata.*

The young leaves of the *F. falcata* plants were harvested from the area of Sitio Tingcob, Digkilaan (8° 1625.5 N, 124° 20' 52.7"E). The leaves are carefully inspected for any signs of damage or discoloration, ensuring that only the young leaves are collected. To prepare the leaves, they are first cleaned with tap water to remove any impurities. After cleaning, the leaves are gently patted dry with a clean cloth. Then the leaves were initially weighed for record keeping. The next step is to air-dry the leaves for 30 days by carefully sorting them and spreading them in a net inside a room away from direct sunlight at 22°C, ensuring that they dry evenly and retain their active compounds (Mediani et al. 2014, as cited by Azlan et al. 2023).

After 30 days, the dried leaves are weighed once again to determine moisture content (Shah 2019). The formula below is used to calculate the moisture content (Bogart 2018) and once the moisture content is taken, they are stored in a clean container to be brought to the laboratory for pulverization.

$$MC = \frac{wet\ weight - weight\ after\ drying}{wet\ weight} x100$$

The researchers have requested permission to use the Pharmacy Department Laboratory at AMCC to carry out the extraction method. The dried leaves that were collected were grinded and sieved through sieve No. 40 and then No. 60 to achieve fine powder particles, and the target weight of dried powder plant material for *F. falcata* is 1200 g (Shah 2019). Using an adapted percolation method, the powdered material is soaked with 98.5% methanol (Larson et al. 2016) in the percolator and collected after 48 hours. To get the right substrate-to-solvent ratio, a 1:10 ratio is used (Kumar et al. 2023).

The crude extract used the Hencik 2022 rotary evaporator and thermostatic water bath HH-2 to evaporate the methanol for the concentration of the sample preparation (Kumar et al. 2023).

The average percentage yield of the crude extract is determined by using the formula below (Panezai 2018).

$$\%\left(\frac{w}{w}\right) yield = \frac{Weight\ of\ the\ extract\ (g)}{Weight\ of\ the\ dried\ leaves\ (g)} x100$$

- *Phase 2. Phytochemical Analysis of the Falcataria Falcata Extract Revealed Secondary Metabolites that have Antibacterial Activities.*

To the best of the researcher's knowledge, there have been no previous studies conducted that included the phytochemical analysis of the leaf parts of the *F. falcata*. It was decided that the researchers would conduct a phytochemical analysis on the crude leaf extract. It was conducted at MSU-IIT Biological Science Laboratory. The most common phytochemical compounds, such as flavonoids, alkaloids, saponins, tannins, and steroids, have been known to show antibacterial properties in plants (Wintola and Afolayan 2015); thus, the conducted tests are decided around these and other minor phytochemical compounds that show

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antibacterial effects. Table 1 shows a summary of the tests for each of the aforementioned phytochemical compounds. These tests are provided alongside the phytochemical analysis.

Table 1: A summary of phytochemical analysis tests conducted (Sahira Banu and Catherine 2015).

Phytochemical Compounds	**Phytochemical Analysis Tests**
Flavonoid	Hydrochloric acid reduction
Alkaloid	Wagner's test
Saponins	Foam test or froth test method
Tannins	Ferric chloride test
Steroids	Salkowski test
Cyanogenic glycosides	Picrate solution test
Anthraquinones	Hexane test

For flavonoids, the crude extracts are transferred into an evaporating dish. It evaporated to almost dryness under a water bath. Cooled, then de-fat with hexane until the hexane washing becomes almost clear. The defatted extract is dissolved in 10 mL of 80% alcohol. It was then filtered into two equal parts. 0.5 mL of 12M HCl is added to the first part while the second part serves as a control. The two test tubes were placed in a hot water bath and the researchers observed the change in color. Red color development in the solution will indicate a positive outcome. Sometimes the development of the red color is very slow, so the researchers observed this reaction within two hours.

For alkaloids, the extracts are transferred in sufficient amounts into an evaporating dish. It evaporated to almost dryness under a boiling bath. When almost dry, the dish is removed from the bath and cooled. With stirring, the researchers add about 5-10 mL of 2M HCl and 0.5 grams of sodium chloride crystals into the dish. It is then placed under a boiling water bath for about five minutes. The researchers cooled the resulting solution and then it was filtered. The residue is washed on the filter paper with a few mL of 2M HCl. The combined filtrate is collected, washed, and divided into two equal parts. The first part was added to the 3-5 drops of Wagner's reagent, while the second part was left with nothing to serve as the control. A positive result was indicated by the formation of a brown precipitate with Wagner's reagent.

For saponins, the researchers added equal volume of water to about 2 mL of the extract in the test tube and they shook it for 30 seconds. The formation of froth, which is about 2 cm tall and lasts for 30 minutes, indicates a positive outcome for saponins.

For tannins, the researchers transferred enough of the crude extract into an evaporating dish. It is evaporated to almost dryness under a boiling water bath. It is cooled and then 20 mL of boiling water is added, followed by 2-3 drops of 10% NaCl solution. The researchers filtered the resulting solution and wash the residue with water if necessary. The combined filtrate was recovered, washed and divided into two equal parts. The first part was added 3-5 drops of 1% Ferric chloride while the second part will serve as a control. A positive result will be indicated by the formation of black or blue-black precipitate with ferric chloride test.

For steroids, the researchers transferred enough crude extract into an evaporating dish and evaporated it to almost dryness under a boiling water bath. The dried crude extract is then cooled and de-fat with hexane. Ferric chloride reagent (3–5 mL) should be added to the extract, then filtered. The filtrate is divided into two equal parts. One ml of concentrated sulfuric acid was slowly added through the wall of the test tube. The formation of brown (or sometimes blue or green) rings at the boundary region of the aqueous extract and sulfuric acid indicates the presence of 2-deoxy sugars.

For cyanogenic glycosides, it was placed into a 20 mL test tube about one mL of the extract and add 4-5 drops of chloroform. A picrate paper is suspended (a filter paper soaked with picrate solution) just above the solution (to ensure that the paper will not touch the wall of the test tube) and the researchers placed the test tube in a hot water bath. The tube is covered with a dropper placed in an inverted position. A positive result is indicated by the immediate formation of red color on the surface of the picrate paper.

For anthraquinones, enough crude extract is transferred into an evaporating dish and was evaporated to almost dry under a boiling water bath. It was cooled and defatted with hexane. 10 mL of distilled water was added to the dish, stir, and filter the resulting solution. The filtrate was extracted twice with 5 mL of benzene. The researchers let it stand for a few minutes to allow complete separation of the aqueous and benzene layers. The benzene layer using a transfer pipet was separated and was placed in a test tube containing one mL of ammonia reagent. The researchers shook it for a few seconds and observed the color in the aqueous layer. The development of reddish pink color in the aqueous layer of the solution indicates the presence of anthraquinones.

- *Phase 3. Preparation of the Stock Solution, Culture Medium, and Bacterial Inoculum.*

After getting the percentage yield calculated, the stock solution is diluted by adding sterile water inside the Biobase Class II A2 biosafety cabinet and by using a sterile 1 mL syringe. This is to maintain sterility of the solution. The amount of water to be added is computed through the calculation using the formula below (Ali et al. 2023; Lab CE 2024). The complete calculations are found in Appendix I.

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$$C1V1 = C2V2$$

The researchers did three replications of each concentration (90%, 75%, 50%, and 25%) of the falcata methanolic leaf crude extract and for the positive and negative control for both bacteria (*S. aureus* and *E. coli)* to get the average measure of the zone of inhibitions; hence, the study will used 12 agar plates. The researchers requested permission to use the Medical Technology Laboratory (AMCC) to cultivate *S. aureus* and *E. coli* bacteria and conducted the susceptibility testing. These bacteria wer acquired at CMU and assisted by a medical technology laboratory technician from the school in the storage and disposal of the bacteria.

In preparation of the agar, using the method from Lab Mal (2019) with slight adjustment, the researchers weighed 14 g of Nutrient Agar and mixed it with 500 mL of distilled water. It is boiled and the medium completely dissolved in the Asahi ES-801 single electric stove hot plate for 15 minutes or until a clear solution is achieved. The mixture was sterilized by autoclaving in Biobase autoclave BKM-Z18N at 121°C for 15 minutes. It was then transferred to the 12 sterilized petri dishes inside the Biobase Class +II A2 biosafety cabinet and store the Nutrient Agar plate in the refrigerator at 3°C.

The preparation of the inoculum bacterial suspension was adapted from Hardy Diagnostics (2023). The reseachers used the red cap microtube to get 1 mL of Normal Saline Solution (NSS) using a sterile disposable 1 mL syringe. The cap is then closed. The inoculating loop was reheated and was let rest for a moment. A small sample of bacteria from the stocks was scraped and was inserted inside the red cap microtube with NSS and swirled around in order to release the bacteria from the inoculating loop. The researchers heat the ends of the test tube, closed the cap, and compared the turbidity using the Wickerham card. Once satisfied, they label the test tube according to the bacteria it is housing, "*S. aureus*" and "*E. coli,"* for identification of the susceptibility testing.

- *Phase 4. Susceptibility Testing.*

Staphylococcus aureus ATCC 25923 and *Escherichia coli* ATCC 25922 were prepared by Central Mindanao University (CMU) on March 19, 2024. The researchers followed the maintenance of the bacteria's in accordance with the protocols provided by Missiakas and Schneewind (2018) for the *S. aureus* and Tuttle et al. (2021) for the *E. coli*.

Following the adapted procedure from Ado et al. (2022); Hardy Diagnostics (2023), after the sterilized cotton swab is prepared, the red cap microtube with the bacterial inoculum is opened, and the swab is soaked into its contents. The cotton swab is stirred inside and gently rubbed on the sides of the test tube to remove excess liquid. The mouth of the test tube is heated and covered.

The cotton swab is gently streaked onto the entire NA plate from left to right, top to bottom. The NA plate is then turned 90° and the streaking is repeated with the same cotton swab. The side of the agar plate is circled with the cotton swab to ensure that no area is left untouched. This is done to all 12 agar plates prepared, 6 plates for *S. aureus* and 6 plates for *E. coli*. The cotton swabs are wrapped in paper and disposed of properly afterward, and the Whatman No. 1 filter paper discs (5- millimeters in diameter) are prepared.

The disc diffusion method is adopted from Habib and Choudhry (2021); Avila (2022), with a slight modification. The Whatman filter paper discs and micropipette tips were sterilized first, and then the filter paper was soaked in a 5 µL volume of the 90%, 75%, 50%, and 25% plant extracts. All paper discs are put in a separate sterilized petri dish to dry. After drying, with the help of the sterile forceps, the filter papers are put onto the media. The control groups [distilled water and TMP-SMX antibiotic disc (1.25/23.75 µg disc)] are placed first to prevent contamination in later procedures. Next is the experimental group (falcata leaf extract in different concentrations) to be placed in the petri dish. This process will be applied to all 12 agar plates, a triplicate experiment to confirm the results, get the best possible results, and clear any uncertainty regarding the results, as shown in Figure 6 and placed inside the incubator for 24 hours before observation at 37°C.

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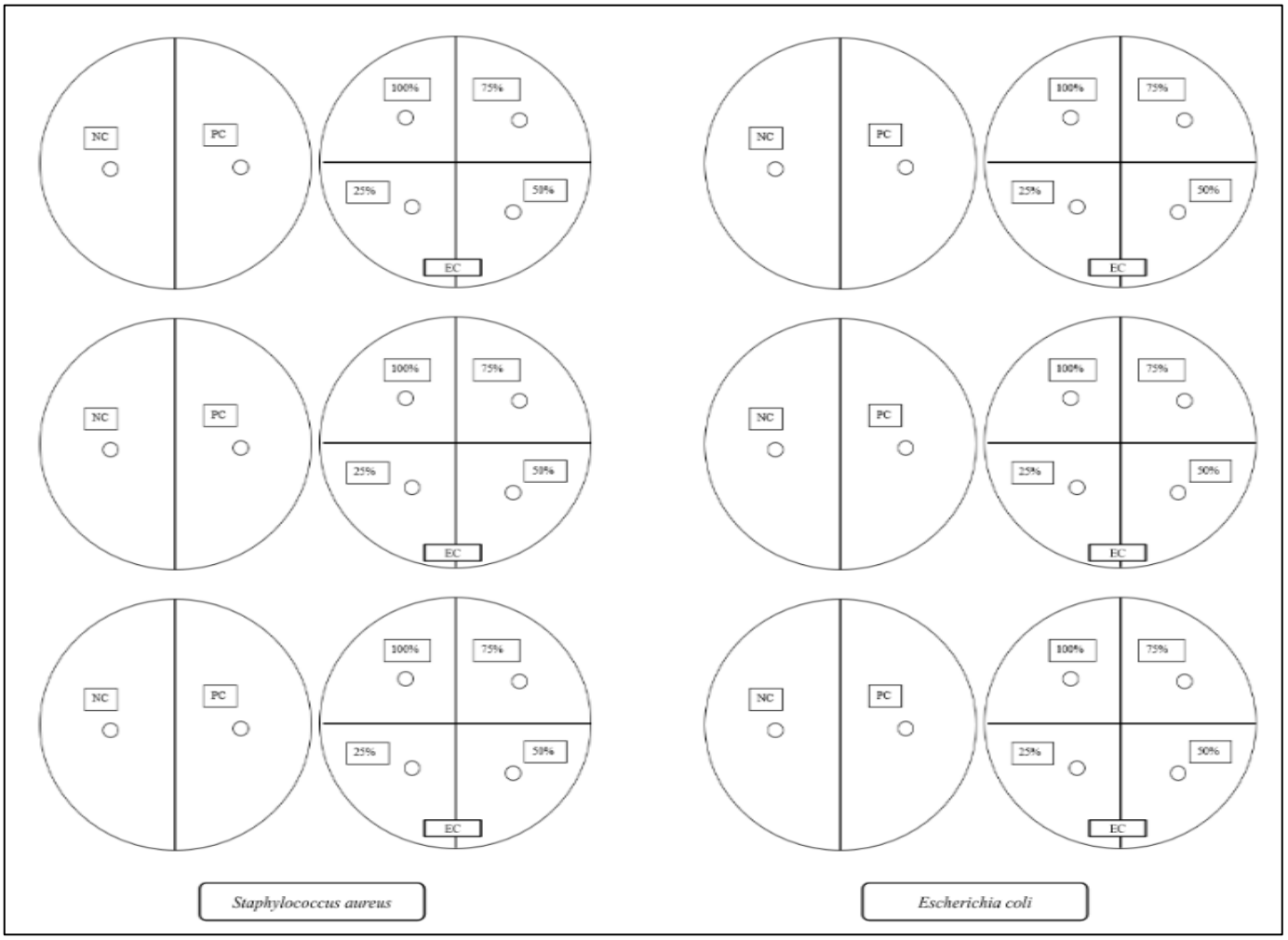

Fig 6: Adapted Experimental Layout of Discs (Ado et al. 2022).

➢ *Phase 5. Determination of the Zone of Inhibition.*

Following a 24-hour incubation period, measure the diameter of the zone of inhibition in millimeters with the use of a ruler (Libre Text Biology 2018). To perform this measurement, the endpoint of the ruler is placed precisely at the edge of the zone of inhibition area towards the other edge of the zone of inhibition to measure the diameter in centimeters and later on converted to millimeters. After gathering data, the researchers disposed of the materials, treatments, and bacteria properly according to the guidelines provided by the Adventist Medical Center College Medical Technologist Laboratory.

The zone of inhibition (in millimeters) was compared to the data obtained from the zone of inhibition in the disc diffusion method or Kirby-Bauer method (Libre Text Biology 2018). The CLSI standards in Table 2 provide the interpretative standards for determining whether the experimental extract is susceptible, intermediate, or resistant to the tested bacterial strains (Humphries et al. 2018).

Below is a summary of the Minimum Inhibitory Concentration (MIC) that inhibits *Staphylococcus aureus* according to the Clinical and Laboratory Standards Institutes (CLSI) with Trimethoprim-Sulfamethoxazole 1.25/23.75 µg (CLSI 2023).

Table 2: Zone of Inhibition Interpretive Standards for *Staphylococcus Aureus* and *Escherichia coli*. (CLSI 2023)

Bacteria	**MIC (µg/mL)**		
	Resistant	**Intermediate**	**Susceptible**
Staphylococcus aureus	≤ 10	11 to 15	≥ 16
Escherichia coli	≤ 10	11 to 15	≥ 16

E. Statistical Treatments

This study's data will be graphed and analyzed using quantitative research software. R programming and Microsoft Excel were used for software in the analysis of the data that are used for deeper inferential analysis of variance to test the significant differences between the four concentrations (90%, 75%, 50%, and 25%) of methanolic leaf crude extract and the control groups (Trimethoprim-sulfamethoxazole) against *Staphylococcus aureus* and *Escherichia coli* bacteria.

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- *Inferential Statistics*

It is described by using a random sample of data taken from a population to describe and make inferences about the population. Inferential statistics were utilized in this study using the Analysis of Variance.

- *Analysis of Variance*

A type of statistical method that separates observed variance data into different components to use for additional tests. A one-way ANOVA is used for three or more groups of data, to gain information about the relationship between the dependent and independent variables. The formula for ANOVA is:

$$F = \frac{MST}{MSE}$$

F = ANOVA coefficient
MST = mean sum of squares due to treatment
MSE = mean sum of squares due to error.

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CHAPTER FOUR
RESULTS AND DISCUSSION

This chapter discussed the result of the percentage yield, phytochemical analysis, and comparison of the experimental group of four concentrations (90%, 75%, 50%, and 25%) of methanolic leaf crude extract with the control groups (Trimethoprim-sulfamethoxazole) against S*taphylococcus aureus* and *Escherichia coli* bacteria. The researchers used the Analysis of Variance as statistical tool.

A. Preparation of the Plant Extract

The crude methanolic leaf extract of *Falcataria falcata* is dark green, semi-solid in mass, and has a strong tea odor (Figure 7).

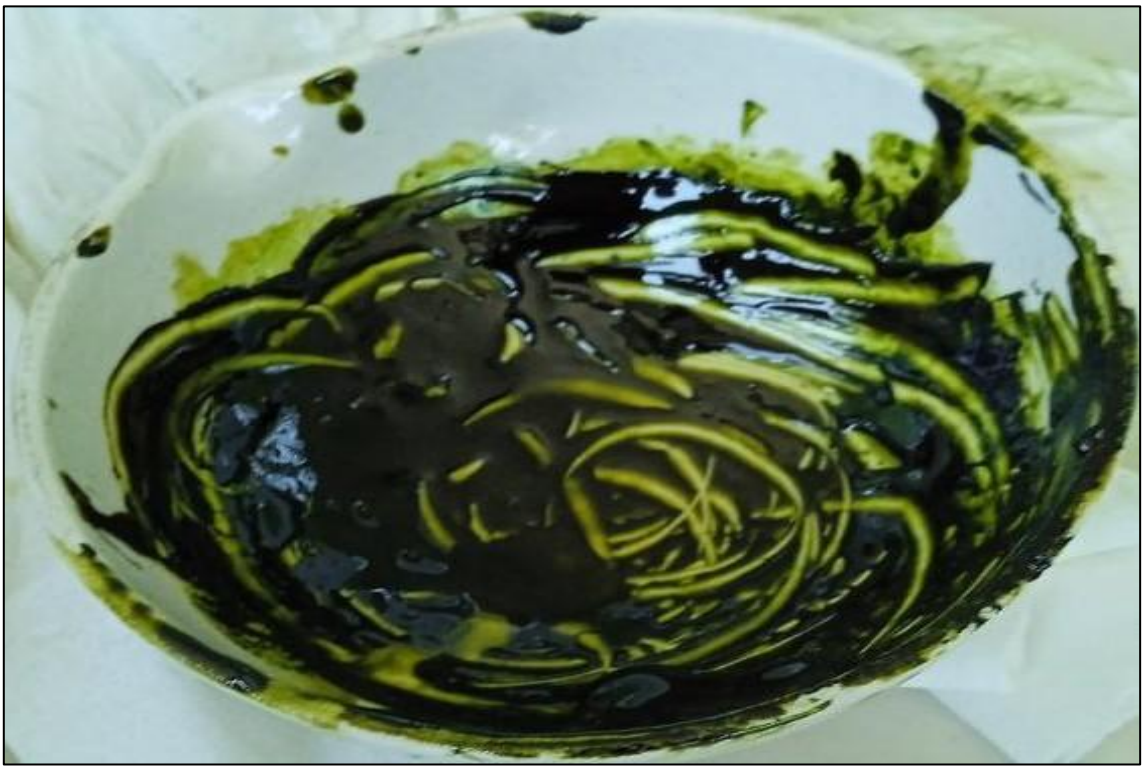

Fig 7: Crude Methanolic Extract of *Falcataria falcate*

The average percentage yield based on the three replicants of 10 grams of dried powdered leaves of *Falcataria falcataria* is 2.67%. Table 3 shows the individual yield obtained from each replicant.

Table 3: The percentage yield of the methanolic crude leaf extract of *Falcataria falcata* (Ali et al. 2023).

Replicant	Weight of dried leaves	Weight of crude extract	Percentage yield
1	10 g	0.1 g	1 %
2	10 g	0.2 g	2 %
3	10 g	0.5 g	5 %
		Mean Value	2.67 %

B. Phytochemical Analysis of Falcataria Falcata Methanolic Leaf Extract of the Secondary Metabolites

The phytochemical screening of secondary metabolites of the methanolic leaf crude extract of *Falcataria falcata* followed the procedure and tests are listed in Table 1 to determine the possible phytochemical compounds such as flavonoids, alkaloids, saponins, tannins, and steroids (Wintola and Afolayan 2015). Table 4 shows the results of the tests conducted.

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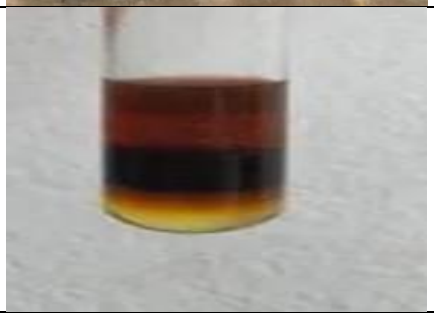
Table 4: Phytochemical Profile of the Methanolic Leaf Extract of *Falcataria falcata*.

TESTS	VISIBLE RESULTS		IMPLICATION
Hydrochloric acid reduction		Red color of solution	Positive of Flavonoids
Wagner's test		Brown precipitate	Positive of Alkaloids
Foam test or froth test method		Formation of froth (2 cm in height) for 30 minutes	Positive of Saponins
Ferric chloride test		Black or blue-black color of precipitate	Positive of Tannins
Salkowski test		Brown (or sometimes blue or green) rings	Positive of Steroids

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Picrate solution test		Immediate formation of red color	Negative of Cyanogenic glycosides
Hexane test		Reddish pink color	Negative of Anthraquinones

To determine the presence of flavonoids, the hydrochloric acid reduction is performed, and the red coloration of the solutions shows that there is a presence of flavonoids. The formation of brown precipitate during Wagner's test indicates the presence of alkaloids. During the foam test, the formation of foam lasted more than 30 minutes and was over 2 cm in height, thus indicating the presence of saponins. In the Ferric chloride test, the development of black precipitate indicated the presence of tannins, and in the Salkowski test, the brown-rings indicated the presence of steroids.

The phytochemical analysis of the methanolic crude extract of *Falcataria falcata* shown in Table 4 presents the outcome of the tests and reveals that the crude extract has the presence of flavonoids, alkaloids, saponins, tannins, and steroids, which may account for the antibacterial activity observed against *S. aureus* and *E. coli*.

Previous studies that have conducted phytochemical screening in the Falcata tree show that its wood has the presence of simple phenols, lignans, flavonoids, and tannins (Louis et al. 2023); its bark has the presence of tannins, flavonoids, alkaloids, saponins, steroids, and triterpenoids (Baihaqi et al. 2020; Arini et al. 2019); and its twigs have the presence of flavonoids, phenolics, steroids, terpenoids, tannins, and saponins (Rumidatul et al. 2021).

C. Antibacterial activity through Disc Diffusion Method

The antibacterial activity of *Falcataria falcata* was determined by using the disc diffusion method. Table 5 shows the results of the six-day experiment, which was evaluated against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

Table 5: Results of the ZOI of the Experimental, Positive, and Control Groups

Groups	**Mean Inhibition Zone (mm)**	
	S. aureus	***E. coli***
Trimethoprim-sulfamethoxazole	22	0
Distilled water	0	0
90% methanolic leaf extract	0	2.33
75% methanolic leaf extract	11.33	5
50% methanolic leaf extract	7 .33	0
25% methanolic leaf extract	6	2.67

After 24 hours of implanting the experimental, positive, and control groups in the agar, the zone of inhibition was measured. Table 5 shows the results of the zones of inhibition of the groups.

The positive control (Trimethoprim-sulfamethoxazole), produced an average inhibition of 22 mm for the inhibition of S. aureus and 0 mm for the inhibition of *E. coli*. According to the CLSI standards, TMP-SXT has been found to have a susceptible effect against S. aureus, while resistant to *E. coli*. The highest concentration in the experimental group that has inhibition to *S. aureus* is 75% with an average inhibition of 11.33 mm, which, according to the CLSI standards, has an intermediate effect against *S. aureus*. The highest concentration in the experimental group that inhibits *E. coli* is 75% with an average of 5 mm, which, according to the CLSI standards, has a resistant effect against *E. coli*.

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The researchers used the Analysis of Variance to test if there was a significant difference in experimental group, positive group and control group towards the zone of inhibition of positive, control and experimental group for *Staphylococcus aureus* with 0.05 level of significance. The researchers provide the test:

- Ho: There is no significant difference in overall result of experimental group, positive group, and control group towards the zone of inhibition.
- Ha: There is a significant difference in overall result of experimental group, positive group, and control group towards the zone of inhibition

Table 6: *Staphylococcus aureus* Overall Result in Comparing Experimental, Positive, and Control vs. Zone of Inhibition.

Analysis of Variance	**Zone of Inhibition**	
	F-value	**P-value**
Distilled Water	n/a	n/a
Trimethoprim-sulfamethoxazole	1200	0.0001**
Experimental (90%, 75%, 50%, 25% Extract of Falcata)	5.417677	0.013719*

Note: Significant if p-value < 0.05* and p-value < 0.01**

Table 6 shows the results of the comparison of *Staphylococcus aureus* among the Experimental, Positive, Control groups, and the Zone of Inhibition. The table presents an ANOVA analysis performed to determine if there were significant differences in the zone of inhibition between different groups: the Experimental group, positive group (Trimethoprim-sulfamethoxazole), and the Control group. A significance level of 0.05 was used to test the null hypothesis. This threshold is a common benchmark for determining whether a result is statistically significant.

The ANOVA results indicated a rejection of the null hypothesis for the Positive group (Trimethoprim-sulfamethoxazole) and the Experimental group (with different concentrations of 90%, 75%, 50%, and 25% of Falcata extract), which have a p-value of 0.013719. This suggests that these groups had a significant effect on the zone of inhibition, as the p-values were below the 0.05 threshold. This implies that these substances (Trimethoprim-sulfamethoxazole and varying concentrations of methanolic leaf crude extract from Falcata) effectively inhibited the growth of *Staphylococcus aureus.*

Additionally, the results showed that distilled water had no significant effect on the zone of inhibition. Since both raw data values were zero, there was no observable variation or effect, which is why "n/a" (not applicable) was indicated in the results.

Therefore, the ANOVA results provide evidence that both the positive (Trimethoprim-sulfamethoxazole) and the experimental group with different concentrations of Falcata extract had a significant effect on the zone of inhibition, demonstrating effectiveness against *Staphylococcus aureus*. Distilled water, however, showed no impact on inhibiting the bacterial growth, confirming its use as a control group with no expected effect.

The study of Kim (2017) provided further evidence in favor of the ANOVA analysis's rejection of the null hypothesis. As stated in the study, when each group is coupled with another to try three paired comparisons, the rise in Type I error becomes a regular occurrence in the comparison of the means of three mutually independent groups that fulfill the normality and equal variance assumptions. Put another way, even if the null hypothesis is correct, there is a greater chance that it will be rejected, which raises the likelihood that the alternative hypothesis—the research hypothesis—will be accepted even if it is not significant. Therefore, the null hypothesis may be rejected if there is a difference between any two of the three groups' means, as shown on Table 6 ANOVA results.

The researchers used the Analysis of Variance to test the significant difference in each concentration in experimental group (90%, 75%, 50%, 25% Extract of Falcata), control group (Distilled Water) and positive group (Trimethoprim-sulfamethoxazole) towards the zone of inhibition of each concentration in experimental group (90%, 75%, 50%, 25% Extract of Falcata) for *Staphylococcus aureus* with 0.05 level of significance. We provide the test:

- Ho: There are no significant differences between 90%, 75%, 50%, 25% methanolic crude leaf extracts of *Falcataria falcata*, against the zone of inhibition of each concentration in the experimental group and the positive control (Trimethoprim-sulfamethoxazole).
- Ha: There is a significant difference between 90%, 75%, 50%, 25% methanolic crude leaf extracts of *Falcataria falcata*, against the zone of inhibition of each concentration in the experimental group and the positive control (Trimethoprim-sulfamethoxazole).

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Table 7: Result of *Staphylococcus aureus* Comparing Each Experimental 90%, 75%, 50%, 25% Extract of Falcata, Distilled Water, Trimethoprim-Sulfamethoxazole vs. ZOI of 90% 75%, 50%, 25% Extract of Falcata and Positive Control

Analysis of Variance	***Staphylococcus aureus***	
	F-value	**P-value**
90 % Extract of Falcata in Zone of Inhibition	8.44	0.007344**
75 % Extract of Falcata in Zone of Inhibition	186.3231	0.0001**
50 % Extract of Falcata in Zone of Inhibition	73.9233	0.0001**
25 % Extract of Falcata in Zone of Inhibition	49.636	0.0001**

Note: Significant if p-value < 0.05* and p-value < 0.01**

The study used an Analysis of Variance (ANOVA) test to determine if there were statistically significant differences among the 90%, 75%, 50%, and 25% concentrations of the methanolic leaf extract of *Falcataria falcata* in terms of their effect on the zone of inhibition against *Staphylococcus aureus*. The positive control used in this comparison was Trimethoprim-sulfamethoxazole. The test was conducted at a 0.05 significance level, which is the typical threshold for determining whether a result is statistically significant. The null hypothesis would be retained if the test statistic (area under the curve) was greater than or equal to the predetermined significance level.

Table 7 shows that there is a significant difference in the zone of inhibition among all four concentrations of the experimental group (90%, 75%, 50%, 25% of the Falcata extract) which has a p-value of 0.007344 for the 90% and the remaining concentration has a p-value of 0.0001 when compared to zone of inhibition of experimental group and the positive control (Trimethoprim-sulfamethoxazole). The p-values for these comparisons were less than 0.05, indicating statistical significance. The rejection of the null hypothesis suggests that each concentration of the experimental group had a significant impact on the zone of inhibition against *Staphylococcus aureus*, similar to the positive control. The significance level of 0.05 indicates a 95% confidence that the results were not due to chance, while the reported 99% level of significance provides even greater confidence in these findings.

The results suggest that the methanolic leaf extract of *Falcataria falcata* at different concentrations (90%, 75%, 50%, 25%) has a significant effect on the zone of inhibition against *Staphylococcus aureus*, similar to or even greater than the positive control, Trimethoprim-sulfamethoxazole. These findings imply that the Falcata extract has potential as an effective agent for inhibiting the growth of *Staphylococcus aureus*. Therefore, the analysis demonstrates the potential efficacy of the methanolic leaf extract of *Falcataria falcata* as an antimicrobial agent, with all tested concentrations showing significant effects against *Staphylococcus aureus* when compared to a positive control.

The researchers used the Analysis of Variance to test if there was a significant difference in experimental group, positive group and control group towards the zone of inhibition of positive, control and experimental group for *Escherichia coli* with 0.05 level of significance. The researchers provide the test:

- Ho: There is no significant difference in overall result of experimental group, positive group, control group towards the zone of inhibition
- Ha: There is a significant difference in overall result of experimental group, positive group, control group towards the zone of inhibition.

Table 8: *Escherichia coli* Overall Result in Comparing Experimental, Positive, Control vs. Zone of Inhibition.

Analysis of Variance	**Zone of Inhibition**	
	F-value	**P-value**
Distilled Water	n/a	n/a
Trimethoprim-Sulfamethoxazole	n/a	n/a
Experimental (90%, 75%, 50%, 25% Extract of Falcata)	4.384	0.02655*

Note: Significant if p-value < 0.05* and p-value < 0.01**

The Analysis of Variance (ANOVA) was used to determine whether there were statistically significant differences in the zone of inhibition among the experimental group, positive control, and control group when using different treatments on *Escherichia coli*. The significance level was set at 0.05. The experimental group consisted of four concentrations (90%, 75%, 50%, 25%) of methanolic leaf crude extract of *Falcataria falcata*. The control group used distilled water, while the positive control group used Trimethoprim-Sulfamethoxazole.

The results showed a significant difference in the zone of inhibition for the experimental group which had the p-value of 0.02655, indicating that the four concentrations of the methanolic leaf extract had a significant effect on *Escherichia coli*. The p-value was less than 0.05, leading to the rejection of the null hypothesis with a 99% confidence level. The positive control group (Trimethoprim-sulfamethoxazole) and the control group (distilled water) had no significant effect on the zone of inhibition, as both groups had zero values, suggesting no antimicrobial activity against *Escherichia coli*.

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The results indicate that the methanolic leaf extract of *Falcataria falcata*, at various concentrations, has a significant impact on inhibiting the growth of *Escherichia coli*. The rejection of the null hypothesis at a 99% confidence level suggests that this effect is not due to chance. The lack of significant effect from the positive control and control group suggests that neither Trimethoprim-Sulfamethoxazole nor distilled water has an observable impact on the zone of inhibition in this context, reinforcing the effectiveness of the experimental treatments.

Therefore, the analysis suggests that the different concentrations of the methanolic leaf crude extract of *Falcataria falcata* have a significant antimicrobial effect on *Escherichia coli*, while the control group (distilled water) and positive control group (Trimethoprim-Sulfamethoxazole) did not show any significant impact on the zone of inhibition.

The outcomes are further supported by Braga et al. (2007) as cited by Batubara et al. (2020), who emphasizes that natural variation and adaptation of plant extracts can cause variations in their composition based on plant genotype, environmental conditions, and extraction methods. Additionally, a particular combination of bioactive compounds in the methanolic extract of *Falcataria falcata* may confer enhanced antibacterial activity against *Escherichia coli* when compared to the positive control group (trimethoprim-sulfamethoxazole) and the control group on the study used by Cowan (1999), as cited by Vaou et al. (2021). The methanolic crude leaf extract of *Falcataria falcata* contains bioactive compounds that have the potential to target multiple pathways involved in bacterial growth and survival. This approach is multifaceted and may lead to a broader and more effective inhibition of bacterial growth in comparison to distilled water (Rios and Rico 2005 as cited by Diniz et al. 2023).

The positive control (Trimethoprim-sulfamethoxazole) in the study showed that it had no significant effect on the zone of inhibition on the bacteria, which is why it was indicated as “n/a” in the results. The antibiotic resistance of the bacteria can be explained by the origin of the strain of *E. coli* which was taken from an animal. A study published in Current Urology examined the *E. coli* isolates and found that about 32.4% of the isolates were resistant to TMP-SXT (Daoud et al. 2020). The study of Jeżak and Kozajda (2022) also states that among the zoonotic bacteria on animal farms environment are: *Staphylococcus* spp.; *Salmonella* spp.; *Campylobacter* spp.; *E. coli*; *Listeria* spp.; *Enterococcus* spp. These bacteria are the most common cause of infections in humans and second constitute a source for the animals that later on can develop antibiotic resistance genes (ARG) due to the excessive use of antibiotics in veterinary.

The researchers used the Analysis of Variance to test the significant difference in each concentration in experimental group (90%, 75%, 50%, 25% Extract of Falcata), control group (Distilled Water) and positive group (Trimethoprim-Sulfamethoxazole) towards the zone of inhibition of each concentration in experimental group (90%, 75%, 50%, 25% Extract of Falcata) for *Escherichia coli* with 0.05 level of significance. The researchers provide the test:

- Ho: There are no significant differences between 90%, 75%, 50%, 25% methanolic leaf extract of *Falcataria falcata*, against zone of inhibition of each concentration in experimental group and positive control (Trimethoprim-sulfamethoxazole).
- Ha: There is a significant difference between 90%, 75%, 50%, 25% methanolic leaf extract of *Falcataria falcata*, against zone of inhibition of each concentration in experimental group and positive control (Trimethoprim-sulfamethoxazole).

Table 9: Result of *Escherichia coli* Comparing each Experimental 90%, 75%, 50%, 25% Extract of Falcata, Distilled Water, Trimethoprim-Sulfamethoxazole vs. ZOI of 90%, 75%, 50%, 25% Extract of Falcata

Analysis of Variance	***Escherichia coli***	
	F-value	**P-value**
90 % Extract of Falcata in Zone of Inhibition	94.057	0.0001**
75 % Extract of Falcata in Zone of Inhibition	94.333	0.0001**
50 % Extract of Falcata in Zone of Inhibition	n/a	n/a
25 % Extract of Falcata in Zone of Inhibition	93.85	0.0001**

Note: Significant if p-value < 0.05* and p-value < 0.01**

The Analysis of Variance (ANOVA) was used to determine whether there were statistically significant differences in the zone of inhibition for *Escherichia coli* among different concentrations of methanolic leaf extract (90%, 75%, 50%, 25%), compared with the positive control (Trimethoprim-Sulfamethoxazole) and the control group (distilled water). The significance level for this analysis was set at 0.05.

The results indicated a statistically significant difference for the 90%, 75%, and 25% concentrations of methanolic leaf extract of *Falcataria falcata* which had the p-value of 0.0001 towards the zone of inhibition. The p-values were less than 0.01, leading to a rejection of the null hypothesis with a 99% level of confidence. This suggests that these concentrations had a significant antimicrobial effect on *Escherichia coli*. The 50% concentration did not show a significant impact on the zone of inhibition, as the data had zero values, indicating no observable effect or variation.

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The significant effect observed in the 90%, 75%, and 25% concentrations suggests that these levels of methanolic leaf extract are effective in inhibiting the growth of *Escherichia coli.* The lack of significance for the 50% concentration implies that this level of the extract does not have a notable antimicrobial impact on *Escherichia coli*. The consistent results with a 99% confidence level underscore the robustness of the analysis, indicating that the observed effects are not due to random variation but rather a genuine difference in antimicrobial activity.

Therefore, the findings suggest that the higher concentrations (90%, 75%, 25%) of the methanolic leaf extract of *Falcataria falcata* can significantly impact the zone of inhibition for *Escherichia coli,* while the 50% concentration shows no such effect. This could have implications for the use of these extracts in antimicrobial applications, especially at concentrations above 50%.

The effectiveness of the methanolic falcata extract can be supported by the phytochemical screening performed in this study, as shown by the result in Table 9. It presents that the flavonoids test has a positive presence, knowing that Shamsudin et al. (2022) state that flavonoids are known to have antibacterial effects, inhibiting the growth of microorganisms. This concludes that the significance of the experimental groups does exist and it can be claimed that this methanolic falcata has antibacterial effects. The absence of effect from the 50% concentration can be supported by the claims of Donayre (2020), who states that lower concentrations may exert more effect than higher concentrations of plants because of the crude extracts' bioactive composition.

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CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

This study was conducted to identify the phytochemical compounds present in the methanolic leaf crude extract of *Falcataria falcata* and to determine the potential antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*.

A. *Summary of Findings*

- The percentage yield of methanolic leaf crude extract of *Falcataria falcate* based on the three replicants of 10 grams of dried powdered leaves is 2.67%.
- The phytochemical compounds of methanolic crude leaf extract of *Falcataria falcata* were flavonoids, tannins, saponins, alkaloids, and steroids.
- The zone of inhibition (ZOI) against *Staphylococcus aureus* and *Escherichia coli* bacteria differs significantly between the 90%, 75%, 50%, and 25% concentrations of the methanolic crude leaf extract of *Falcataria falcata*, the positive group (Trimethoprim-sulfamethoxazole), and the negative group (distilled water), with the statistical values: *S. aureus* (90% - 0.007344, 75% - 0.0001, 50% - 0.0001, 25% - 0.0001). Its overall results show that there is a significant difference of 0.013719 for the experimental group when compared to TMP-SMX - 0.0001 and distilled water - n/a. It indicates that there is an effectiveness based on zone of inhibition of the *S. aureus* bacteria; *E. coli* (90% - 0.0001, 75% - 0.0001, 50% - n/a, 25% - 0.0001). Its overall results show that there is a significant different of 0.02655 for the experimental group when compared to TMP-SMX - n/a, distilled water - n/a. It indicates that the 90%, 75% and 25% have a significant impact on inhibiting the growth of *E. coli*.
- The antibacterial resistance, susceptibility, or intermediate activity of the methanolic crude leaf extracts of *Falcataria falcata* against *Staphylococcus aureus* and *Escherichia coli* is as follows: *S. aureus* (90% - resistant, 75% - intermediate, 50% - resistant, 25% - resistant); *E. coli* (90% - resistant, 75% - resistant, 50% - resistant, 25% - resistant)

B. *Conclusions*

The current study's findings demonstrate the antibacterial activity of *Falcataria falcata* methanolic leaf crude extract. The extract has been found to have antibacterial activity, which is shown in the varying degrees of antibacterial action on *Escherichia coli* and *Staphylococcus aureus*. The researchers can then conclude to reject the null hypothesis. The presence of secondary metabolites such as flavonoids, tannins, saponins, alkaloids, and steroids may be responsible for its activity. Ultimately, this investigation has shown that *Falcataria falcata* has exceptional antibacterial activity at a 75% concentration that effectively limits the growth of bacteria, especially *Staphylococcus aureus*. This discovery offers scientific evidence for the inclusion of *Falcataria falcata* in the list of antibacterial medicinal plants, which may serve as a point of reference for future drug development.

C. *Recommendations*

➢ *Based on the Result of the Study, the Following Recommendations are Hereby Forwarded:*

- Since there are only few studies of leaf extracts of *Falcataria falcata*, it is suggested that further study on its other possible potential health applications due to its presence of flavonoids and steroids. The plant may show anti-inflammatory properties, saponins and tannins may also show anthelmintic properties; and some of the actions of alkaloids are known for their analgesic effects or any other related studies.
- Further study on the mechanisms of action of *Falcataria falcata* to determine how the plant interacts with bacteria by conducting the Minimum Inhibitory Concentration or Bactericidal vs Bacteriostatic Assay must also be conducted.
- Synergistic studies are also a possibility to determine other plants that might enhance the antibacterial efficacy of *Falcataria falcata*.
- Conduct solubility testing in various solvents to identify suitable solvents for other formulations. Compatibility testing should also be conducted for the selection of excipients to analyze the physical or chemical changes. This should be followed by preparation of formulations and evaluation of formulations such as appearance and color, pH level to ensure the soap is skin friendly, free alkali content and total fatty matter, lathering ability, cleaning efficiency, skin feel, shelf-life stability assay of API, and dissolution testing. Tests such as toxicity testing, skin irritation testing, and ocular irritation testing should be conducted for viable soap formulations.
- Due to the presence of saponins, it is recommended that future researchers will formulate a soap formulation that may contain *Falcataria falcata* extracts.
- Conducted studies for oral toxicity to determine the pharmacokinetics of the plant extract of *Falcataria falcata*. Tests such as subacute toxicity testing to determine the no observed adverse effect level and chronic testing can be conducted to assess long-term toxicity and potential cumulative effects of the extract.
- Isolation testing is to be conducted in order to identify the specific compounds present based on the phytochemical screening in the extract. Partitioning or liquid-liquid extraction can be conducted to analyze the presence of these compounds. Column chromatography and thin layer chromatography can also be conducted to identify the specific compounds to be tested.

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APPENDIX A

Certificate of Authentication

DEPARTMENT OF BIOLOGICAL SCIENCES
COLLEGE OF SCIENCE AND MATHEMATICS

CERTIFICATE OF IDENTIFICATION AND AUTHENTICATION OF BOTANICAL SPECIMENS

This is to certify that the BOTANICAL specimen/s herein listed and presented by the persons/s herein noted were identified and authenticated by this office.

Name: **Laliefe B. Arnan**
Katherine Joy S. Escueta
Nor Rashida P. Rashid
Elyca M. Tachobo
Queenie Rose M. Tinoy

School/Institution: **Adventist Medical Center College-Iligan City**

Family	Scientific Name	Local/Common Name
Fabaceae	*Falcataria falcata* (L.) Greuter & R.Rankin	Moluccan albizia (Eng), Falcata (Tag)

Collected from: Sitio Tingcob, Digkilaan, Iligan City
Date of Collection: 13 December 2023

Muhmin Michael E. Manting, MSc.
Faculty, Department of Biological Sciences
MSU-Iligan Institute of Technology

Influencing the Future

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APPENDIX B

Results of Phytochemical Analysis

MSU- Iligan Institute of Technology
College of Science and Mathematics
Department of Chemistry
Andres Bonifacio Avenue, Iligan City 9200

RESULTS OF ANALYSIS

Name of Student: Arnan, Laliefe, Tacbobo, Elyca, Rashid, Nor Rashida, and Co.
Name of School: Adventist Medical Center College
Name of Analysis: Phytochemical Screening
Sample Tested: Crude extract of Falcataria Falcata Leaves
Date Analyzed: February 22, 2024

Sample Code	Alkaloids	Anthra-quinones	Cyanogenic-Glycosides	Flavonoids	Saponins	Steroids	Tannins
Falcata leaves	++	-	-	+++ (very rich)	+++	+++	+++

Remarks:
Legend:
(-) – presence is below detection
(+) – presence is in small or in trace amount
(++) – presence is moderate amount
(+++) – presence is in large amount

Analyzed by:

ENJELYN C. GOMEZ, RCh.
PRC Reg. No. 0007319

Noted:

for:
Dr. CHARLIE A. LAVILLA JR.
Chairman, Chemistry Department

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APPENDIX C

Certificate of Analysis

Liofilchem®

DICHIARAZIONE DI CONFORMITÀ CE

La società Liofilchem® S.r.l., con Sede Legale in Via Scozia, 64026 Roseto degli Abruzzi (TE) Italia, in qualità di fabbricante dei dispositivi medico-diagnostici *in vitro* elencati nella tabella sotto riportata Revisione 37.4 del 22.05.2022

dichiara sotto la propria responsabilità

1. che i dispositivi sottoindicati soddisfano tutte le disposizioni applicabili della Direttiva 98/79/CE (Allegato III) recepita nella Legislazione Italiana dal Decreto Legislativo n° 332 del 8 settembre 2000;
2. che i dispositivi sottoindicati non sono inclusi nell'Allegato II, lista A e B della Direttiva 98/79/CE
3. che la documentazione tecnica di cui all'allegato III della direttiva Direttiva 98/79/CE è a disposizione delle autorità nazionali presso la sua sede e sarà conservata per 5 anni dall'ultima data di fabbricazione del prodotto;
4. che il processo di fabbricazione segue adeguati principi di assicurazione della qualità;
5. di aver attivato e di mantenere aggiornato, un sistema di sorveglianza post-produzione per il monitoraggio dei prodotti;
6. che i dispositivi sottoindicati sono stati messi in commercio muniti di marcatura CE.

EC DECLARATION OF CONFORMITY

The company Liofilchem® S.r.l., registered office in Via Scozia, 64026 Roseto degli Abruzzi (TE) Italy, as a manufacturer of the in vitro medical-diagnostic devices listed in the table below, Revision 37.4 of 22.05.2022

hereby certifies under its own responsibility

1. that the below mentioned devices comply with all the applicable provisions of Directive 98/79/EC (Annex III) and its relevant transposition into national law;
2. the below mentioned devices are not included in Annex II, List A and B of Directive 98/79/EC;
3. that the technical documentation referred to at Annex III of the Directive 98/79/EC is available for the national authorities in its facility and that this documentation shall be kept for 5 years after the last product has been manufactured;
4. that the manufacturing process follows suitable principles of quality assurance;
5. that, has implemented and keep up to date, a post-production surveillance system for monitoring the products;
6. that the below mentioned devices, were introduced into the market provided with CE mark.

Roseto degli Abruzzi (TE),
22.05.2022

Signature:

LIOFILCHEM s.r.l.
BACTERIOLOGY PRODUCTS
Via Scozia
64026 Roseto degli Abruzzi (TE)
Cod. Fisc. e Partita IVA 00530130073

Technical Director
(Dr. Silvio Brocco)

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Liofilchem®

CODE	DESCRIPTION
9162/1	Streptomycin S 300 µg
9129	Sulbactam SU 20 µg
9129/1	Sulbactam SU 20 µg
9150	Sulfadiazine SUZ 300 µg
9150/1	Sulfadiazine SUZ 300 µg
9041	Sulfafurazole SF 300 µg
9041/1	Sulfafurazole SF 300 µg
9187	Sulfamethoxazole SMX 100 µg
9187/1	Sulfamethoxazole SMX 100 µg
9084	Sulfamethoxazole SMX 50 µg
9084/1	Sulfamethoxazole SMX 50 µg
9132	Sulfaprim SXT 50 µg
9132/1	Sulfaprim SXT 50 µg
9126	Sulfonamide S3 300 µg
9126/1	Sulfonamide S3 300 µg
9243/1	Tedizolid TZD 2 µg
9243	Tedizolid TZD 2 µg
9050	Teicoplanin TEC 30 µg
9050/1	Teicoplanin TEC 30 µg
9186	Temocillin TMO 30 µg
9186/1	Temocillin TMO 30 µg
9043	Tetracycline TE 30 µg
9043/1	Tetracycline TE 30 µg
9094	Tiamulin T 30 µg
9094/1	Tiamulin T 30 µg
9070	Ticarcillin TC 75 µg
9070/1	Ticarcillin TC 75 µg
9096	Ticarcillin-clavulanic acid TTC 85 µg
9096/1	Ticarcillin-clavulanic acid TTC 85 µg
9147	Tigecyclin TGC 15 µg
9147/1	Tigecyclin TGC 15 µg
9044	Tobramycin TOB 10 µg
9044/1	Tobramycin TOB 10 µg
9163	Tobramycin TOB 30 µg
9163/1	Tobramycin TOB 30 µg
9042	Trimethoprim – Sulfamethoxazole SXT 25 µg
9042/1	Trimethoprim – Sulfamethoxazole SXT 25 µg
9083	Trimethoprim TM 2.5 µg
9083/1	Trimethoprim TM 2.5 µg
9110	Trimethoprim TM 5 µg
9110/1	Trimethoprim TM 5 µg
9082	Tylosin TY 30 µg
9082/1	Tylosin TY 30 µg
9045	Vancomycin VA 30 µg
9045/1	Vancomycin VA 30 µg
9164	Vancomycin VA 5 µg
9164/1	Vancomycin VA 5 µg
9168	Voriconazole VO 1 µg
9168/1	Voriconazole VO 1 µg
99002	ESBL disc kit (acc. to EUCAST)
99003	KPC&MBL disc kit (acc. to EUCAST)
99004	ESBL disc kit (acc. to EUCAST)
99005	ESBL disc kit (acc. to CLSI)
99006	ESBL (Chromos. Ind. AmpC) disc kit (acc. to EUCAST)
99007	KPC&MBL&OXA-48 disc kit (acc. to EUCAST)
99008	ESBL+AmpC screen disc kit
99009	AmpC disc kit

CODE	DESCRIPTION

Antibiotic disc in canister

CODE	DESCRIPTION
9004/2	Amikacin AK 30 µg
9133/2	Amoxicillin AML 10 µg
9191/2	Amoxicillin + Clavulanic acid AUG 3 (2+1) µg
9179/2	Amoxicillin AmL 25 µg
9005/2	Amoxicillin AML 30 µg
9048/2	Amoxicillin-clavulanic acid AUG 30 µg
9137/2	Amphotericin B AMB 10 µg
9071/2	Amphotericin B AMB 20 µg
9151/2	Amoxicillin AML 2 µg
9255/2	Amoxicillin-clavulanic acid AUG 7.5 µg
9006/2	Ampicillin AMP 10 µg
9115/2	Ampicillin AMP 2 µg
9031/2	Ampicillin-sulbactam AMS 20 µg
9122/2	Ampliclox (Ampicillin-cloxacillin) ACL 30 (25+5) µg
9105/2	Azithromycin AZM 15 µg
9007/2	Azlocillin AZL 75 µg
9008/2	Aztreonam ATM 30 µg
9051/2	Bacitracin BA 10 IU
9009/2	Carbenicillin CAR 100 µg
9165/2	Caspofungin CAS 5 µg
9010/2	Cefaclor 30 µg
9052/2	Cefadroxil CDX 30 µg
9014/2	Cefamandole MA 30 µg
9015/2	Cefazolin KZ 30 µg
9143/2	Cefepime + Clavulanic acid FEL 40 µg
9220/2	Cefepime FEP 10 µg
9104/2	Cefepime FEP 30 µg
9266/2	Cefiderocol FDC 30 µg
9089/2	Cefixime CFM 5 µg
9016/2	Cefoperazone CFP 30 µg
9108/2	Cefoperazone CFP 75 µg
9203/2	Cefotaxime + Clavulanic acid + Cloxacillin CTLC
9182/2	Cefotaxime + Clavulanic acid CTL 40 (30+10) µg
9224/2	Cefotaxime + Cloxacillin CTC
9017/2	Cefotaxime CTX 30 µg
9152/2	Cefotaxime CTX 5 µg
9081/2	Cefotetan CTT 30 µg
9018/2	Cefoxitin FOX 30 µg
9064/2	Cefpodoxime PX 10 µg
9185/2	Cefpirome CR 30 µg
9190/2	Cefpodoxime + Clavulanic acid PXL 11 (10+1) µg
9112/1	Cefprozil CPR 30 µg
9053/2	Cefsulodin CSD 30 µg
9198/2	Ceftaroline CPT 30 µg
9195/2	Ceftaroline CPT 5 µg
9205/2	Ceftazime-avibactam CZA 50 µg
9145/2	Ceftazidime + Clavulanic acid CAL 40 (30+10) µg
9204/2	Ceftazidime + Clavulanic acid + Cloxacillin CALC
9225/2	Ceftazidime + Cloxacillin CAC
9153/2	Ceftazidime CAZ 10 µg
9019/2	Ceftazidime CAZ 30 µg
9206/2	Ceftazime-avibactam CZA 14 µg
9101/2	Ceftibuten CTB 30 µg
9054/2	Ceftizoxime CZX 30 µg

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APPENDIX D

Statistician's Certificate

Adventist Medical Center College Department of Pharmacy

CERTIFICATION

This is to certify that the research entitled "**THE POTENTIAL ALTERNATIVE ANTIBACTERIAL ACTIVITY OF FALCATA (*Falcataria falcata*) LEAF METHANOLIC EXTRACT AGAINST *Staphylococcus aureus* AND *Escherichia coli*"** by **LALIEFE B. ARNAN, KATHERINE JOY S. ESCUETA, NOR RASHIDA P. RASHID, ELYCA M. TACBOBO, AND QUEENIE ROSE M. TINOY**. Has been checked and statistically corrected by the undersigned.

This certification is issued to ensure that Adventist Medical Center College received quality research work. Signed this 3rd day of May in the year, 2024 at AMCC.

JAYSON PAYLA, MSc.
Statistician

JAYSON PAYLA, MSc.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG722

APPENDIX E

Certificate of Specimen

Republic of the Philippines
CENTRAL MINDANAO UNIVERSITY
University Town, Musuan, Bukidnon

College of Veterinary Medicine

Microbiology Research Laboratory

CERTIFICATION

To whom it may concern:

This is to certify the purity and viability of the following active microbial cultures which were purchased from the Microbiology Research Laboratory, Central Mindanao University by Adventist Medical Center College, Iligan City (Department of Pharmacy) in the person of Queenie Rose Tinoy:

Escherichia coli

Staphylococcus aureus

AIRISH JANE RISMA MIRANDA, RMT
Science Research Analyst
Microbiology Research Laboratory

The Academic Paradise of the South

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG722

APPENDIX F

Certificate of Proofreading

Adventist Medical Center College Department of Pharmacy

CERTIFICATION

This is to certify that the research entitled "THE POTENTIAL ALTERNATIVE ANTIBACTERIAL ACTIVITY OF FALCATA (*Falcataria falcata*) LEAF METHANOLIC EXTRACT AGAINST *Staphylococcus aureus* AND *Escherichia coli*" by LALIEFE B. ARNAN, KATHERINE JOY S. ESCUETA, NOR RASHIDA P. RASHID, ELYCA M. TACBOBO, AND QUEENIE ROSE M. TINOY. Has been checked and proofread for appropriate English language usage, grammar punctation, and spelling by a professional native English-speaking editor.

This certification is issued to ensure that Adventist Medical Center College received quality research work. Signed this 28th day of May in the year, 2024 at AMCC.

ANGIEROSS SHARON VALENZUELA
Proofreader

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG722

APPENDIX G

Documentation

A. Collection of the Plant Samples and Preparation of the Methanolic Extract of Falacata Falcataria

Fig 8: Collection of Falcataria falcata Leaf Materials

Fig 9: Separating Twigs and Other Materials from the Leaves

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Fig 10: Pulverization of Plant Material using a Blender

Fig 11: Sieving (No. 60) the Plant Material

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Fig 12: Using the percolation method for extraction

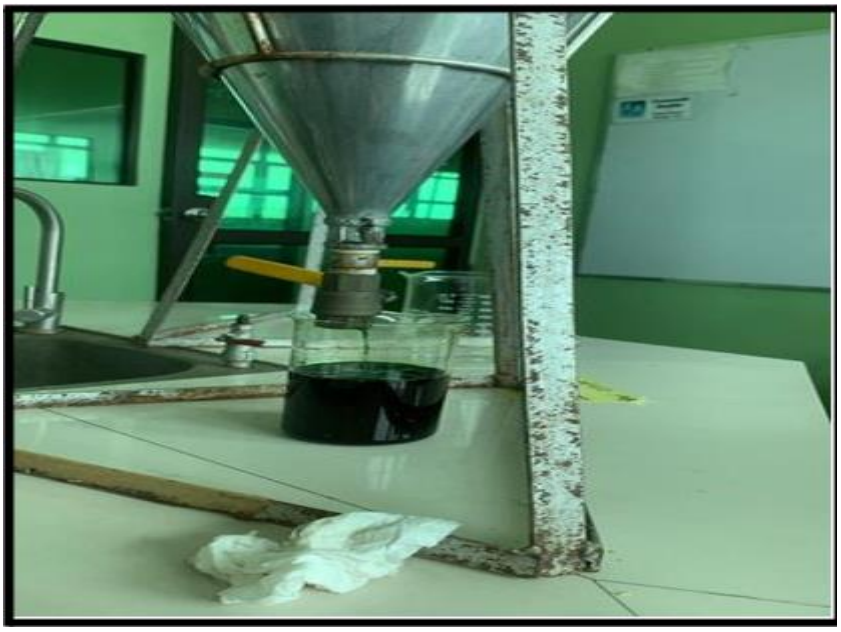

Fig 13: The Marc Collected after 48 Hours

Fig 14: The Crude Extract Being Rotary Evaporated

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Fig 15: A Water Bath was Used on the Plant Material to Evaporate Further Impurities

B. Phytochemical Analysis of the Falcataria Falcata Extract Revealed Secondary Metabolites that have Antibacterial Activities.

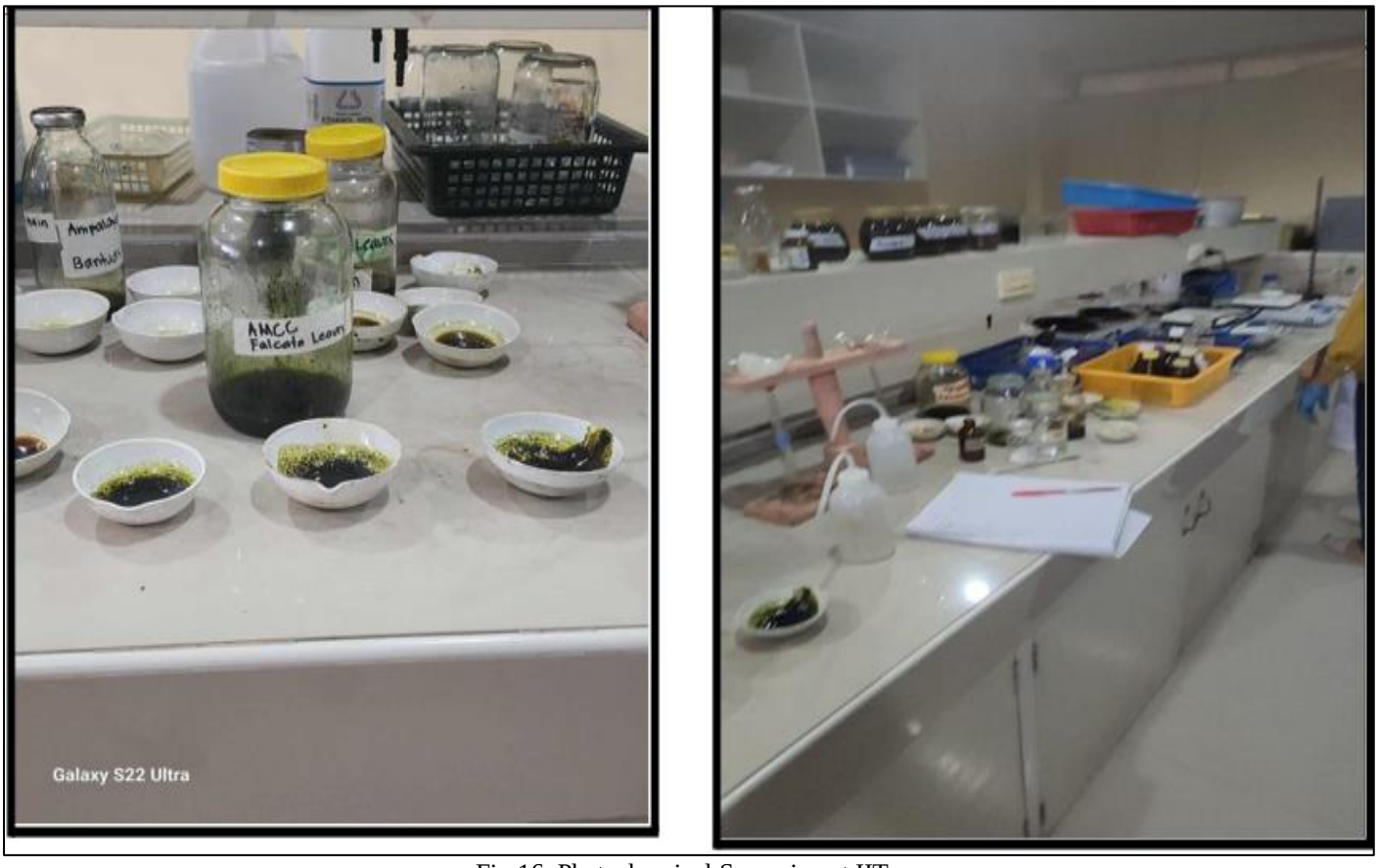

Fig 16: Phytochemical Screening at IIT.

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C. Preparation of the Culture Medium and Bacterial Inoculum

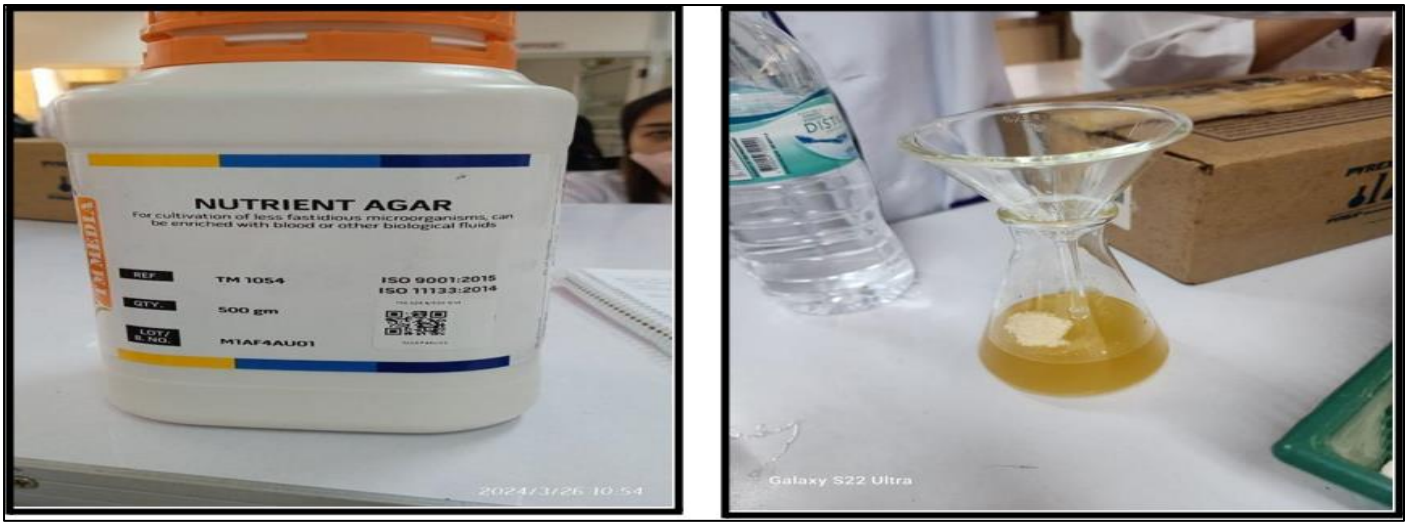

Fig 17: Nutrient Agar was used in the Experiment

Fig 18: Melting and Sterilization of the Agar

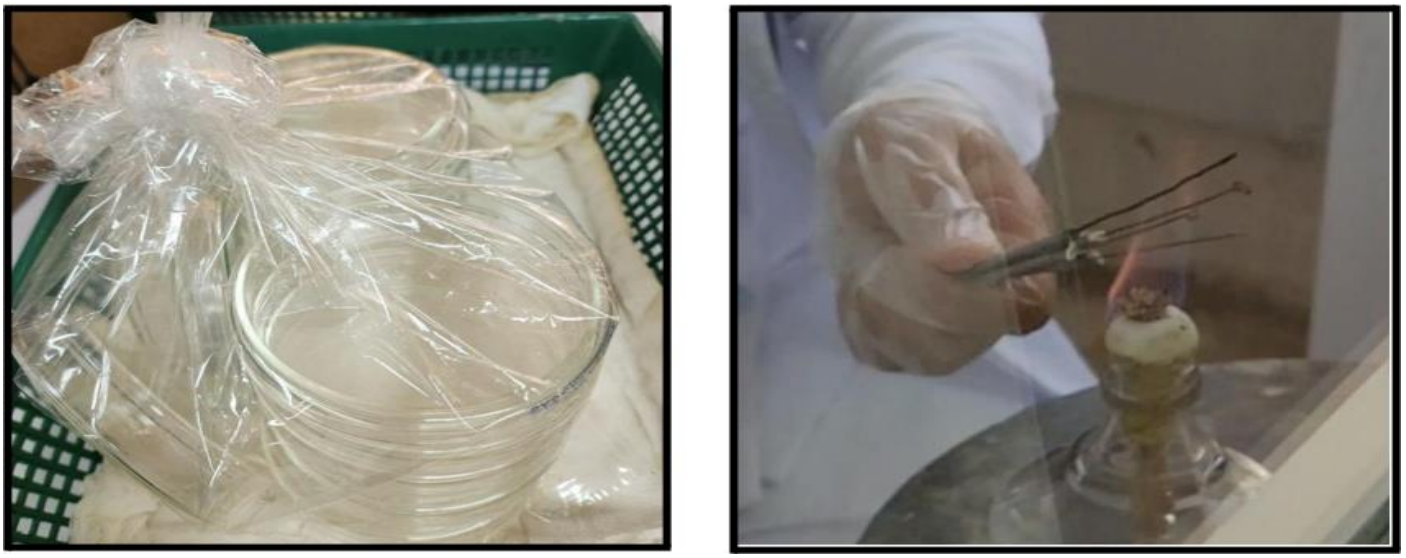

Fig 19: Sterilization of the Materials to be Used in the Experiment

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Fig 20: Preparation of Bacterial Inoculum and Streaking

D. Susceptibility Testing

Fig 21: Making the Stock Solution

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Fig 22: Soaking the Whatman Filter Paper with Extracts and Distilled Water with a Micropipette

E. Determination of the Zone of Inhibition

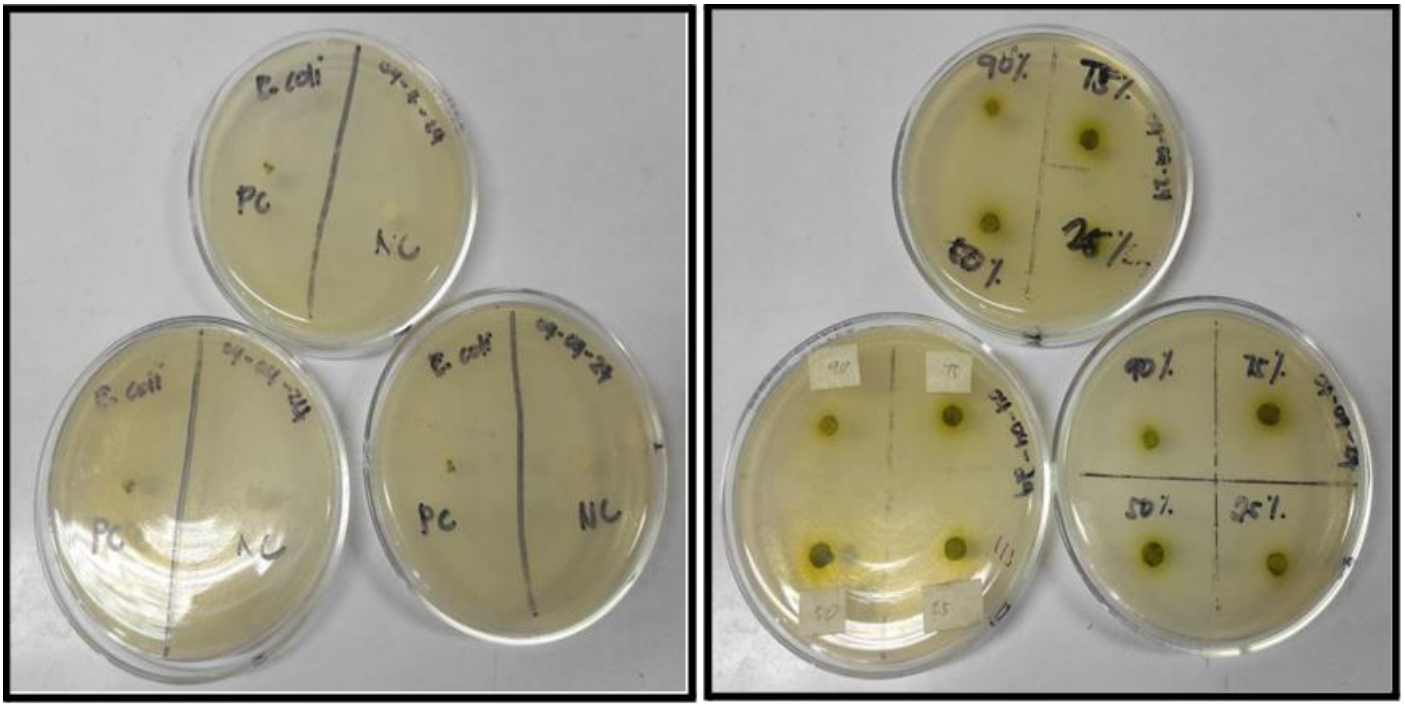

Fig 23: The Zone of Inhibition Results in the E. coli Bacteria

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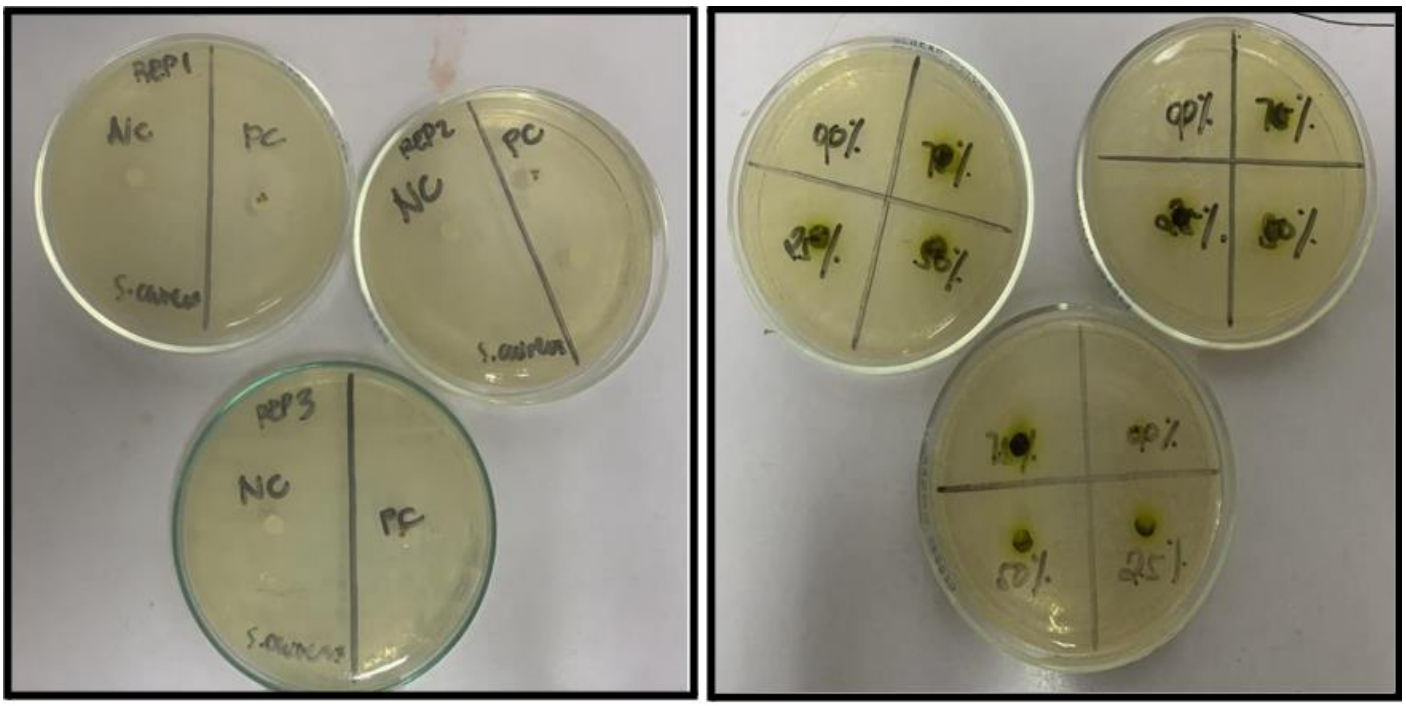

Fig 24: The Zone of Inhibition Results in the S. Aureus Bacteria

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APPENDIX H

Zone of Inhibition of the experimental group and **Trimethoprim-sulfamethoxazole and comparison of the 75%, 50% and 25% crude methanolic extracts against *Staphylococcus aureus*.**

- *Staphylococcus Aureus*

Experimental Group (Rep 1, 2, and 3) vs. the Zone of Inhibition

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Experimental1StaphAu	4	2.2	0.55	0.25
Experimental2StaphAu	4	2.4	0.6	0.24
Experimental3StaphAu	4	2.8	0.7	0.226667
ZOIExperiementalStaphAu	4	24.66667	6.166667	22.03704

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	92.45417	3	30.81806	5.417677	**0.013719**	3.490295
Within Groups	68.26111	12	5.688426			
Total	160.7153	15				

Note: Significant if p-value < 0.05* and p-value < 0.01**

Trimethoprim-Sulfamethoxazole Vs. Zone of Inhibition of Trimethoprim-Sulfamethoxazole

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
TriSulfStaphAu	3	6	2	1
ZOITrimetSulfStaphAu	3	66	22	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	600	1	600	1200	**4.14E-06**	7.708647
Within Groups	2	4	0.5			
Total	602	5				

Note: Significant if p-value < 0.05* and p-value < 0.01**

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Comparing 90% Extract of Falcata, Distilled Water and Trimethoprim-Sulfamethoxazole vs. ZOI of 90% Extract of Falcata

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
90%ExpStaphAu	3	0	0	0
WaterStaphAu	3	0	0	0
TriSulfStaphAu	3	6.6	2.2	1.72
ZOI90%ExpStaphAu	3	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10.89	3	3.63	8.44186	**0.007344**	4.066181
Within Groups	3.44	8	0.43			
Total	14.33	11				

Note: Significant if p-value < 0.05* and p-value < 0.01**

Comparing 75% Extract of Falcata, Distilled Water and Trimethoprim-Sulfamethoxazole vs. ZOI of 75% Extract of Falcata

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
75%ExpStaphAu	3	3.4	1.133333	0.013333
WaterStaphAu	3	0	0	0
TriSulfStaphAu	3	6.6	2.2	1.72
ZOI75%ExpStaphAu	3	33.99	11.33	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	242.22	3	80.74001	186.3231	**9.7E-08**	4.066181
Within Groups	3.466667	8	0.433333			
Total	245.6867	11				

Note: Significant if p-value < 0.05* and p-value < 0.01**

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Comparing 50% Extract of Falcata, Distilled Water and Trimethoprim-Sulfamethoxazole vs. ZOI of 50% Extract of Falcata

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
50%ExpStaphAu	3	2.2	0.733333	0.053333
WaterStaphAu	3	0	0	0
TriSulfStaphAu	3	6.6	2.2	1.72
ZOI50%ExpStaphAu	3	21.99	7.33	1.18E-30

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	98.31803	3	32.77268	73.92333	**3.57E-06**	4.066181
Within Groups	3.546667	8	0.443333			
Total	101.8647	11				

Note: Significant if p-value < 0.05* and p-value < 0.01**

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
50%ExpStaphAu	3	2.2	0.733333	0.053333
WaterStaphAu	3	0	0	0
TriSulfStaphAu	3	6.6	2.2	1.72
ZOI50%ExpStaphAu	3	21.99	7.33	1.18E-30

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	98.31803	3	32.77268	73.92333	**3.57E-06**	4.066181
Within Groups	3.546667	8	0.443333			
Total	101.8647	11				

Note: Significant if p-value < 0.05* and p-value < 0.01**

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Comparing 25% Extract of Falcata, Distilled Water and Trimethoprim-Sulfamethoxazole vs. ZOI of 25% Extract of Falcata

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
25%ExpStaphAu	3	1.8	0.6	0.04
WaterStaphAu	3	0	0	0
TriSulfStaphAu	3	6.6	2.2	1.72
ZOI25%ExpStaphAu	3	18	6	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	65.52	3	21.84	49.63636	**1.63E-05**	4.066181
Within Groups	3.52	8	0.44			
Total	69.04	11				

Note: Significant if p-value < 0.05* and p-value < 0.01**

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APPENDIX I

Summary of the zone of Inhibition of the experimental group and comparison of the 90%, 75%, and 25% crude methanolic extracts against *Escherichia coli*.

Escherichia coli

Experimental Group (Rep 1, 2, and 3) vs. the Zone of Inhibition

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Experimental1Ecoli	4	2.3	0.575	0.5225
Experimental2Ecoli	4	0.7	0.175	0.1225
Experimental3Ecoli	4	0	0	0
ZOIExperimentalEcoli	4	10	2.5	4.185185

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.8825	3	5.294167	4.384235	**0.026555**	3.490295
Within Groups	14.49056	12	1.207546			
Total	30.37306	15				

Note: Significant if p-value < 0.05* and p-value < 0.01**

Comparing 90% Extract of Falcata, Distilled Water and Trimethoprim-Sulfamethoxazole vs. ZOI of 90% Extract of Falcata

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
90%ExpEcoli	3	0.7	0.233333	0.163333
WaterEcoli	3	0	0	0
TriSulfEcoli	3	0	0	0
ZOI90%ExpEcoli	3	6.99	2.33	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11.52203	3	3.840675	94.05735	**1.41E-06**	4.066181
Within Groups	0.326667	8	0.040833			
Total	11.84869	11				

Note: Significant if p-value < 0.05* and p-value < 0.01**

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Comparing 75% Extract of Falcata, Distilled Water and Trimethoprim-Sulfamethoxazole vs. ZOI of 75% Extract of Falcata

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
75%ExpEcoli	3	1.5	0.5	0.75
WaterEcoli	3	0	0	0
TriSulfEcoli	3	0	0	0
ZOI75%ExpEcoli	3	15	5	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	53.0625	3	17.6875	94.33333	**1.39E-06**	4.066181
Within Groups	1.5	8	0.1875			
Total	54.5625	11				

Note: Significant if p-value < 0.05* and p-value < 0.01**

Comparing 25% Extract of Falcata Distilled Water and Trimethoprim-Sulfamethoxazole vs. ZOI of 25% Extract of Falcata

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
25%ExpEcoli	3	0.8	0.266667	0.213333
WaterEcoli	3	0	0	0
TriSulfEcoli	3	0	0	0
ZOI25%ExpEcoli	3	7.98	2.66	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.0161	3	5.005367	93.85063	**1.42E-06**	4.066181
Within Groups	0.426667	8	0.053333			
Total	15.44277	11				

Note: Significant if p-value < 0.05* and p-value < 0.01**

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APPENDIX J

Calculations used in the Study

➢ *Calculation for the Moisture Content*

Formula used: (Bogart 2018)

$$MC = \frac{wet\ weight - weight\ after\ drying}{wet\ weight} x100$$

➢ *Calculations:*

$$MC = \frac{1169\ g - 1028\ g}{1169\ g} x100;\ \text{MC} = 12.06\%$$

Calculation for the Stock solution

Formula used: (Ali et al. 2023; LabCE 2024)

$C1V1 = C2V2$

➢ Calculations:

- 90% of crude methanolic leaf extract of *Falcataria falcata*.
- $\frac{90\%}{1\ mL} = \frac{0.3\ g}{x}$; x = 0.33 mL total stock solution of *F. falcata* extract.
- 75% of crude methanolic leaf extract of *Falcataria falcata*.
- $\frac{75\%}{1\ mL} = \frac{0.2\ g}{x}$; x = 0.3 mL total stock solution of *F. falcata* extract.
- 50% of crude methanolic leaf extract of *Falcataria falcata*.
- $\frac{50\%}{1\ mL} = \frac{0.2\ g}{x}$; x = 0.4total stock solution of *F. falcata* extract.
- 25% of crude methanolic leaf extract of *Falcataria falcata*.
- $\frac{25\%}{1\ mL} = \frac{0.1\ g}{x}$; x = 0.4 mL total stock solution of *F. falcata* extract.

Table 10: A Summary of Concentrations of *F. falcata* Methanolic Extract

Concentrations	***F. falcata* methanolic crude extract collected**	**Sterile water is to be added**
90%	0.3 g	0.03 mL
75%	0.2 g	0.1 mL
50%	0.2 g	0.2 mL
25%	0.1 g	0. 3 mL

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APPENDIX K

Gantt Chart

Research objectives	2023					2024				
	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1 Proposal writing										
2 Plant collection and preparation										
3 Crude leaves extraction										
4 Phytochemical analysis										
5 Susceptibility testing										
6 Data collection and analysis writing										
7 Research defense										

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APPENDIX L

Budget Schedule

Item	Price	Quantity	Total Price
Methanol	1,398	5	6,991
Bacteria samples	600	2	1,200
Plant samples	300	2	600
Phytochemical analysis	1,300	1	1,300
Transportation (all fees/expenses)	5,200	1	5,200
Medical technology laboratory payment	4,874	1	4,874
Payment for the Statistician	2,000	1	2,000
Lab apparatus (1)NBeaker	239	1	239
Lab apparatus (2) Petri dishes	357	5	1,785
3x4 Poster presentation	273	1	273
Hard bounding	350	3	1,050
CD burning	200	1	200
Miscellaneous expenses	5,260	1	5,260
Total			**30972**

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We are deeply grateful to Professor Samson Mangin, Professor Mae Lee Tumaneng-Agoo and Professor Junnin Gay Garay for being our thesis advisors and to Arlee Sinahon, Jayson Payla and Angieross Sharon Valenzuela for their invaluable contributions to this research. Their unwavering support, insights, and expertise have been instrumental in shaping this study from its inception to its completion. We are also deeply grateful to Adventist Medical Center College for providing us with the resources or facilities that were essential for our work.

Specifically, we would like to express our sincere appreciation to our research panel members for their exceptional guidance and mentorship. Their unwavering belief in our abilities has been a source of constant inspiration. To our colleagues for their collaborative spirit, intellectual discussions, and hands-on assistance during the research project. Their dedication to ensuring the success of this research has been truly remarkable.

Finally, we would like to thank our families and friends for providing the necessary funding, resources, unwavering support, encouragement, and understanding throughout this challenging yet rewarding journey. Their generous support has been essential to the advancement of our knowledge in this field. Without the contributions of those mentioned above, this project would not have been possible.

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EDUCATIONAL BACKGROUND

Level of Education	Institution Attended	Year Graduated
B.S. Pharmacy	Adventist Medical Center College	Present
High School	Digkilaan National High School	2021
Elementary	Sardab Elementary School	2015

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EDUCATIONAL BACKGROUND

Level of Education	Institution Attended	Year Graduated
B.S. Pharmacy	Adventist Medical Center College	Present
B.S. Nursing	Iligan Medical Center College	2012
High school	Saint Michael's College- Basic Education Department	2007
Elementary	St. Augustine Academy La Salle Academy	2001
PROFESSIONAL QUALIFICATION		
Registered Nurse	License Number 0774632	2001

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EDUCATIONAL BACKGROUND

Level of Education	Institution Attended	Year Graduated
B.S. Pharmacy	Adventist Medical Center College	Present
High school	La Salle Academy	2021
Elementary	United Methodist Development Academy	2015
	Life Giver Christian Learning Academy	2013

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EDUCATIONAL BACKGROUND

Level of Education	Institution Attended	Year Graduated
B.S. Pharmacy	Adventist Medical Center College	Present
High school	Adventist Medical Center College	2021
Elementary	St. Michael's College- Basic Education Department	2019
	Iligan City East Central School (ICECS)	2015

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EDUCATIONAL BACKGROUND

Level of Education	Institution Attended	Year Graduated
B.S. Pharmacy	Adventist Medical Center College	Present
High school	Sta. Felomina Central School	2021
Elementary	Sta. Felomina Central School	2015