



UNIVERSITI PUTRA MALAYSIA

***NUTRITIONAL VALUE AND ANTIBACTERIAL
ACTIVITY OF YEAST FERMENTED HERBS
AS AN ANTIBIOTIC SUPPLEMENT FOR FISH***

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**NUTRITIONAL VALUE AND ANTIBACTERIAL ACTIVITY OF YEAST-
FERMENTED HERBS AS AN ANTIBIOTIC SUPPLEMENT FOR FISH**

By

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**A Project Report Submitted in Partial Fulfillment of the Requirement for the Bachelor
of Science in Aquaculture With Honours in the
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DEDICATION

This thesis is dedicated to my beloved father, Barudi Linggong, relatives, and not to forget, to all my friends for their unconditional love, prayers, and support through this journey.



ABSTRACT

Medicinal herbs are widely employed in treating diverse human ailments and present promising potential as supplements for fish, enhancing their growth. However, the overuse of antibiotics has spurred the proliferation of resistant microbes, posing a threat to ecosystems and all living organisms, including fish, terrestrial animals, and humans. To tackle this concern, five specific herbs namely: *Syzygium polyanthum*, *Curcuma longa*, *Andrographis paniculata*, *Orthosiphon stamineus*, and *Euphorbia hirta*, have been chosen for this study. This research aims to assess variations in nutrient and mineral compositions and compare the antibacterial properties of both yeast-fermented and non-fermented forms of these herbs. The fermentation process involved the addition of *Lactobacillus casei* from Yakult and commercial yeast, *Saccharomyces cerevisiae*. The study employed the agar well diffusion assay method to examine the inhibitory effects of these herbs against pathogenic bacteria including *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus gallolyticus*, and *Aeromonas hydrophila*. Analyzing the nutritional composition using the AOAC standard procedure revealed that yeast-fermented herbs displayed higher protein content but lower levels of carbohydrates, fats, crude fiber, ash, and energy compared to their non-fermented counterparts. Moreover, the yeast-fermented herbs exhibited reduced levels of potassium, magnesium, calcium, copper, and zinc compared to the non-fermented ones. In antibacterial activity tests, yeast-fermented *Curcuma longa* proved highly effective against *Aeromonas hydrophila* and *Streptococcus gallolyticus*. Hence, the potential application of yeast-fermented *Curcuma longa* as an alternative and safe feed additive for sustainable fish production, serving as a natural antibiotic, is underscored by these findings.

ABSTRAK

Herba perubatan digunakan secara meluas dalam merawat pelbagai penyakit manusia dan berpotensi sebagai makanan tambahan untuk ikan, untuk meningkatkan tumbesarannya. Walau bagaimanapun, penggunaan antibiotik secara berlebihan telah mendorong pembiakan mikrob yang mempunyai rintangan terhadap antibiotik serta boleh menimbulkan ancaman terhadap ekosistem dan semua organisma hidup, termasuk ikan, haiwan darat dan manusia. Untuk menangani kebimbangan ini, lima herba khusus iaitu: *Syzygium polyanthum*, *Curcuma longa*, *Andrographis paniculata*, *Orthosiphon stamineus*, dan *Euphorbia hirta*, telah dipilih untuk kajian ini. Penyelidikan ini bertujuan untuk menilai variasi dalam komposisi nutrien dan mineral serta membandingkan sifat antibakteria bagi herba yang ditapai dan herba yang tidak ditapai. Proses penapaian melibatkan penambahan *Lactobacillus casei* daripada Yakult dan yis komersial, *Saccharomyces cerevisiae*. Kajian ini menggunakan kaedah “agar well diffusion assay” untuk mengkaji kesan perencatan herba terhadap bakteria patogen seperti *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus gallolyticus*, dan *Aeromonas hydrophila*. Menganalisis komposisi nutrient menggunakan prosedur piawai AOAC mendedahkan bahawa herba yang ditapai yis menunjukkan kandungan protein yang lebih tinggi tetapi tahap karbohidrat, lemak, serat kasar, abu dan tenaga yang lebih rendah berbanding dengan herba yang tidak ditapai. Selain itu, herba yang ditapai yis mempamerkan paras kalium, magnesium, kalsium, kuprum dan zink yang semakin berkurangan berbanding dengan herba yang tidak ditapai. Dalam ujian aktiviti antibakteria, *Curcuma longa* yang ditapai yis terbukti sangat berkesan terhadap *Aeromonas hydrophila* dan *Streptococcus gallolyticus*. Oleh itu, penggunaan *Curcuma longa* yang ditapai yis berpotensi menjadi makanan alternatif dan makanan tambahan yang

selamat untuk pengeluaran ikan yang mapan, dan berfungsi sebagai antibiotik semula jadi, seperti yang telah digariskan oleh penemuan ini.



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APPROVAL SHEET

I certify that this research project report entitled “Nutritional Value and Antibacterial Activity of Yeast-fermented Herbs as an Antibiotic Supplement for Fish” has been examined and approved as a partial fulfillment of the requirement for the Bachelor of Science In Aquaculture with Honours in the Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus.

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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	iv-v
ACKNOWLEDGEMENTS	vi
APPROVAL SHEET	vii
TABLE OF CONTENTS	viii-x
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF PLATES	xiii
LIST OF ABBREVIATIONS/GLOSSARY OF TERMS	xiv
CHAPTER	
1 INTRODUCTION	1
1.1 Background	1-3
1.2 Problem Statement	3-4
1.3 Objectives	4
2 LITERATURE REVIEW	5
2.1 Medicinal Plants/herbs	5-7
2.1.1 <i>Euphorbia hirta</i> (Asthma Plant)	8-9
2.1.2 <i>Syzygium polyanthum</i> (Bay leaf)	10-11
2.1.3 <i>Curcuma longa</i> (Turmeric)	12-13
2.1.4 <i>Andrographis paniculata</i> (“Hempedu bumi”)	13-17
2.1.5 <i>Orthosiphon stamineus</i> (“Misai kucing”)	17-18

2.2 Fermentation	18-20
2.2.1 Benefit of fermentation	20-22
2.2.2 The Effluent of Fermentation on Herbs	22-23
2.2.3 The Microorganisms Involved in Herbs Product Fermentation	23-24
2.3 Antibacterial Activity for Fish	25-27
2.3.1 Antibiotic	27-29
3 MATERIALS AND METHODS	30
3.1 Description of the Study Area	30
3.2 Preparation and Characterization of Yeast-Fermented Herbs	30
3.2.1 Collection and Identification of Plant Materials	30
3.2.2 Sample Preparation	30
3.2.3 Fermentation Process	30-31
3.2.4 pH Determination	31
3.3 Nutritional Analysis	31-32
3.4 Mineral Profiling	32
3.5 Herbs Extraction	32
3.6 Solvent Reconstitution	33
3.7 Antibacterial Activity Test	33
3.7.1 Preparation of Mueller Hinton Agar/Broth	33
3.7.2 Standardization of Bacteria Culture	33-34
3.7.3 Agar Well Diffusion Assay	34
3.8 Statistical Analysis	34-35
4 RESULTS AND DISCUSSION	36
4.1 Nutrient Composition	36-43
4.2 Mineral Composition of Non-Fermented and Yeast-Fermented	44-58

Herbs	
4.3 Antibacterial Activity	58-62
6 CONCLUSION	63
REFERENCES	64-82
APPENDICES	80-82
PUBLICATION OF THE PROJECT UNDERTAKING SHEET	83



LIST OF TABLES

Table	Page
3.2.4 Initial and final pH of five yeast-fermented herbs	29
4.1 Nutrient composition of non-fermented and yeast-fermented herbs (g/100g)	33
4.2 Mineral composition of non-fermented and yeast-fermented herbs (g/100g)	40



LIST OF FIGURES

Figure		Page
4.3	Pathogenic bacteria's susceptibility to fermented and non-fermented herbs extracts.	52



LIST OF PLATES

Plate	Page
2.1.1 <i>Euphorbia hirta</i> (Barudi, 2023).	7
2.1.2 <i>Syzygium polyanthum</i> (National Parks Board, 2021).	9
2.1.3 <i>Curcuma longa</i> (Happy Valley Seeds, 2023).	11
2.1.4 <i>Andographis paniculata</i> (Alamy, 2019).	12
2.1.5 <i>Orthosiphon stamineus</i> (Naturmed Scientific, 2022).	15

LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percentage
μL	Microlitre
AOAC	Association of Official Analytical Chemists
CFU	Colony forming unit
g	gram
GI	Glycemic index
kcal	Kilocalories
L	Litre
LAB	Lactic acid bacteria
mcg	Microgram
mg	Milligram
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
mL	Millilitre
mm	Millimetre
pH	Potential of hydrogen
RM	Ringgit Malaysia
rpm	Revolutions per minute
SCFA	Short-chain fatty acids
NDF	Neutral detergent fibre

CHAPTER 1

INTRODUCTION

1.1 Background

The aquaculture industry is an essential source of food for hundreds of millions of people worldwide, and it is considered one of the fastest-growing animal protein industries (Tkachenko et al. 2022). An innovative approach that consistently and reliably raises the quality of aquatic feed is the administration of bioactive immunostimulants (Vijayaram et al., 2022; Ragunath & Ramasubramanian, 2022; Firmino et al., 2021).

Vijayaram et al. (2022) and Alfred et al. (2020) have highlighted microbial diseases,—caused by bacteria, fungi, and viruses, as major challenges in aquaculture production, posing significant threats to the industry. These diseases can eventually result in catastrophic financial losses. To address these concerns, a variety of bioactive active molecules, including those with growth-promoting, antimicrobial, antioxidant, and immunostimulant properties, are used to improve the health of aquatic animals (Vijayaram et al., 2022; Ragunath & Ramasubramanian, 2022; Firmino et al., 2021).

While antibiotics and chemotherapeutics play a crucial role in promoting growth, boosting immunity, and treating bacterial-induced diseases in fish, their widespread use in aquaculture raises significant issues. These include the risk of increasing the number of resistant pathogens, issues with antibiotic residues accumulating in treated fish, and unfavourable environmental effects (Tkachenko et al., 2022). The presence of antibiotics biologically active chemicals that can harm aquatic ecosystems due to their extensive presence in aquaculture systems and the lack of standards for monitoring them (Tkachenko et al., 2022; Pepi &

Focardi, 2021). Tkanchenko et al. (2022), explained that good aquaculture management techniques focus on minimizing the use of antimicrobials in fish to prevent the emergence and spread of antimicrobial resistance in aquaculture environments.

Over the last two decades, there has been a lot of interest in using medicinal herbs in aquaculture to provide safe, and environmentally friendly substances to replace antibiotics and other chemotherapeutic medications, as well as to boost fish immune systems and control fish illnesses (Tkachenko et al., 2022; Gabriel and González-Redondo, 2019). Based on Tkachenko et al. (2022), herbs are high in secondary metabolites and phytochemicals, which have antiviral, antibacterial, and antiparasitic properties in fish. The key advantage of medicinal herbs is that they are naturally occurring, and most herbs do not endanger human health, fish, or the environment (Tkachenko et al., 2022). Antibacterial capabilities of medicinal herbs are by far the most well-studied of biological activities, with numerous in-vitro studies demonstrating antibacterial activity in various herbs against both gram-positive and gram-negative marine bacteria (Tkachenko et al., 2022).

Fermentation induces changes in antioxidant activity by converting conjugated phenolic molecules into additional hydroxyl groups (Darwesh et al. 2023; Eweys et al. 2022). Darwesh et al. (2023) and Zhao et al. (2021) revealed how the structural breakdown of cereal cell walls, during fermentation, allowing for the synthesis and release of various bioactive compounds. This process enables microorganisms to modify herb elements. Furthermore, Darwesh et al. (2023) demonstrated that enzymes such as cellulases, inulinase, tannase, amylase,

and glucosidase can be created during fermentation and can break down the herb cell wall, resulting in the production of bioactive substances.

A study conducted by Chen et al. (2023) shows that combining fermented Chinese herbs with *Saccharomyces cerevisiae* and *Lactobacillus casei* enhances the growth of weaned piglets. This enhancement is achieved through increased secretion of intestinal digestive enzymes, alterations in intestinal microbial diversity, regulation of intestinal short-chain fatty acid contents, promotion of intestinal health, and improvement in nutrient digestibility. Moreover, mannoprotein from *L. plantarum* and *S. cerevisiae* yeast cell walls applied to the food has been demonstrated to improve serum and mucosal immunity, disease resistance against infections, and fish and prawn survival rates (Van Doan et al., 2019).

1.2 Problem Statement

In response to the growing concern about antibiotic resistance, Fiedler et al. (2019) conducted tests to assess the prevalence of antibiotic resistance in *Bacillus cereus* isolates obtained from fresh food. *B. cereus* strains, attributed to the development of β -lactamase enzymes, were confirmed to be generally resistant to penicillin G, ampicillin, cefotaxime, and the β -lactamase inhibitor combination of amoxicillin and clavulanic acid. Conversely, other antibiotics, including tetracycline, erythromycin, chloramphenicol, gentamicin, ciprofloxacin, and imipenem, demonstrated general effectiveness against these strains. Highlighting the broader environmental implications, Tkachenko et al. (2022) underscored that approximately 80% of antimicrobials used in aquaculture enter the environment, posing risks to fish, terrestrial animals, humans, and the overall ecosystem. The use of antibiotics in

aquaculture has been identified as a potential contributor to the emergence of antibiotic-resistant microorganisms in affected areas (Tkachenko et al., 2022; Pepi and Focardi, 2021; Rangel-López et al., 2022). In light of these concerns, this study seeks to investigate the potential of fermented herbs as a natural antibiotic source. The objective is to explore the feasibility of replacing synthetic antibiotic growth promoters in the aquaculture industry, offering a sustainable alternative to mitigate the environmental and health risks associated with antibiotic use in this sector.

1.3 Objectives

The specific objectives of this study are:

- i. To assess variations in the nutrient and mineral composition between yeast-fermented and non-fermented herbs.
- ii. To investigate and compare the antibacterial activity of yeast-fermented and non-fermented herbs.

CHAPTER 2

LITERATURE REVIEW

2.1 Medicinal plants/herbs

According to Hasani (2022), many plants are needed for a wide range of products, including food, medicine, and cosmetics. Several species of herbs and specific plants with therapeutic properties are included in the term "medicinal plants." According to estimates from the World Health Organisation, 80% of people, particularly those in developing nations, get their medical care from traditional medicines, which are primarily derived from plants (Nordin et al., 2019).

It is believed that these medicinal herbs provide a rich source of ingredients for the creation and production of pharmaceuticals (Degla et al., 2021; Bastampoor et al., 2021; Faheem et al., 2022). There is nothing new about the concept of employing herbs as medications to cure a range of human conditions, such as bacterial infections, cancer, and wounds (Nordin et al., 2019).

Medicinal plants and traditional healers are the main sources of healthcare for many civilizations in undeveloped nations (Idowu et al., 2019). Plant extracts show great potential as supplements for fish farming due to their accessibility, affordability, and eco-friendliness (Park et al., 2019). Samtiya et al. (2021) stated that vitamins and other important micronutrients are abundant in plant-based diets.

Plants have long been thought to be excellent sources of medication for a wide range of illnesses. According to Parveen et al. (2020), Sahin et al. (2021) and Faheem et al. (2022), other drugs believed to be produced from plants are artemisinin, aspirin, atropine, digoxin, reserpine, colchicine, physostigmine, taxol, pilocarpine, ephedrine, morphine, quinine, quinidine, vinblastine, vincristine, and tubocurarine.

In developing nations, aquaculture is crucial to the livelihood, jobs, and local economic growth of coastal communities (Fujaya et al., 2022). Farmers suffered significant losses as a result of an enormous rise in disease cases and crop failures brought on by the aquaculture sector's quick intensification (Waiho et al., 2021). China is also one of the countries that currently uses plants in aquaculture (Xie et al., 2019). Using naturally occurring herbs with therapeutic properties is one option. Growth promoters that are natural or non-antibiotic are among the active components found in natural herbs that are proven to help improve aquaculture production performance (Fujaya et al., 2022).

Mbokane and Moyo (2022), reported that pharmacological qualities that most medicinal plants have include antistress, growth promotion, hunger stimulation, immunostimulation, aphrodisiac, antibacterial, antifungal, and antiparasitic effects that may be utilized to supplement fish feed. Plants can improve fish illness resistance and growth performance (Reverter et al., 2021). For *Oreochromis mossambicus* and *Clarias gariepinus*, some plants have been studied for their ability to promote growth. It has been observed that bioactive chemicals found in medicinal plants can increase the activity of digestive enzymes.

Mbokane and Moyo (2022), explained that certain plants have been shown to improve gonadal development in certain fish species. Additionally, plants can be employed to improve or regulate fish fertility. Research conducted by Gabriel (2019) indicates that certain substances present in plants can regulate tilapia reproduction. Still, most of the plants that have been demonstrated to boost fish fertility also include bioactive components that have been found to enhance fish health in general by detoxifying and scavenging free radicals, which lowers oxidative stress in fish (Mbokane & Moyo, 2022).

Due to the abundance of bioactives including saponin, phenols, flavonoids, and polysaccharides that these medicinal herbal preparations contain, it has been claimed that they improve tilapia species' development, appetite, immunological responses, antioxidants, hepatoprotective action, and control reproduction (Gabriel, 2019). It has been demonstrated by Rashidian et al. (2020), that they improve the performance and growth of cultivated fish. They can also strengthen the immune system of fish, leading to less severe illnesses with lower death rates and financial losses.

Anjusha et al. (2019) mentioned that medicinal herbs are a safe and effective substitute for chemotherapy and antibiotics. Due to their ability to promote the synthesis of blood cells and other haematological indices, most herbs can boost both specific and non-specific immune systems. Thus, phytotherapy is an inexpensive, safe way to cure fish illnesses. Fish disorders can already be cured or prevented with medicinal plant extracts, and can even be used to stimulate reproduction in fish (Bilen and Elbeshti, 2019; Bilen et al., 2019; Bilen et al., 2020a; Bilen et al., 2020b; Sonmez et al., 2019).

Therefore, five types of medicinal plants such as *Syzygium polyanthum* (Bay leaf), *Curcuma longa* (Turmeric), *Andrographis paniculata* ("Hempedu Bumi"), *Orthosiphon stamineus* ("Misai kucing"), and *Euphorbia hirta* (Asthma Plant) were tested against four pathogenic bacteria in this study. The four pathogenic bacteria are *Escherichia coli*, *Streptococcus gallolyticus*, *Staphylococcus aureus*, and *Aeromonas hydrophila*.

2.1.1 *Euphorbia hirta* (Asthma Plant)



Figure 2.1.1: *Euphorbia hirta* (Barudi, 2023).

The plant species is a member of the Euphorbiaceae family. Open waste areas, meadows, watercourse banks, roadsides, and pathways are common places to view it. According to a previous study conducted by Idowu et al. (2019), the antibacterial activity of this plant extract was demonstrated against *Staphylococcus aureus* and *Escherichia coli*. Furthermore, it has been demonstrated by Salosso et al. (2021) that the antibacterial activity of *E. hirta* against fish diseases such as *Aeromonas hydrophila* and *V. alginolyticus*.

With its distinct chemical structure and potent effects on the body, *E. hirta* is mostly utilized as a medicinal herb that has the potential to be employed as an antimicrobial to improve survival and immunological competence. Fish treated with *E. hirta* will have a stronger immune system against bacterial illnesses and haemoglobin levels rise (Salosso et al., 2023). Traditionally, it has been used to treat feminine diseases, respiratory ailments such as cough, coryza, bronchitis, asthma, worm infestations in infants, diarrhea, jaundice, acne, gonorrhea, digestive issues, and tumors. According to reports, the herb has been used to cure conjunctivitis, intestinal parasitosis, diarrhea, and dysentery (Idowu et al., 2019).

Additionally, the plant species *E. hirta* has compounds that have pharmacological properties like antibacterial, antioxidant, and anti-inflammatory properties (Windari et al., 2021; Salosso et al., 2023). Previous research on the aqueous leaf extract of *E. hirta* demonstrated the existence of various bioactive compounds, including phenol, tannins, saponins, alkaloids, and flavonoids. (Idowu et al., 2019). According to Salosso et al. (2023), by breaking down the bacterial cell wall, phenolic derivatives like tannin and flavonoids have antibacterial properties.

The *E. hirta* methanolic extract showed the highest antibacterial activity against *A. hydrophila*, with the lowest minimum inhibitory concentration (MIC). These findings suggest that *E. hirta* may prove to be a useful dietary supplement for strengthening *Clarius gariepinus*' resistance to *A. hydrophila* contamination. Dao et al. (2020) explained that it was shown tannins had a major role in the antioxidant and antibacterial qualities of the plant extracts from *E. hirta*.

The outcomes demonstrated the effectiveness of *E. hirta* in treating grouper infections caused by *V. alginolyticus*. After being treated with *E. hirta*, the grouper hybrid's erythrocyte increases. It suggests that the erythrocytes have stopped hemolyzing, which means that *V. alginolyticus* has stopped producing hemolytic toxin and the fish have recovered. *E. hirta*'s ability to have antibacterial activity is probably what led to this recovery (Salosso et al., 2023).

2.1.2 *Syzygium polyanthum* (Bay leaf)



Fig. 2.1.2: *Syzygium polyanthum* (National Parks Board, 2021).

Syzygium polyanthum is a naturally occurring evergreen shrub that is a member of the Myrtaceae family (Nordin et al., 2019). Malaysians are familiar with *Syzygium polyanthum* as "salam," "serai kayu," or "samak kelat." *S. polyanthum* is an ethnomedicinal herb that is currently attracting interest for its many pharmacological potentials (Ismail, 2019). Ismail (2019) added that traditional uses of *S. polyanthum* include medicinal and non-medicinal uses for its leaves, fruits, and bark. Apart from its medical function, *S. polyanthum* is eaten raw as a salad, or as the Malay call "ulam." Mature leaves can also be added to improve the flavour of the Malay cuisines.

Many advantageous medicinal properties of *S. polyanthum* have been found, including the capacity to inhibit dental plaque, act as an acetylcholinesterase inhibitor, have lipase inhibitory activity, and exhibit antihypertensive, antibacterial, antidiabetic, antidiarrheal, antioxidant, anticancer, and antitumor properties (Ismail, 2019). According to Nordin et al. (2019), comprehensive approaches, such as utilising vitamins or herbs throughout treatment, are not new and can be helpful in a variety of ways. These herbs can also be used in concert with commercially accessible ones to improve the prognosis of the treatment.

A study conducted by Nordin et al. (2019) demonstrates that the *S. polyanthum* extract exhibits the greatest zone of inhibition against *S. aureus*, measuring 13.5 mm in diameter, at a dosage of 1000 mg/ml. In dairy cattle, mastitis can be prevented and controlled by using the plant, according to the MBC of *S. polyanthum*, which was found to be 125 mg/ml. According to the study's findings, *S. aureus*, *S. hyicus*, and *S. intermedius* can be inhibited by the hydromethanolic extract of *S. polyanthum*.

It is intriguing to consider this plant as a dietary supplement for the management and avoidance of obesity (Ismail, 2019). Prior studies by Chalabi et al. (2020) documented that the decoction or extract of bay leaf is an efficient antidiabetic medication for lowering blood glucose. Tannins are substances that occur naturally in plant tissue and have been shown to have a variety of positive health impacts, including antiviral and antibacterial properties (Halim & Maryani, 2022).

The primary possible markers of medicinal use in tea made from bay leaf were their tannin and flavonoid contents (Halim & Maryani, 2022). Furthermore, longer-brewed tea made from *S. polyanthum* included higher levels of condensed tannin, flavonoid, and phenolic components.

2.1.3 *Curcuma longa* (Turmeric)



Fig. 2.1.3: *Curcuma longa* (Happy Valley Seeds, 2023).

Curcuma longa, a member of the Zingiberaceae family, has been utilised for ages and has exceptional therapeutic benefits. Since ancient times, Indians have used turmeric, which is derived from the rhizome, as a spice, food preservative, and colouring agent (Park et al., 2019).

Classical Unani literature has described the pharmacological activities and applications of *C. longa* in a variety of fields, including gastrointestinal, cosmetics, and respiratory systems. In the Unani medical system, it is recognised for its importance either as a stand-alone medication or as a component of several formulations that cure a wide range of illnesses (Ansari et al., 2020).

According to Ansari et al. (2020), the active ingredient, curcumin, also known as diferuloylmethane, is a yellow pigment with a broad range of biological effects, including anti-inflammatory, hepatoprotective, anti-cancer, anti-fungal, and neuroprotective properties. In addition, the fact that curcumin is a cheap, natural substance and safe alternative to many medications suggests that it might be useful in the prevention and treatment of a wide range of illnesses.

For thousands of years, *C. longa* has been used as a medication to treat a wide range of ailments, including blood, liver, and stomach problems, infectious diseases,

and inflammation. Since curcumin can alter several signalling pathways implicated in the pathophysiology of various diseases, it is regarded as a privileged structure. Curcumin is the primary bioactive chemical present in the rhizome of *C. longa* (Tung et al., 2019).

According to research on animals, curcumin may be effective in treating a variety of human conditions, such as diabetes, obesity, cancer, neurological and mental disorders, and long-term illnesses affecting the kidneys, liver, eyes, lungs, gastrointestinal tract, and cardiovascular system. Curcumin is being sold as a supplement in many countries and it comes in a variety of forms, such as pills, energy drinks, capsules, tablets, soaps, and cosmetics (Tung et al., 2019).

The numerous health advantages of curcumin have drawn attention from all over the world. According to Tung et al. (2019), these benefits seem to be mainly attributed to their anti-inflammatory and antioxidant properties. Curcumin may be able to assist with the treatment of metabolic syndrome, oxidative and inflammatory diseases, arthritis, anxiety, and hyperlipidemia, according to research. It may also aid in the control of inflammation and stiffness in the muscles brought on by exercise, improving recuperation and performance in those who engage in physical activity.

2.1.4 *Andrographis paniculata* (“Hempedu bumi”)



Fig. 2.1.4: *Andrographis paniculata* (Alamy, 2019).

Several Asian countries have employed *A. paniculata* as a supplementary treatment for COVID-19. Its active components have antiviral, anti-inflammatory, immunomodulatory, and antipyretic properties that may be useful in the treatment of COVID-19 (Intharuksa et al., 2022). The Ministry of Public Health in Thailand (2021) has designated *A. paniculata* as one of the essential medicinal herbs to be included in the Thailand National List of Essential Medicines, which is used in hospitals and public health services.

As opposed to prevention, *A. paniculata* is a promising natural source for drug discovery on COVID-19 post-infectious treatment. *A. paniculata* and its active ingredients have good antiviral, anti-inflammatory, immunomodulatory, and antipyretic properties that make them potentially useful for treating COVID-19 (Intharuksa et al., 2022). *A. paniculata* is advised to be used in conjunction with contemporary medicine in many nations, including China and Thailand.

To prevent the progression of mild symptoms to severe symptoms, patients with COVID-19 infection were advised to take an oral dosage of *A. paniculata* and its extract, together with 180 mg of andrographolide, every day (National Drug Selection Committees. Thai National List of Essential Medicine, 2021). Ghosh et al. (2020) stated that *A. paniculata* should be used in an Ayurvedic process for treating COVID-19 and boosting immunity, according to a recommendation made by the Ministry of AYUSH in India.

Intharuksa et al. (2022) stated that recent in silico and in vitro models have chosen and analysed *A. paniculata* extract and derivatives of andrographolide as natural lead drugs with the potential for COVID-19 treatment. It was shown that the active component extracted from *A. paniculata* had a higher propensity to interact

with target proteins specific to the virus. The pharmacological properties of andrographolide include anti-inflammatory, anti-leishmanial, antiviral, antipyretic, analgesic, hepatoprotective, anti-allergic, antibacterial, anticancer, anti-diabetic, anti-dyslipidemic, and neuroprotective effects (Kandanur et al., 2019; Kumar et al., 2020; Luo et al., 2020; Liu et al., 2020; Geng et al., 2019; Wang et al., 2020; Zhang et al., 2019; Paemanee et al., 2019).

According to Intharuksa et al. (2022), herbal medicine *A. paniculata* has been used historically to treat respiratory illnesses and the flu because of its potent antiviral, anti-inflammatory, and immunomodulatory properties. For generations, people have used *A. paniculata* to cure and prevent the common cold, the flu, and associated symptoms. This use has been widespread in many nations, particularly in China, India, Southeast Asia, and Scandinavia. According to scientific research conducted by Intharuksa et al. (2022), *A. paniculata* and its active ingredients have strong antipyretic effects.

A. paniculata is included as a dietary supplement in the US pharmacopeia and is mentioned in several official materia medica and national pharmacopeia, including the Chinese, Indian, Thai, and British herbal pharmacopeias (Intharuksa et al., 2022).

According to instructions from the China National Health Commission, patients in Xiyanping, China, who have progressive stages of COVID-19 infection may benefit from a commercially available injection of *A. paniculata* extract that contains andrographolides (Zhang et al., 2021). Inflammation was reduced, viral clearance was improved, and associated symptoms like fever, coughing, and lung rales were all improved by the Xiyanping injection programme (Zhang et al., 2021; Wang et al., 2021).

Based on Intharuksa et al. (2022) journal article, both in Asia and Europe, the aerial portions and roots of *A. paniculata* are used for a variety of medical reasons in numerous nations. The plant is said to contain a large number of flavonoids, lactones, and diterpenoids. Furthermore, the results of this investigation show that throughout the previous thirty years, several flavonoids have been identified. Although flavonoids were also discovered in the plant's aerial sections, their separation and elucidation were mostly accomplished utilising the root (Intharuksa et al., 2022).

All parts of the plant, including the leaves, stems, and roots, contain andrographolide, the primary active ingredient in *A. paniculata* (Intharuksa et al., 2022). In addition, Maiti et al. (2019) found that xanthone, polysaccharide, quinic acid, and its derivative, sitosterol, and others were identified as minor chemical components in *A. paniculata*.

According to the review conducted by Shi et al. (2020), *A. paniculata* and Andrographolide's ethanolic extracts are efficient at preventing the formation of infectious virion and may even work in concert with contemporary medications. The plant *A. paniculata* has the potential to yield innovative antiviral drugs due to its overall virucidal bioactivity against a wide range of viruses from different families through a variety of methods (Intharuksa et al., 2022).

It has been known for more than 20 years that *A. paniculata* and its components, diterpene lactones, display immunomodulatory actions via a variety of mechanisms. Dai et al. (2019) mentioned that some investigations revealed that andrographolide had anti-pulmonary inflammatory properties. *A. paniculata* has the potential to be a promising herbal remedy that can be used in conjunction with its

anti-pulmonary inflammatory characteristic as an additional or alternative method of treating COVID-19 disease development (Intharuksa et al., 2022).

2.1.5 *Orthosiphon stamineus* (“Misai kucing”)



Fig. 2.1.5: *Orthosiphon stamineus* (Naturmed Scientific, 2022).

O. stamineus is a Lamiaceae species known as "Misai Kucing" in Malaysian communities. According to Silalahi (2019), the name “Misai Kucing” was derived from its flower structure, which features a stamen structure like the cat's whiskers. The aerial portions, or dried stems and leaves, are sometimes prepared as tea to treat a range of conditions, including urogenital tract infections and inflammatory illnesses (Gimbun et al., 2019).

This plant is widely used in Southeast Asian traditional medicine to treat a variety of conditions, including gonorrhea, diabetes, hypertension, gallstones, tonsillitis, epilepsy, rheumatic illnesses, menstrual disorders, lithiasis, renal calculus, edema, influenza, hepatitis, jaundice (Gimbun et al. 2019). *O. stamineus* extracts are thought to have antidiabetic effects because of how they affect the metabolism of glucose. According to reports, this plant is also a well-liked alternative antidiabetic treatment for type II diabetes.

The Southeast Asian population has long been aware of *O. stamineus* leaf extract's diuretic properties. Because of this advantage, infusions of *O. stamineus* have been utilised in traditional medicine to treat a range of kidney conditions, including infections and renal calculi (Gimbun et al., 2019). Although the exact

quantity needed for effective treatment is unknown, fresh leaf decoction taken twice a day is typically used to treat urinary tract infections. A decoction made from dried leaves is frequently used to treat dysuria and stranguria.

According to Chung et al. (2020), the plant's secondary metabolites have a variety of effects, including anti-inflammatory and gastroprotective properties, as well as anorexic and antihypertensive properties. *O. stamineus* is naturally endowed with rich primary metabolites, which include proteins, lipids, and carbohydrates, in addition to secondary metabolites.

O. stamineus may also be used as a possible therapy for conditions affecting the central nervous system (CNS). According to the published research, *O. stamineus* may be able to treat CNS diseases such as oxidative stress, neurodegeneration, and neuroinflammation. It may also be a possible neuroprotective drug (Chung et al., 2020). Beyond averting memory loss, *O. stamineus* might also be able to improve memory. The synthesis of catecholamine neurotransmitters depends on two important enzymes: dopamine and noradrenaline (Chung et al., 2020).

2.2 Fermentation

Main biochemical alterations to the food matrix are accomplished through fermentation, a processing technique that uses microorganisms and their enzymes (Samtiya et al., 2021). Fermented foods are connected to the dietary state of their population as well as the cultural ties between various countries.

Researchers become quite interested in fermentation to learn more about its mechanism and to commercialise food items. Sheikha and Hu, (2020) mentioned that one kind of metabolic action called fermentation releases energy by oxidising carbohydrates when there are no external electron acceptors present.

Traditional food processing techniques include fermentation, boiling, soaking, puffing, and germination to lower antinutritional components and improve protein digestion (Samtiya et al., 2020; Kumari & Platel, 2020). Additionally, by generating the enzyme phytase, which breaks down the phytic acids in plant-based diets, fermentation increases the mineral bioavailability (Samtiya et al., 2021).

Commercial microbial enzymes have been used extensively to increase the functional value of plant phytochemicals; however, fermentation by lactic acid is preferred to increase the nutraceutical significance of food because it is less costly while simultaneously improving the overall nutritional and organoleptic properties (Rollán et al., 2019).

In addition, fermentation is employed as a microbiological and enzymatic food processing technique. By reducing polyphenol and phytic acids and increasing safety, antinutrient elimination, longer shelf life, appealing flavour, and nutritional enrichment, fermentation improves the quality of raw food components (Kumari & Platel, 2020). However, Rousseau et al. (2022) mentioned that it may also increase the bioavailability of minerals by producing organic acids.

Aquaculturists have previously employed probiotics and synbiotics to improve immune systems, growth, and disease resistance; they also lower feeding costs, repair the production environment, and aid in the evolution of sustainable aquaculture (Nargesi et al., 2020; Verschuere et al., 2000; Amenyogbe et al., 2020; Yılmaz et al., 2020).

Furthermore, relatively little energy is needed for fermentation and no complex equipment is needed. Consequently, as it increases the nutritional content of food goods, it may be a practical way for developing countries to increase the value

of locally produced raw materials for better local economies (Tsafrakidou et al., 2020). It may also be an effective way to combat malnutrition.

Developing technology to make fermentation simpler, using analytical technologies such as monitoring systems to assess temperature, pH, pressure, and dissolved oxygen, and using automated fermenters to control the fermentation process are some of the strategies that have been explored (Terefe & Augustine, 2020).

2.2.1 Benefit of fermentation

The antioxidant properties of these plants in vitro were greatly enhanced by fermentation. These results suggest that fermented plants with enhanced antioxidant abilities might be useful as potential additives for dog foods (Park et al., 2019). By presenting an aroma that dogs enjoy, these additions made from medicinal plants may increase the acceptance and preference of the food.

According to Terefe and Augustine (2019), eating fermented food is often linked to many health advantages. Probiotic-containing fermented meals improve nutritional qualities by generating bioactive molecules and increasing mineral bioavailability.

Synbiotics are a well-balanced blend of probiotics and prebiotics that can significantly boost aquaculture output by promoting fish growth and strengthening their immune systems, facilitating better digestion and absorption, managing diseases, and regulating water quality (Nguyen et al., 2019; Waagbo and Remo, 2020). For instance, in the catfish *Clarias gariepinus*, extracts of hairy aubergine *Solanum ferox* combined with probiotic *Lactobacillus casei* enhanced both growth indices and immunity (Hardi et al. 2022).

However, studies in the literature demonstrate that fermentation can increase the nutritional and mineral bioavailability of plant meals (Samtiya et al., 2021). Doan et al.'s (2019) study saw a comparable outcome when probiotic *Lactobacillus plantarum* and pectin generated from orange peels were fed to Nile tilapia. This resulted in significant changes in the fish's development, mucus production on the skin, and immunological response in the serum.

In general, the number of probiotics rose while the quantity of infections dropped. Intestinal microbiota in dogs saw a favourable composition shift when we fed them diets containing a fermented product (Park et al., 2019). By giving lactic acid and probiotic LAB to the feeding animals as well as enhancing the bioavailability of polyphenols, LAB fermentation is known to improve the biological qualities of plant matrices, which may be advantageous to the health of the hosts.

For those fed fermented foods, the faecal microbiota shifted in a generally positive way. Dogs' gut microbiome may benefit from the inclusion of fermented medicinal herbs in their diet. These findings conducted by Park et al. (2019) indicate that fermented turmeric, glasswort, Ganghwa mugwort, and their combination could be included in dog diets as nutritionally useful foods because of their increased antioxidant activity.

According to Fujaya et al. (2022), high concentrations of flavonoids, alkaloids, potassium, vitamin C, sodium, lipase, and protease were found in the fermented plant extract. Following 35 days of incubation, tilapia-fed feed coated with fermented herb extract showed improved weight increase, specific growth rate, and feed efficiency. Furthermore, the number of goblet cells, lymphocytes, and improved length and density of intestinal villi, as well as the retention of nutrients to support

growth, all had a favourable impact on the feed efficiencies of treatments with fermented herb extract.

According to the study's findings, fermented herb extract is a functional feed additive that has the potential to boost tilapia development and increase feed efficiency (Fujaya et al., 2022). The tilapia fingerlings in this study benefited from the fermentation method applied to the herbal extracts.

2.2.2 The effluent of fermentation on herbs

Researchers identified the fermented herbal extract's phytochemical and biochemical characteristics. By employing ultraviolet-visible (UV-Vis) spectrophotometry, the Integrated Research and Testing Laboratory of Gadjah Mada University in Indonesia was able to determine the presence of alkaloids, saponins, and flavonoids, while sitosterol and curcumin were examined using thin-layer chromatography (Fujaya et. al, 2022).

Endogenous enzymes are triggered during food fermentation because of the decreased pH, which helps to lower phytic acid. By generating phytases that lessen the acidic components in plant-based diets, fermentation increases the bioavailability of minerals such as calcium, zinc, and ferum (Song et al., 2019). Due to the conversion of ferric iron into the more easily absorbed ferrous iron, fermentation lowers pH, which improves iron absorption (Samtiya et al., 2021).

Additionally, there is increased mineral bioavailability if the food ingredients are ground before fermentation begins. Castro-Alba et al. (2019) mentioned the benefits of grinding come from the food material's cellular structure being broken up and its larger surface area facilitating phytase dispersion for a more efficient breakdown of phytate. According to research conducted by Mohapatra et al. (2019)

and Ketnawa and Ogawa, (2019), fermentation may change the amount of protein and amino acids in food.

According to earlier research conducted by Adejuwon et al. (2020), the fermentation process activates enzymes that break down starch into simple sugars and maltodextrins, like maltase and α -amylase. Furthermore, research has shown that active α -amylase and maltase cause a rise in glucose during the early fermentation phase. It makes sense to conclude that foods with low glycemic index are those that have a low glucose level since the natural fermentation of sugars and carbohydrates produces lactic and propionic acids (Ng et al., 2019; Ashaolu et al., 2021).

Furthermore, fermentation leads to significant pH decreases, increased generation of ethanol and carbon dioxide, and decreased starch content in many types of millet. It also facilitates the synthesis of the enzyme phytase (Castro-Alba et al., 2019).

2.2.3 The microorganisms involved in herbs product fermentation

Fermentation increases microbial activity eliminates anti-nutritional substances and increases the bioavailability of macro and micronutrients, including phytochemicals (Samtiya et al., (2021). The microbial metabolism during fermentation increases the digestibility of macromolecules and raises the bioavailability of phytochemicals and macro/micronutrients.

Lactic acid bacteria (LAB) are a group of bacteria that belong to several genera and produce lactic acid as a main metabolite (Samtiya et al., 2021). LAB is used to manufacture fermented foods. LAB is the most widely utilised microbe for food fermentation because it produces lactic acid, which improves food preservation. Some of these genera include *Streptococcus*, *Leuconostoc*, *Lactobacillus*, and

Pediococcus. According to Rollan et al. (2019), LAB are aerobic, fermentative, cytochrome-deficient, non-spore-forming, acid-tolerant, catalase-negative, and Gram-positive microorganisms.

Food made from plant fermentation usually improves the micronutrient bioavailability and food shelf life by enhancing the gut microbial ecology and promoting health. The primary cause of these health advantages is the fermentation-producing probiotic microorganisms, particularly the lactic acid-producing variety (Chileshe et al., 2020).

Enzymes such as phytase, protease, lipase, and amylase, are produced by microorganisms utilised in fermentation and are capable of hydrolyzing proteins, fats, and carbohydrates into easily digested components with acceptable texture and flavour. Additional antinutritional that may be broken down by microbial enzymes include tannins, phytates, and protease inhibitors (Dhull et al., 2021). The International Scientific Association for Probiotics and Prebiotics (ISAPP) expert panel report defines fermented food as "food made through desired microbial growth and enzymatic conversions of food components." (Samtiya et al., 2021).

According to Samtiya et al. (2021), due to the action of microbes during the metabolism and hydrolysis of lipids and carbohydrates as an energy source, the fermentation process causes relative changes through dry matter loss.

2.3 Antibacterial activity for fish

In the aquaculture industry, illnesses result in a 50% loss of production (Gabriel, 2019). According to Gupta et al. (2021), infectious illnesses, which result in significant economic losses globally, are currently one of the limiting issues in

aquaculture. A disease-related loss of almost 300,000 metric tonnes of tilapia occurred in 2019 (FAO, 2021).

Fish pathogens are primarily prevented or treated using antibiotics (Corum et al., 2020; Terzi et al., 2020). According to Suphoronski et al. (2019), one major factor contributing to the rise in the frequency of diseases brought on by resistant bacteria is the use of antibiotics and other synthetic substances for treatment and growth stimulation. Antibiotics have several negative impacts on human health and the environment, yet they are mostly employed to control these infections.

To maintain sustainability and environmental friendliness, the aquaculture industry needs to use synthetic growth boosters and substitute antibiotics. Using naturally occurring herbs with therapeutic properties is one option. There is evidence that natural herbs, whether used as natural or non-antibiotic growth promoters, have active components that can enhance aquaculture production performance (Fujaya et al., 2022).

A previous study conducted by Gabriel and González-Redondo (2019), explains that there has been considerable interest in the use of medicinal herbs in aquaculture over the last two decades, to provide safe, environmentally friendly compounds to replace antibiotics and other chemotherapeutic drugs, as well as improve the fish immune system and control fish diseases.

Medicinal plants can replace pharmaceutical medications in aquaculture if they are administered properly (Gabriel, 2019). As immunostimulants and substitutes for antibiotics or chemotherapeutic treatments, medicinal plants have several benefits in aquaculture, as this review conducted by Gupta et al. (2021) highlights.

Secondary metabolites and phytochemical substances found in herbs are effective against viral, bacterial, and parasite illnesses in fish (Tkachenko, 2022). Moradi and Hadi (2021) mentioned that these unprocessed or extracted compounds have antibacterial properties that can reduce infections and strengthen fish immune systems, despite not being a drug. According to Faheem et al. (2022), numerous plants enhance fish development and general health; some boost immunity to ward against infections, while others stimulate hunger by directly affecting the fish's neuroendocrine system.

It has been demonstrated that bioactive plant chemicals stimulate the innate immune response in fish by activating molecules and cells in the immune system (Mbokane & Moyo, 2022). Bioactive substances that have antibacterial, antifungal, and antiparasitic properties have also been found in plants. The fish's immune system may be assisted in getting rid of disease-causing substances by the presence of substances having these qualities, which may prevent or limit the growth of infections. Vijayaram et al. (2022), mentioned that certain plant-derived chemicals, like thymol and carvacrol, have been demonstrated to enhance intestinal immunity and gut health.

Based on the antiprotozoal activity of medicinal plants, new antiparasitic medications have been developed. Furthermore, aquaculture producers find medicinal plants to be a desirable alternative due to their affordability and easy availability (Özil, 2023). A diet rich in medicinal plant compounds also helps the kidneys and liver, which enhances the general health of fish. Additionally, improved feed absorption and digestion result in improved growth due to the increased synthesis of digestive enzymes. Fish that consume medicinal plants also benefit from reduced oxidative stress and immune system regulation, which improves illness

resistance. It is reasonable to think of medicinal plants as a long-term substitute for controlling bacterial illnesses in aquaculture (Faheem et al., 2022).

2.3.1 Antibiotic

One of the biggest risks to public health in the twenty-first century is bacterial antimicrobial resistance, or AMR, which is the result of bacterial mutations that make antibiotics less effective in treating infections (Murray et al., 2022). Certain bacteria produce digestive enzymes that break down and eliminate antibiotic molecules (Nordin et al., 2019).

The overuse of antibiotics without proper supervision has led to a problem with antibiotic resistance, which has made it difficult to treat mastitis and increased operating costs, particularly for farmers who lack access to effective antimicrobial medications (Nordin et al., 2019).

In developing nations, aquaculture is crucial to the livelihood, jobs, and local economic growth of coastal communities (Fujaya et al., 2022). Farmers suffered significant losses as a result of an enormous rise in disease cases and crop failures brought on by the aquaculture sector's quick intensification (Waiho et al., 2021).

Fish pathogens are primarily prevented or treated using antibiotics (Corum et al., 2020; Terzi et al., 2020). Tkachenko et al. (2022) stated that antibiotics are biologically active chemicals that can harm aquatic ecosystems. Antimicrobial drug use in aquaculture may develop resistance to harmful bacteria. It is believed that 80% of antimicrobials used in aquaculture end up in the environment, where they can harm fish, terrestrial animals, humans, and the environment in general.

Therefore, it has become more and more important in recent decades to cultivate aquatic creatures sustainably by avoiding the use of synthetic chemical

compounds and antibiotics as growth boosters (FAO, 2020). In fish feed, several artificial growth promoters, hormones, antibiotics, and antioxidants, including hydroxyanisole, butylated hydroxytoluene, and ethoxyquin, have been used to increase fish production and prevent damage from oxidative stress (Merel et al., 2019; Bahadir et al., 2019; Faheem et al., 2022). But the health of fish and consumers has been seriously threatened by their bioaccumulation (Jabeen et al., 2021; Faheem et al., 2022).

According to the Food and Agriculture Organization (2020), antibiotic application in aquaculture resulted in the establishment of antibiotic-resistant bacterial strains and the presence of drug residue in treated fish diets. Because bacteria become resistant to synthetic chemicals, vaccines, and antibiotics and because of their high residues, their usage is not sustainable. Furthermore, several nations have outlawed the use of specific chemicals that are used to treat fish infections because they pose a risk to human health (Faheem et al., 2022).

Due to their high cost and unavailability to impoverished rural farmers, these medications have negative effects on human health and the environment. Additionally, some harmful bacteria become resistant to antibiotics, resulting in residues in the final products (Gabriel, 2019). The use of therapeutic herbal extracts as an alternative to chemical agents in aquaculture to increase productivity is receiving a lot of attention these days (Gabriel, 2019).

Mbokane and Moyo (2022) stated that small-scale farmers are particularly impacted because they frequently lack the money to pay for costly commercial diets, antibiotics, and artificial hormones that are necessary to control reproduction. Fish growth performance, illness resistance, and reproduction can all be improved by adding medicinal herbs to feed, which is the most promising of the suggested

treatments. Medicinal plants are more accurate than chemotherapy drugs due to their wide range of actions, affordability, and ability to prevent disease in an environmentally acceptable manner. Fish health can be enhanced and their haematological profile altered by incorporating medicinal herbs into their diets (Anjusha et al., 2019).

As a potential substitute for antibiotics, medicinal plants have been shown to have many positive effects on fish health, including immune system regulation (Gupta et al., 2021). Reverter et al. (2021), demonstrated that many substances, including polyphenols, carbohydrates, amino acids, flavonoids, alkaloids, tannins, organic acids, volatile oils, polysaccharides, minerals, and vitamins, are found in plants; some of these substances are essential for fish and other animal growth, immunity, and general well-being. Many scientific investigations found that fish-fed herbal plants had significantly higher haemoglobin status, leucocyte counts, and red blood cell counts (Anjusha et al., 2019).

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of the study area

The study was conducted in Makmal Akuatik 2 and Makmal Parasitologi, Universiti Putra Malaysia Kampus Bintulu, Sarawak.

3.2 Preparation and characterization of yeast-fermented herbs

3.2.1 Collection and identification of plant materials

Five types of plants were used in this study which are *Syzygium polyanthum*, *Curcuma longa*, *Andographis paniculata*, *Orthosiphon stamineus*, and *Euphorbia hirta*. Commercial plants except *E. hirta* were purchased from the local market, at RM30 for 500 grams. Fresh samples of *E. hirta* were collected in March 2023 from Ladang Kongsu 2, Universiti Putra Malaysia Bintulu Campus, Sarawak.

3.2.2 Sample preparation

The samples of *S. polyanthum*, *C. longa*, *A. paniculata*, *O. stamineus*, and *E. hirta* were washed with distilled water and dried in the oven at 60°C for 72 hours. The plant was ground into a fine powder using the electrical grinder and stored in an air-tight plastic at room temperature.

3.2.3 Fermentation process

The five herbal extracts of *S. polyanthum*, *C. longa*, *A. paniculata*, *O. stamineus*, and *E. hirta* were fermented with probiotics, *Lactobacillus casei*, and commercial yeast, *Saccharomyces cerevisiae* at equal concentrations. 50 grams of each powdered plant is inoculated with 25 mL of *S. cerevisiae* and *L. casei* obtained from Yakult with an initial count of 8.23 log CFU/mL. Yakult provided the probiotic strain of *L. casei*, while instant dried yeast, *S. cerevisiae*, a commercial yeast, was utilised for the

fermentation process (Fujaya et al., 2022). 10 mL of molasses and distilled water is added to the inoculum. According to the study conducted by Utama et al. (2021), the plants which included *C. longa*, and *S. polyanthum* were mixed with molasses and *L. casei*. The inoculum was incubated at 28°C for 14 days anaerobically in polyethylene bags.

3.2.4 pH determination

The initial and final pH of the fermented plant extract was recorded in triplicate. 5 mL of each of the five fermented plant extracts is diluted with 50 mL of sterile distilled water. The pen-type pH meter (PH-20W) was used to examine the initial and final fermented herb extract. The average of the initial and final pH readings of the five fermented herbs are as follows:

Table 3.2.4: Initial and final pH of five yeast-fermented herbs

HERBS	BEFORE	AFTER
<i>Andrographis paniculata</i>	5.03 ± 0.06	4.5 ± 0.00
<i>Syzygium polyanthum</i>	4.07 ± 0.00	4.37 ± 0.06
<i>Curcuma longa</i>	4.87 ± 0.12	4.47 ± 0.12
<i>Euphorbia hirta</i>	4.63 ± 0.06	4.33 ± 0.06
<i>Orthosiphon stamineus</i>	4.67 ± 0.06	4.27 ± 0.06

3.3 Nutritional Analysis

The nutritional composition of the five different herb samples, both yeast-fermented and non-fermented, was assessed using the standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2005). The samples underwent a muffle furnace heating process at 500°C for approximately 5–6 hours to determine their ash content. To assess the crude fat content of the sample extract, a Soxhlet apparatus and petroleum ether (60–80°C) were employed as the extraction solvent. The micro-Kjeldahl technique was applied to determine the crude protein content. Utilizing the conventional biochemical analysis method, estimates for crude fibre

content, total soluble sugars, reducing sugars, non-reducing sugars, and total carbohydrates were calculated. It is important to note that the term "crude fibre" does not pertain to dietary fibre; instead, it denotes the residue left behind after food is processed with acid and alkali, primarily consisting of cellulose and lignin. The crude mixture of fat-soluble material in a sample is referred to as crude fat, typically extracted using the dry Soxhlet extraction method in anhydrous ether.

3.4 Mineral Profiling

A di-acid mixture of nitric acid and perchloric acid (9:4) was combined with 1g of each sample extract individually for mineral profiling. After leaving the combination alone for the entire night, it was digested at 115–118°C until a translucent, watery liquid was produced. After filtering, the liquid was topped off with 50 mL of double-distilled water. The final step involved the measurement of nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), copper (Cu), Zinc (Z), and Sodium (Na) in each sample using Atomic Absorption Spectroscopy (AA 800, Perkin-Elmer, Rodgau, Germany).

3.5 Herbs Extraction

50 g of each fermented and non-fermented herbs sample on a dry weight basis is soaked with 20 mL of 96% absolute ethanol in a 50 mL conical flask in the orbital shaker for 40°C at 150 rpm for 24 hours. The samples are filtered using 150 mm Whatman filter paper to allow the maximum quantity of bioactive compounds from the herbs. The herbs-filtered bioactive compound is extracted using the rotary vacuum evaporator at 40°C for three hours and the herb extract is then stored in the chiller at -20°C for further analysis.

3.6 Solvent reconstitution

10% of 96% ethanol (EtOH) (*Ethanol 96% EMSURE®* Reag. Ph Eur) was used as the negative control. This EtOH solution is prepared by dissolving 10 mL of EtOH with 90 mL of distilled water. This 10% of EtOH was proven not to kill all microorganisms tested in this study. 10% herb extract was prepared by diluting 10 mg of the herb extract with 1000 µL of EtOH. 30 mcg of Tetracycline discs was used as the positive control in this study. It was kept in the chiller at 4°C for further use.

3.7 Antibacterial Activity Test

3.7.1 Preparation of Mueller Hinton Agar/Broth

The media of agar/broth is prepared based on the following manufacturer instructions provided on the media bottle (Oxoid Ltd. United Kingdom). These are the following general instructions for preparing MHA and MHB. The Mueller Hinton Agar (MHA) is prepared by dissolving 38 grams of MHA powder into 1L of distilled water in a 2L Schott bottle. While for the MHB, 21 grams of the MHB powder is dissolved into 1L of distilled water in a 2L Schott bottle. Both MHA and MHB solutions were autoclaved for 15 minutes at 121°C. The sterilized MHA solution is then aseptically poured into the sterile Petri dishes for about 20 mL each. The Petri dish is left at room temperature to solidify and the MHB was kept at room temperature to let it cool down. Then, the poured MHA and MHB were kept in the chiller for further usage.

3.7.2 Standardization of bacteria culture

The bacteria inoculum was prepared by following the McFarland turbidity standard with a slight modification. A clinical strain of *Escherichia coli*, *Streptococcus gallolyticus*, *Staphylococcus aureus*, and *Aeromonas hydrophila* were obtained from

the Faculty of Animal Science and Fisheries, Universiti Putra Malaysia Bintulu Campus, Sarawak. Overnight antimicrobial assays were carried out by subculturing the bacteria strain to the Mueller-Hinton broth (MHB) and were incubated at 37°C for 18 hours to obtain bacteria culture in the log phase and was standardized at 0.5 McFarland at 600 nm by using a spectrophotometer (DLAB SP-UV1100 Spectrophotometer). The turbidity of the McFarland will be equivalent to 1.5×10^8 CFU/mL. Spectrophotometer UV will be used for the absorbance measurement (Toh et al., 2023).

3.7.3 Agar Well Diffusion Assay

According to Toh et al. (2023), sterilized agar was divided into 4 sections and labelled with types of plant extract. 1ml of each bacteria strain from the cultured MHB was cultured on MHA using a sterilized cotton swab with 3 directions and let dry for 15 minutes. Each bacteria culture was triplicate (144 petri dishes were used). 4 mm sterilized bores were punched through the seeded cultured MHA and 50 µL of fermented and non-fermented different herb extract, and 10% of 96% ethanol (EtOH) (*Ethanol 96% EMSURE® Reag. Ph Eur*) were transferred into it. A 30 mcg Tetracycline disc was placed on top of MHA for the positive control, which is the commercial antibiotics. The cultured were dried at room temperature for 15 minutes before being incubated at 37°C for 24 hours. Antimicrobial activity was measured by using a ruler (mm).

3.8 Statistical analysis

The SAS statistical programme (v9.4, SAS Institute Inc., Cary, NC, USA) was used to perform the analysis of variance. The statistical significance at the 95%

significance level was evaluated using Duncan's multiple-range tests (Park et al., 2019).



CHAPTER 4

RESULTS AND DISCUSSION

4.1 Nutrient composition

In the current study, the comparative nutritional analysis of non-fermented and fermented five different types of herbs demonstrated variable results as presented in Table 4.1.

Table 4.1: Nutrient composition of non-fermented and yeast-fermented herbs (g/100g)

Herbs	Parameters					
	Carbohydrate (g)	Protein (g)	Fat (g)	Crude Fiber (g)	Ash (%)	Energy (kcal)
<u>Non-fermented</u>						
<i>Curcuma longa</i>	43.94 ± 0.70 ^b	6.36 ± 0.21 ^{cd}	2.51 ± 0.17 ^a	15.10 ± 0.39 ^d	12.78 ± 0.13 ^a	253.99 ± 11.62 ^b
<i>Syzygium polyanthum</i>	53.21 ± 0.36 ^a	3.44 ± 0.16 ^f	2.37 ± 0.12 ^{ab}	22.84 ± 0.18 ^c	7.17 ± 0.27 ^c	308.75 ± 3.82 ^a
<i>Andographis paniculata</i>	43.96 ± 0.40 ^b	5.69 ± 0.08 ^e	1.38 ± 0.20 ^{cd}	32.60 ± 0.74 ^a	6.15 ± 0.24 ^d	303.77 ± 9.11 ^a
<i>Euphorbia hirta</i>	53.28 ± 0.25 ^a	5.79 ± 0.11 ^{de}	2.15 ± 0.12 ^b	14.38 ± 0.42 ^d	9.05 ± 0.53 ^b	291.16 ± 12.29 ^a
<i>Orthosiphon stamineus</i>	42.99 ± 0.43 ^b	6.42 ± 0.20 ^{cd}	1.72 ± 0.13 ^c	28.89 ± 0.32 ^b	5.44 ± 0.18 ^e	290.45 ± 2.32 ^a
<u>Yeast-fermented</u>						
<i>Curcuma longa</i>	26.54 ± 0.38 ^f	10.14 ± 0.03 ^a	1.57 ± 0.03 ^c	8.70 ± 0.44 ^g	7.62 ± 0.00 ^c	147.15 ± 0.31 ^f
<i>Syzygium polyanthum</i>	36.66 ± 0.47 ^c	6.59 ± 0.02 ^c	2.27 ± 0.11 ^{ab}	9.96 ± 0.29 ^f	2.09 ± 0.02 ^g	195.55 ± 3.47 ^c
<i>Andographis paniculata</i>	24.77 ± 0.03 ^g	6.61 ± 0.13 ^c	1.12 ± 0.02 ^d	13.19 ± 0.19 ^e	12.72 ± 0.07 ^a	156.33 ± 0.73 ^{ef}
<i>Euphorbia hirta</i>	32.18 ± 0.48 ^d	6.32 ± 0.50 ^{cd}	1.20 ± 0.02 ^d	10.21 ± 0.14 ^f	3.47 ± 0.02 ^f	174.35 ± 2.36 ^{de}
<i>Orthosiphon stamineus</i>	30.59 ± 0.44 ^f	7.57 ± 0.10 ^b	1.07 ± 0.06 ^d	12.33 ± 0.12 ^e	3.57 ± 0.03 ^f	175.93 ± 1.44 ^d

Results are expressed as mean ± SE and are the average of four replicates. Different letters within the same column differ significantly (P<0.05).

The findings underscore a significant disparity in carbohydrate content between non-fermented and yeast-fermented herbs. Notably, all five non-fermented

herbs exhibited substantially higher carbohydrate levels compared to their yeast-fermented counterparts. Within the non-fermented herb group, *E. hirta* emerged as the leader in carbohydrate content, boasting an impressive 53.28g. Following closely behind, *S. polyanthum* demonstrated a similar richness in carbohydrates at 53.21g. *A. paniculata* and *C. longa* showcased slightly lower but still substantial carbohydrate content at 43.96g and 43.94g, respectively. *O. stamineus* recorded a comparatively lower but notable 42.99g of carbohydrates among the group.

This aligns with existing research that emphasizes the impact of fermentation on carbohydrate composition. Fermentation processes, catalyzed by microorganisms like *Lactobacillus* species, play a pivotal role in altering the carbohydrate makeup of foods. Every distinct plant-based product has an associated glycemic index (GI). By transforming glucose produced during starch breakdown into short-chain organic acids like propionic, acetic, and lactic ones, fermentation lowers GI (Knez et al., 2023). Through their conversion to polysaccharides, monosaccharides are also reduced by fermentation. Energy is needed by lactic acid bacteria (LAB) for growth, and it is obtained by metabolism and absorption of several substances, including sugar, minerals, fatty acids, and amino acids (Emkani et al., 2022). Following four days of fermentation, the fermented food accumulated exclusively malto-oligosaccharides (G3/maltotriose), whereas the non-fermented sample contained no such oligosaccharide (Hor et al., 2021). Studies indicate that the carbohydrate content in fermented foods tends to be lower than in non-fermented variants due to enzymatic breakdown during the fermentation process. Additionally, fermentation's acidification, particularly the production of lactic acid, influences the transformation of rapidly digested starch (RDS) into slowly digested starch (SDS), impacting GI levels (Hor et al. 2020). Based on a previous study by Xu et al. (2020), carrot juices

that had undergone fermentation showed a similar correlation, with 52% more polysaccharides and 27% fewer monosaccharides found. This was most likely brought by the enzyme fructosyltransferases, which are generated by starter microorganisms like *Lactobacillus gasseri*, converting simple carbohydrates to insulin. According to Sharma et al. (2020), enzymatic hydrolysis plays a crucial role in fermented foods by reducing the amounts of anti-nutrients such as tannins and phytic acid which are broken down by phytases, which increases the bioavailability of simple sugars or polysaccharides (amylases). Moreover, the presence of microbial enzymes, acids, and short-chain organic acids generated during fermentation not only alters carbohydrate structures but also contributes to the probiotic properties essential for gut health (Emkani et al., 2022). *Lactobacillus* species, prominent in lactic acid fermentation, drive these beneficial changes in carbohydrates and contribute to the overall health-promoting attributes of fermented foods (Dalile et al., 2019).

The protein content of yeast-fermented *C. longa* (10.14 ± 0.03), *O. stamineus* (7.57 ± 0.10), *A. paniculata* (6.61 ± 0.13), and *S. polyanthum* (6.59 ± 0.02) was significantly higher than that of the non-fermented herbs. There was no significant difference between the protein content of yeast-fermented and non-fermented *E. hirta* (6.32 ± 0.50). Among these, *C. longa* exhibited the highest protein content at 10.14g. Studies have shown that fermenting legume proteins with specific bacteria enhances their water-holding capacity due to bacterial proteolysis (Xing et al. 2020). This process modifies the protein structure, exposing more hydrophilic areas, thereby increasing water retention. Additionally, lactic acid fermentation has been found to reduce antinutritional chemical levels and improve bean protein digestibility (Emkani et al. 2022).

However, it's important to note that the impact of fermentation on protein characteristics can vary. While some studies demonstrate positive effects, like increased digestibility in lentils and improved protein content in fermented yam bean flour, others, like Arteaga et al. (2021), observed decreased emulsifying capacity in fermented pea protein. This reduction in emulsifying ability was linked to protein aggregation and interactions with fermentation byproducts, potentially hindering their ability to interact with oil molecules. Furthermore, incorporating specific bacteria like *Bacillus subtilis* natto into bean fermentation might enhance essential amino acid content and improve plant protein digestibility, which could be particularly beneficial for vegans seeking well-rounded protein sources (Knez et al., 2023).

The fat content of non-fermented herbs of *C. longa* (2.51 ± 0.17), *E. hirta* (2.15 ± 0.12), and *O. stamineus* (1.72 ± 0.13) was much higher compared to the three yeast-fermented plants. There is no significant between both yeast-fermented and non-fermented *S. polyanthum* (2.27 ± 0.11 and 2.37 ± 0.12) and *A. paniculata* (1.12 ± 0.02 and 1.38 ± 0.20). The non-fermented *C. longa* (2.51 ± 0.17) has the highest number of fats followed by *E. hirta* (2.15 ± 0.12), and *O. stamineus* (1.72 ± 0.13). The role of lipids in biological processes is indeed multifaceted, extending from membrane structure to energy provision, and their modulation via fermentation processes has garnered significant attention in recent research. Fermentation, as indicated by Knez et al. (2023), not only reduces fat content but also impacts protein levels. Emkani et al. (2022) highlighted the energy needs of lactic acid bacteria (LAB) for growth, which involves metabolizing various substances, including fatty acids. Moreover, the presence of microbial enzymes and acids during fermentation can induce changes in lipid conformation and structure. Enzymatic hydrolysis during

fermentation, noted by Sharma et al. (2020), leads to decreased anti-nutrients like tannins and phytic acid while enhancing the bioavailability of free fatty acids. Hor et al. (2021) demonstrated that fermented products exhibited higher quantities of certain beneficial fatty acids such as octanoate, dodecanoate, pentadecanoate, and eicosenoate compared to non-fermented ones. Chen et al. (2022) observed that fermented soybeans contained increased levels of short-chain fatty acids (SCFA), primarily acetic acid. This rise in SCFA content during fermentation, particularly acetic acid, illustrates the dynamic changes in fatty acid profiles. Overall, the impact of fermentation on lipids spans various aspects, including composition alterations, changes in fatty acid profiles, and potential implications for health and disease management, highlighting the diverse and promising facets of this process.

The comparison between non-fermented and yeast-fermented herb samples highlights a significant disparity in crude fiber content. Among the non-fermented herbs, *A. paniculata* displays the highest average crude fiber content at 32.60g, followed by *O. stamineus* at 28.89g, *S. polyanthum* at 22.84g, *C. longa* at 15.10g, and *E. hirta* at 14.38g. However, intriguingly, the yeast-fermented herb samples demonstrate a contrasting trend, exhibiting the lowest crude fiber content compared to their non-fermented counterparts. This notable decrease in crude fiber content following yeast fermentation across all herbs indicates a significant impact of this fermentation process on the fiber composition of these plants. The reduction in crude fiber content post-fermentation suggests that yeast fermentation might play a role in breaking down or altering fiber structures within these herbs, potentially leading to a decrease in the measured fiber content. The discrepancy in crude fiber content between non-fermented and yeast-fermented herbs can be attributed to the microbial activity during fermentation, as indicated by Zhuang et al. (2021). They suggest that

the degradation of fiber during fermentation, primarily by Bacteroidetes, contributes to this reduction. Interestingly, diets rich in fiber are linked to the enrichment of these bacteria. However, as microbes utilize fiber as an energy source during fermentation, there's a consequent decrease in its presence in the final fermented product. Moreover, Xiao et al.'s (2022) study highlighted *A. paniculata*'s higher levels of crude fiber, neutral detergent fibre (NDF), and acid detergent fibre (ADF). This observation suggests the potential utilization of these herbs as dietary fibre supplements to promote animal intestinal health due to their rich fibre content. The fermentation process brings about notable changes, as indicated by Zhuang et al. (2021), including increased lactic acid, acetic acid, and propionic acid content, alongside a drop in pH to 3.89. These alterations signify an improved fermentation quality, inhibiting the growth of undesirable bacteria. Additionally, this process leads to enhanced crude protein levels and reduced NDF content, reflecting a transformation in the nutritional profile of the fermented herbs.

The comparison between non-fermented and yeast-fermented herbs highlights a notable difference in ash content. Among the non-fermented herbs, *C. longa* demonstrates the highest ash content at 12.78%, followed closely by yeast-fermented *A. paniculata* at 12.72%. However, the trend shifts for the other three herbs, where the non-fermented versions display higher ash content compared to their yeast-fermented counterparts. This divergence in ash content between non-fermented and yeast-fermented herbs suggests that the fermentation process might influence the mineral composition differently across various herb species. While non-fermented *C. longa* and yeast-fermented *A. paniculata* exhibit comparable high ash content, the pattern differs for the remaining herbs. Understanding the specific mechanisms underlying these variations in ash content between fermentation processes could

provide insights into how different herbs respond to microbial activity and fermentation conditions.

The comparison of average energy content between yeast-fermented and non-fermented herbs indicates a consistent trend: non-fermented herbs possess higher energy content than their yeast-fermented counterparts. Among the non-fermented herbs, *S. polyanthum* exhibits the highest average energy content at 308.75 kcal, followed closely by *A. paniculata* (303.77 kcal), *E. hirta* (291.16 kcal), *O. stamineus* (290.45 kcal), and *C. longa* (253.99 kcal). The observed decrease in energy content following fermentation aligns with the concept that fermentation processes, while enhancing other qualities of food, might contribute to a reduction in certain nutrients or energy-providing components. This phenomenon might be attributed to the utilization of components like fibre as an energy source by microbes during fermentation, leading to a decrease in their presence in the final product. Samtiya et al. (2021) perspective emphasizes fermentation as a cost-effective method of food preservation that not only improves organoleptic qualities but also enhances digestibility and assimilation of bioactive compounds. However, this improvement might come at the expense of certain nutrients or energy content, which could be consumed or utilized by fermenting microorganisms for their growth and metabolic processes. Hor et al. (2021) demonstrated that compared to milled rice, fermented rice has a lower overall energy content. There is a strong correlation between the amount of carbohydrates and the decrease in energy content. As a result, fermented rice has more nutrients and health benefits than unfermented rice due to microbial interaction. However, there are drawbacks to fermentation as well, particularly to both fibre and vitamin C. During this process, one of the most common changes is a drop in both fibre and vitamin C. Microorganisms get their energy from fibre. As a

result, fermented veggies have a reduced concentration of this ingredient. But there is a drop in starch and a rise in resistant starch, which lowers the GI (Knez et al., 2023).



4.2 Mineral composition

Table 4.2 shows the mineral composition analysis results comparing five types of fermented and non-fermented herbs in the current study.

Table 2: Mineral composition of non-fermented and yeast-fermented herbs (g/100g)

Herbs	Nutrient element							
	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Copper (ppm)	Zinc (ppm)	Sodium (ppm)
<u>Non-fermented</u>								
<i>Curcuma longa</i>	2.31 ± 0.06 ^{cd}	0.33 ± 0.02 ^c	5.89 ± 0.25 ^a	0.44 ± 0.01 ^b	0.92 ± 0.11 ^e	6.74 ± 0.52 ^e	77.88 ± 0.56 ^b	308.00 ± 2.48 ^{def}
<i>Syzygium polyanthum</i>	1.26 ± 0.09 ^h	0.23 ± 0.02 ^{fg}	1.78 ± 0.00 ^e	0.23 ± 0.01 ^c	1.54 ± 0.02 ^d	3.94 ± 0.13 ^g	55.26 ± 0.11 ^d	352.50 ± 2.10 ^{cd}
<i>Andrographis paniculata</i>	1.14 ± 0.02 ^h	0.21 ± 0.01 ^{gh}	2.52 ± 0.03 ^c	0.52 ± 0.01 ^a	1.42 ± 0.07 ^d	9.07 ± 0.12 ^a	125.98 ± 2.10 ^a	373.00 ± 5.48 ^c
<i>Euphorbia hirta</i>	1.46 ± 0.05 ^g	0.15 ± 0.01 ⁱ	2.17 ± 0.22 ^d	0.24 ± 0.02 ^c	2.86 ± 0.09 ^a	8.17 ± 0.10 ^{bc}	72.05 ± 2.89 ^c	2365.75 ± 54.05 ^a
<i>Orthosiphon stamineus</i>	1.62 ± 0.10 ^f	0.18 ± 0.01 ^h	2.19 ± 0.02 ^d	0.26 ± 0.02 ^c	0.97 ± 0.01 ^e	8.33 ± 0.10 ^{bc}	56.38 ± 0.52 ^d	324.50 ± 1.94 ^{cde}
<u>Yeast-fermented</u>								
<i>Curcuma longa</i>	3.26 ± 0.01 ^a	0.49 ± 0.00 ^a	5.14 ± 0.01 ^b	0.41 ± 0.00 ^b	0.76 ± 0.01 ^f	6.04 ± 0.35 ^f	68.73 ± 0.37 ^c	255.00 ± 5.77 ^f
<i>Syzygium polyanthum</i>	2.18 ± 0.01 ^{de}	0.25 ± 0.00 ^{ef}	0.78 ± 0.00 ^f	0.11 ± 0.00 ^e	0.97 ± 0.01 ^e	3.31 ± 0.03 ^g	45.43 ± 0.01 ^e	312.50 ± 4.33 ^{cdef}
<i>Andrographis paniculata</i>	2.14 ± 0.01 ^e	0.28 ± 0.00 ^d	2.48 ± 0.00 ^{cd}	0.41 ± 0.00 ^b	2.40 ± 0.01 ^b	8.54 ± 0.23 ^{ab}	128.50 ± 5.21 ^a	330.00 ± 1.73 ^{cde}
<i>Euphorbia hirta</i>	2.43 ± 0.03 ^{bc}	0.27 ± 0.00 ^{de}	1.62 ± 0.01 ^e	0.19 ± 0.00 ^c	1.82 ± 0.00 ^c	7.22 ± 0.05 ^{de}	57.05 ± 0.07 ^d	2082.25 ± 28.72 ^b
<i>Orthosiphon</i>	2.46 ±	0.36 ±	2.27 ±	0.19 ±	0.72 ±	7.82 ±	46.58 ±	275.50 ±

<i>stamineus</i>	0.03 ^b	0.00 ^b	0.00 ^{cd}	0.00 ^d	0.01 ^f	0.13 ^{cd}	0.34 ^e	6.06 ^{ef}
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Different letters within the same column differ significantly (P<0.05)



The study (**Table 2**) showed that yeast-fermented herbs exhibited notably higher nitrogen levels compared to their non-fermented counterparts. Across various herbs like *Curcuma longa*, *Syzygium polyanthum*, *Andrographis paniculata*, *Euphorbia hirta*, and *Orthosiphon stamineus*, the nitrogen content was observed to peak during yeast fermentation, indicating a significant increase from non-fermented states.

However, it is important to note that nitrogen supply plays a crucial role in yeast fermentation. Insufficient nitrogen levels can lead to reduced yields and prolonged fermentation times. To address this, Ma et al. (2019) highlighted the necessity of yeast extract addition during fermentation to enhance nitrogen sources. Cairns et al. (2021) further emphasized the importance of boosting yeast assimilable nitrogen (YAN) sources, especially in cider fermentation. Their findings suggest that augmenting nitrogen levels typically accelerates fermentation, impacting both the rate of fermentation and potentially the aroma of the final product. Nitrogen addition typically speeds up fermentation, although the extent of these benefits varies depending on the type of yeast. In general, adding YAN to cider fermentations within the concentration limits will increase the maximum rate of fermentation and possibly have an impact on the aroma of the finished product (Cairns et al., 2021). The addition of YAN, such as yeast extract, during fermentation proves instrumental in improving the fermentation process, maximizing its speed, and potentially influencing the aroma profile of the end product.

The yeast-fermented herbs were found to possess a higher percentage (%) of P than the non-fermented herbs. The yeast-fermented herbs that contain the highest phosphorus content are *C. longa*, followed by *O. stamineus*, *A. paniculata*, *E. hirta*, and lastly *S. polyanthum*. P is a crucial nutrient needed for plant development. Reduced yields and delayed plant maturity are caused by inadequate P consumption.

Chakraborty & Prasad (2019) emphasized the indispensability of phosphorus for plants, as it plays a pivotal role in numerous essential processes. From aiding in the absorption and storage of solar energy to the conversion of this energy into vital biomolecules like adenosine triphosphate (ATP), phosphorus is integral to biochemical reactions, including pivotal ones like photosynthesis. Moreover, phosphorus is a key component of DNA, RNA, and various other essential biomolecules that govern regular plant functions, such as carbohydrate metabolism, protein synthesis, lipid formation, and nucleic acid synthesis. Mustafa et al.'s (2022) study on oat bran highlighted that yeast-fermentation resulted in an increase in phosphorus content. Raw oat bran contained a phosphorus level of 531 ± 6.4 mg/100 g, whereas yeast-fermented oat bran exhibited a higher phosphorus content of 569 ± 7.8 mg/100 g. This finding underscores the potential of yeast fermentation to enhance the phosphorus content in certain food sources like oat bran.

The comparison between non-fermented and yeast-fermented herbs revealed interesting variations in potassium (K) content. Non-fermented herbs like *C. longa*, *E. hirta*, and *S. polyanthum* exhibited higher potassium levels compared to their yeast-fermented counterparts. However, there wasn't a significant difference observed between non-fermented *A. paniculata* and *O. stamineus* when compared to their yeast-fermented forms. The process of fermentation appeared to decrease the potassium content in the investigated herbs, leading to a reduction in major potassium components. Recent studies conducted by Sustr et al. (2019), Srivastava et al. (2019) have shown that K has a significant direct impact on a plant's ability to photosynthesize, its growth in various functional mechanisms that allow it to respond to various stresses, and its ability to maintain homeostasis and metabolic regulation.

A sufficient K supply promotes better nutritional absorption and photosynthetic assimilation (Sustr et al., 2019).

It was observed that there is no significant difference between yeast-fermented and non-fermented *C. longa* (0.41 ± 0.00 and 0.44 ± 0.01). However, a great significant difference is shown by non-fermented *A. paniculata* (0.52 ± 0.01), *O. stamineus* (0.26 ± 0.02), *E. hirta* (0.24 ± 0.02), and *S. polyanthum* (0.23 ± 0.01) compared to the yeast-fermented herbs. During yeast fermentation, it was also discovered that Mg, an element essential to life and bodily function, decreased. Hence, if the concentration of Mg is low, yeast growth may have a negative impact. On the other hand, adding more Mg can ensure yeast development without causing any major changes. It follows that, upon mineral supplementation, yeast can, without altering its development, boost the creation of aromas, including esters and higher alcohols (Riberio-Filho et al., 2022). As a result, Mg plays a role in flavour formation by inducing enzymes.

4.2.5 Calcium (Ca)

There was a significant difference between the non-fermented *E. hirta* (2.86 ± 0.09), *S. polyanthum* (1.54 ± 0.02), *O. stamineus* (0.97 ± 0.01), and *C. longa* (0.92 ± 0.11) against the yeast-fermented herbs with 1.82 ± 0.00 , 0.97 ± 0.01 , 0.72 ± 0.01 , and 0.76 ± 0.01 . As for the *A. paniculata*, there is a significant difference between the yeast-fermented (2.40 ± 0.01) and the non-fermented (1.42 ± 0.07) herbs.

As a result, calcium plays a crucial part in the development and expansion of plant cells as well as in the process of cell division. Based on a study conducted by Roosta et al. (2023), it is therefore plausible that the pennyroyal plant requires high Ca, and

that improving physiological features and therefore increasing output can be achieved by applying Ca by foliar application. Enhancing crop quality and plant development, controlling nutrient shortages, and effectively managing plant nutrition are the three main benefits of foliar fertilisation.

As per the findings of Roosta et al.'s study from 2023, the plants in the greenhouse culture method that used calcium carbonate foliar spraying had the largest shoot fresh mass and had the maximum level of total phenol as compared to the plants that did not utilise it. Proline and other phenolic compounds were shown to be enhanced in lemongrass under Ca foliar treatment, according to Vafadar and Ehsanzadeh (2023).

It was discovered by Singhal et al. (2021) that important minerals like Ca are reduced during fermentation. In fermented bamboo shoots, reduced concentrations of components including Ca were observed (Singhal et al., 2021).

Additionally, lactic acid is produced during fermentation, which alters pH and creates the ideal environment for microbial enzyme activation, which amplifies the activity of the enzymes (Sharma et al., 2020; Tachie et al., 2023). Studies conducted by Castro-Alba et al. (2020) have verified this mechanism by demonstrating a connection between lower levels of phytate and greater Ca availability in fermented quinoa flour.

Furthermore, combining *L. plantarum* with ground quinoa seeds for 4 or 10 hours at 30°C during fermentation greatly lowered the phytic acid concentration and increased the minerals' bioavailability, including Ca (Castro-Alba et al., 2019).

4.2.6 Copper (Cu)

There is a significant difference between both non-fermented and yeast-fermented *E. hirta* and *C. longa*. As for *A. paniculata*, *O. stamineus*, and *S. polyanthum*, both yeast-fermented and non-fermented herbs show no significant difference.

Cu plays a vital part in signal transduction, respiration, photosynthesis, and the antioxidant system. According to Chen et al. (2022), one of the crucial trace elements needed to keep plants growing and developing regularly is Cu. It functions as a cofactor and is the active centre of many different enzymes. In biology, it is involved in many different processes, including respiration/photosynthesis, hormone signal transduction, protein transport, and cell wall metabolism (Gong et al., 2021).

C. longa is one type of herb with high Cu content. Turmeric may aid with allergies, heart protection, arthritic symptoms, and inflammation reduction in the human body, according to research conducted by The Botanical Institute, (2023). Furthermore, turmeric might possess antibacterial properties.

According to The Botanical Institute (2023), copper is one of the fundamental minerals and is necessary for numerous body processes. It promotes red blood cell production, lung elasticity, cardiovascular system integrity, collagen, connective tissue, and brain neurotransmitters (The Botanical Institute, 2023; Harvard T.H. Chan, 2022). Furthermore, Cu is important for angiogenesis, the breakdown of superoxide radicals, and the healing processes (Bellu et al., 2021; Sandoval et al., 2022). Additionally, Cu aids in the metabolism and absorption of iron and it helps different enzymes that the body uses to make energy (Harvard T.H. Chan, 2022).

Cu protects proteins, lipids, and DNA from damage caused by free radicals by collaborating with an antioxidant enzyme. A lack of Cu can cause several illnesses,

including anaemia, immunological problems, skin pigmentation loss, osteoporosis, and cardiac problems (The Botanical Institute, 2023). In a previous study conducted by Sandoval et al. (2022), Cu has better biocompatibility than other metallic ions, which encourages skin regeneration and improves skin quality.

Due to its antibacterial qualities, low toxicity, natural occurrence in biological systems, and increased cost-effectiveness, copper is receiving attention (Antonio-Pérez et al., 2023). Another theory contends that the stability and folding of proteins are impacted by copper ions, which encourages protein aggregation in anaerobic environments (Zuily et al., 2022).

Due to their capacity to harm pathogen cell membranes and interfere with their metabolic activities, copper nanoparticles (CuNPs) are effective as a control agent against phytopathogens (Kashyap et al., 2023). Food-borne bacteria and other microbes that cause spoiling are unable to reproduce when CuNPs are used to produce packaging materials. This means that the nutritional value of perishable food can be extended because there is a lower chance of food contamination which may increase food safety (Dash et al., 2022; Zhang et al., 2023).

The impact of key inorganic element supplementation, such as Cu was assessed by Ribeiro-Filho et al. (2021) in a previous study. They found that during fermentation, taste components such as ethanol, glycerol, and higher alcohols and esters had significantly increased.

For adults, the recommended daily allowance (RDA) is 0.9 mg. According to Jakubczyk et al. (2022), the levels of Cu after day 14 of fermentation varied from about 0.06 mg/L to 0.25 mg/L, ranging from 7% and 28% of the RDA for both sexes.

The brew prepared from black tea on the 14th day of fermentation had the highest concentration of Cu.

Products for protecting plants, fertilisers, insecticides, and fungicides all contain minerals like Cu. The product's application in agriculture and the elements present in phytosanitary goods may be connected to their presence in it (Antoniewicz et al., 2021).

4.2.7 Zinc (Zn)

There is a significant difference between both non-fermented and yeast-fermented *C. longa*, *E. hirta*, *O. stamineus*, and *S. polyanthum*. Non-fermented *C. longa*, *E. hirta*, *O. stamineus*, and *S. polyanthum* show higher Zn content than yeast-fermented herbs. However, there are no significant differences between the non-fermented and yeast-fermented *A. paniculata*.

Zn is an important micronutrient for plants because it plays a role in several vital cellular activities, including ion homeostasis, enzyme activity, and thermoregulation (Yang et al., 2020; Alsafran et al., 2022). Furthermore, Zn is involved in biophysicochemical reactions (Noman et al., 2019; Zaheer et al., 2022). These functions include protein synthesis, carbohydrate metabolism, gene regulation and activation, and morphological and anatomical involvement in bio-membranes (Zaheer et al., 2020). According to Alatawi et al. (2022), Zn boosts the synthesis of cytochrome and is necessary for seed development.

Additionally, Zn is necessary for a wide range of physiological processes in plants, including the regulation of hormones and the synthesis of tryptophan, a precursor to indole acetic acid, as well as the transmission of signals via mitogen-activated protein kinases (Bhantana et al., 2021; Kaur and Garg, 2021; Ahmad et al., 2022).

Plant biochemistry and metabolism are significantly impacted by Zn. It supports several cellular and physiological processes in plants and encourages their growth, development, and yield. Zn has a crucial role in the structural, enzymatic, and regulatory aspects of numerous proteins and enzymes. In light of this, optimising Zn utilisation efficiency which comprises strengthening root system architecture, organic acid absorption of zinc complexes, and plant uptake and translocation mechanisms is the most successful tactic.

Zn plays an important role in a variety of functions, including immune system regulation, cell development and differentiation, and learning and memory processes (Chruściel et al., 2021). For men, the suggested daily consumption is 11 mg, while for women, it is 8 mg. In a previous study conducted by Huang et al. (2022), on the 14th day of black tea fermentation, zinc levels varied between 0.36 mg/L to 2.08 mg/L, and 3% to 19% of the required amount for men as well as 5% to 26% for women. It has also been shown that zinc and chromium are biotransformed by *Acetobacter acetic* bacteria, increasing their concentrations. Because of the quality of the hypoglycemic impact, diabetes is treated with it.

Additionally, LAB is produced during fermentation, which alters pH and creates the ideal environment for microbial enzyme activation, which amplifies the activity of the enzymes (Sharma et al., 2020; Tachie et al., 2023). Studies conducted by Castro-Alba et al. (2020) have verified this mechanism by demonstrating a connection between lower levels of phytate and greater Zn availability in fermented quinoa flour.

Fertilisers, fungicides, insecticides, and plant protection treatments all contain minerals like Zn. According to Antoniewicz et al. (2021), their inclusion in the product could be connected to its application in agriculture and the components these

phytosanitary products contain. Zn concentration rose dramatically during the fermentation process. Ivanišová et al. (2019) further stress that Kombucha is safe to eat because it does not contain any dangerous ingredients.

In addition, adding *L. plantarum* to ground quinoa seeds for 4 or 10 hours at 30°C greatly decreased the amount of phytic acid and increased the minerals' bioavailability, especially zinc (Castro-Alba et al., 2019).

Prior research by Ribeiro-Filho et al. (2021) assessed the impact of supplementing critical inorganic elements, such as Zn, and found that fermentation significantly increased the number of taste compounds, such as ethanol, glycerol, and higher alcohols and esters.

4.2.8 Sodium (Na)

There is a significant difference between non-fermented and yeast-fermented *E. hirta* while no significant difference between both non-fermented and yeast-fermented herbs of *A. paniculata*, *S. polyanthum*, *O. stamineus*, and *C. longa*.

Na is not a necessary element for plants, but like micronutrients, it can be utilised in small quantities to support chlorophyll synthesis and metabolism. It helps with stomates opening and shutting, which helps control internal water balance, and it can be utilised in some plants as an alternative for K (Bloodnick, 2022).

The fermented peas with a lower salt content had a higher total titratable acidity (TTA) and a lower pH. This is likely because adding salt lowers water activity, which combines with other factors to reduce microorganism activity and their ability to perform chemical reactions (Gunasekaran et al., 2020). Growing bacteria that produce biogenic amines (BA) are also inhibited by higher salt content, ultrasound treatment, and lower water activity in solid-state fermentation (SSF).

The explanation for the colour variations in peas can be found in the acidification of the substrate that occurs during fermentation. Organic acids have an impact on oxidation processes that can result in colour changes and texture production. The water-holding capacity and water activity of proteins is controlled by the salt level, which also affects these changes (Gunasekaran et al., 2020).

4.3 Antibacterial activity

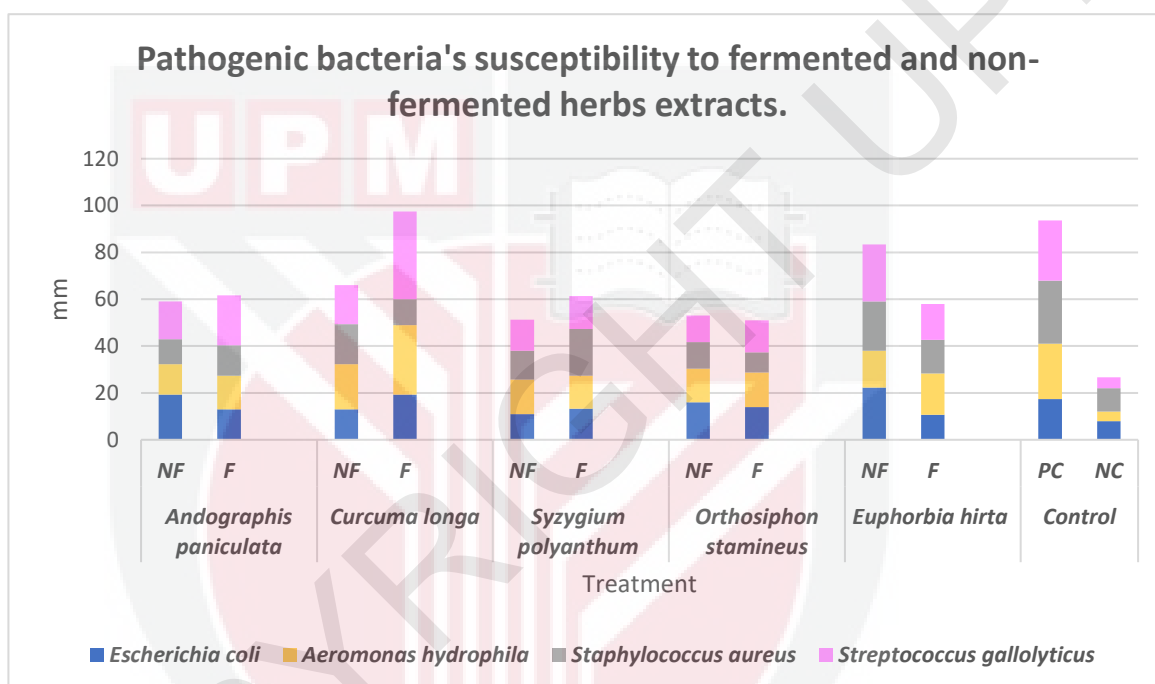


Figure 4.3: Pathogenic bacteria's susceptibility to fermented and non-fermented herbs extracts.

Figure 4.3 demonstrates a significant difference, showcasing larger inhibition zones for yeast-fermented *C. longa*. This suggests that the fermentation process enhances *C. longa*'s antibacterial activity against *Streptococcus gallolyticus* and *Aeromonas hydrophila*, indicating its potential as an effective antibacterial agent against this pathogen. Syawal et al. (2021) study investigating the effects of fermented medicinal herbs, including turmeric (*C. longa*), in combination with molasses on striped catfish revealed positive impacts on various health parameters. The improvement in the catfish's growth rate, feed efficiency, feed conversion ratio, and survival rate were attributed to secondary metabolites present in fermented *C. longa*, such as

flavonoids, vitamin C, essential oils, tannins, and curcuminoids. These compounds are known for their potential to stimulate the immune system and contribute to enhancing physiological and hematological profiles. The antioxidant properties of curcuminoids and flavonoids are particularly noteworthy. Curcuminoids have been found to impact cell membranes, reducing oxidative damage to erythrocyte membranes. Similarly, flavonoids act as natural antioxidants, capable of neutralizing both free radicals and anti-free radicals. Furthermore, yeast-fermented *C. longa* displays higher inhibition zones against *Streptococcus gallolyticus* and *Aeromonas hydrophila* compared to the positive control, Tetracycline. Although no significant difference is observed between yeast-fermented *C. longa* and Tetracycline, the enhanced antibacterial activity of fermented *C. longa* against *Streptococcus gallolyticus* and *Aeromonas hydrophila* underscores its potential as an effective alternative or complementary antibacterial agent. However, the 96% ethanol which represents the negative control shows the lowest inhibition zone among all the treatments against *S. gallolyticus* and *A. hydrophila*. As for *S. gallolyticus*, there is no significant difference between the negative control with both non-fermented and yeast-fermented *S. polyanthum*, *O. stamineus*, yeast-fermented *E. hirta*, non-fermented *C. longa*, and *A. paniculata*. While, as for the *A. hydrophila*, there are large and obvious significant differences between all the extracts compared with the negative control.

The comparison between non-fermented and yeast-fermented *E. hirta* against *E. coli* reveals a significant difference, with the non-fermented *E. hirta* exhibiting a larger inhibition zone than its yeast-fermented counterpart. This indicates that the non-fermented form of *E. hirta* possesses stronger antibacterial activity against *E. coli* compared to the fermented version. Research conducted by Parisa et al. (2019) and

Anggrahini et al. (2021) has explored *E. hirta* as a potential antibacterial and anthelmintic agent. Active compounds like flavonoids, phenols, tannins, terpenes, sterols, and saponins present in *E. hirta* are attributed to its antibacterial properties. Additionally, specific compounds like flavonoids, tannins, and saponins are recognized for their anthelmintic action. Moreover, Gupta & Gupta (2019) reported that *E. hirta* leaf extract can inhibit the growth of *S. aureus* and *E. coli*. Interestingly, although non-fermented *E. hirta* exhibits a larger inhibition zone compared to Tetracycline against *E. coli*, no significant difference is observed between these treatments. This suggests that while non-fermented *E. hirta* shows potent antibacterial activity against *E. coli*, it performs comparably to Tetracycline, a known antibacterial agent, in this particular test. Additionally, both non-fermented and yeast-fermented *C. longa*, *A. paniculata*, and *O. stamineus* show no significant differences in their effects against *E. coli*, indicating a consistent antibacterial efficacy regardless of fermentation status. The negative control, represented by 96% ethanol, exhibits the smallest inhibition zone, confirming the validity of the experiment. These findings underscore the potential of non-fermented *E. hirta* as a strong antibacterial agent against *E. coli* and highlight its comparable effectiveness to established antibacterial treatments like Tetracycline. The varying responses of different herbs and their fermentation status against bacterial strains provide insights into their diverse antibacterial potentials. Furthermore, the non-fermented *E. hirta* has the highest inhibition zone (22.33 ± 2.85) among all the extracts against *E. coli*. The non-fermented *E. hirta*, non-fermented *A. paniculata* (19.33 ± 6.17), and the yeast-fermented *C. longa* (19.33 ± 1.76) show a higher inhibition zone than the positive control which represents the Tetracycline (17.33 ± 2.67) but there are no significant

differences between them. The listed herbs above show potent antibacterial activity against *E. coli*.

Additionally, non-fermented *A. paniculata* (19.33 ± 6.17) displays higher inhibition zones against *E. coli* compared to the positive control, Tetracycline (17.33 ± 2.67). Although no significant difference is observed between non-fermented *A. paniculata* and Tetracycline, the enhanced antibacterial activity of non-fermented *A. paniculata* against *E. coli* underscores its potential as an effective alternative or complementary antibacterial agent.

The comparison between both non-fermented and yeast-fermented *O. stamineus* against *E. coli* reveals no significant difference, with the non-fermented *O. stamineus* exhibiting a larger inhibition zone than its yeast-fermented counterpart. This indicates that the non-fermented form of *O. stamineus* possesses stronger antibacterial activity against *E. coli* compared to the fermented version.

There are no significant differences between the yeast-fermented and non-fermented *S. polyanthum* although the yeast-fermented herbs (13.33 ± 1.33) show a slightly larger inhibition than the non-fermented herbs (11.00 ± 1.15).

There are no significant differences between non-fermented and yeast-fermented of all 5 medicinal herbs, positive and negative control against *S. aureus*. While for *S. gallolyticus* and *A. hydrophila*, there are significant differences between yeast-fermented and non-fermented *C. longa*. The yeast-fermented *C. longa* has a higher inhibition zone compared to the non-fermented *C. longa* and the positive control. According to Yong et al. (2019), fermentation with lactic acid bacteria raises the amount of curcumin in turmeric without increasing its cytotoxicity and strengthens its pharmacological activity, indicating its potential use as a functional food

ingredient. Syawal et al. (2021) demonstrate that the immune system is stimulated and the haematological and physiological profile of catfish is improved by the secondary metabolites of fermented *C. longa*, which contain flavonoids, vitamin C, essential oils, tannins, and curcuminoids. However, there are no significant differences between the yeast-fermented *C. longa* and the Tetracycline against both *S. gallolyticus* and *A. hydrophila*. As for the negative control against both *S. gallolyticus* and *A. hydrophila*, it inhibits the smallest zone than the other extracts. Other than that, the rest of the yeast-fermented and non-fermented herbs such as *E. hirta*, *A. paniculata*, *S. polyanthum*, and *O. stamineus* show no significant differences against *S. gallolyticus* and *A. hydrophila*.

CHAPTER 5

CONCLUSION

To conclude, the current study was conducted to assess variations and mineral composition between yeast-fermented and non-fermented herbs. Based on the results for nutrient composition, carbohydrate, fat, crude fibre, ash, and energy is higher in non-fermented herbs while yeast-fermented herbs have higher protein content. As for the mineral composition, yeast-fermented herbs have a higher phosphorus content compared to non-fermented herbs. Non-fermented herbs have a higher content of nitrogen, potassium, magnesium, calcium, copper, zinc, and sodium than yeast-fermented herbs. For the antibacterial activity test, yeast-fermented *Curcuma longa* was the most effective against *Aeromonas hydrophila* and *Streptococcus gallolyticus*, while non-fermented *Euphorbia hirta* shows potent antibacterial activity against *Escherichia coli*. Both yeast-fermented *Curcuma longa* and non-fermented *Euphorbia hirta* underscores its potential as an effective alternative or complementary antibacterial agent. Thus, as modern cultures and scientific advances spread around the world, modern and advanced technology is needed to improve the experiment and research qualities in terms of providing accurate and precise information. Therefore, further study on the nutritional value and antibacterial activity of yeast-fermented herbs as an antibiotic supplement should be performed.

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APPENDICES

Appendix 1: Sample Preparation



Fresh Herbs



Dried Herbs



Grounded Herbs

Appendix 2: Fermentation Process



L. casei, *S. cerevisiae*, and molasses were added to the herbs.



L. casei, *S. cerevisiae*, and molasses were mixed with the herbs.



The inoculum was incubated at 28°C for 14 days anaerobically.

Appendix 3: pH Determination

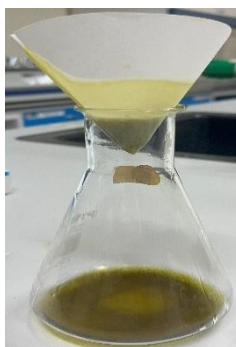


Initial and final pH readings of the yeast-fermented herbs were measured.

Appendix 4: Herbs Extraction



50 g herb samples were soaked with 20 mL absolute ethanol for 24 hours.

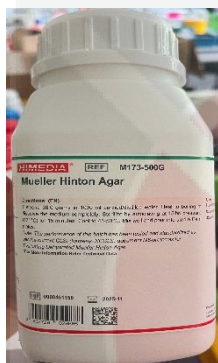


The samples are filtered.



The herbs-filtered bioactive compound is extracted.

Appendix 5: Preparation of Mueller Hinton Agar/Broth



MHA is prepared based on the manufacturer's instructions.



MHA solutions were autoclaved



Sterilized MHA is poured into the Petri dish.

Appendix 6: Standardization of Bacteria Culture



The bacteria strains were subcultured.

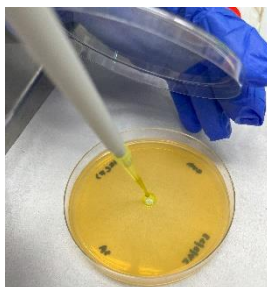


Bacteria cultures were standardized.

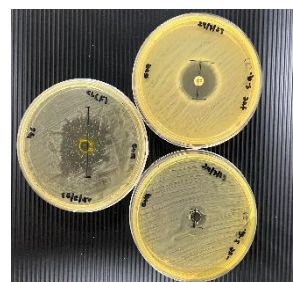
Appendix 7: Agar Well Diffusion Assay



1 mL of bacteria strain was cultured on MHA by swabbing.



Extracts were transferred.



Antimicrobial activity was measured and compared.



PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publishing the project entitled “Nutritional Value and Antibacterial Activity of Yeast-Fermented Herbs as an Antibiotic Supplement for Fish” by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Sarawak Campus, and published in the form approved by the Faculty.



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Date: 7th January 2024