GAS CHROMATOGRAPHY-MASS SPECTROMETRY PROFILING AND ANTIBACTERIAL ACTIVITY OF ALIM [*Melanolepis multiglandulosa* (Reinw.ex Blume) Rchb. & Zoll] ETHANOLIC LEAF EXTRACT AGAINST *Pseudomonas aeruginosa*

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In Partial Fulfillment
of the Requirement of the Degree
Bachelor of Science in Medical Technology

MARY JOSETH M. SARMIENTO
NJ MARK L. SEDENIO
JOEL ANDRE R. SUSADA
JHUNNABIE E. TABAMO

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Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

DEPARTMENT OF MEDICAL TECHNOLOGY

CERTIFICATE OF PANEL APPROVAL

The research paper attached hereto, entitled **"GAS CHROMATOGRAPHY-MASS SPECTROMETRY PROFILING, AND ANTIBACTERIAL ACTIVITY OF ALIM [*Melanolepis multiglandulosa* (Reinw.ex Blume) Rchb. & Zoll] ETHANOLIC LEAF EXTRACT AGAINST *Pseudomonas aeruginosa* "**, prepared and submitted by **MARY JOSETH M. SARMIENTO, NJ MARK L. SEDENIO, JOEL ANDRE R. SUSADA,** and **JHUNNABIE E. TABAMO,** in partial fulfillment of the requirements for the degree **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY,** is hereby recommended for approval.

MELIZA PARBA, PhD
Adviser
7/2/26
Date

Approved by the Defense Panel Committee with a grade of ***Passed***.

MARIE ROSELYNN C. ENGUITO, MSc. Bio.
Member
7/2/26
Date

ATTY. ANTHONY L. AWA, MSc. Bio.LPT
Member
7/2/26
Date

KARL MAXEL O. LAO, RMT, MSc. MLS
Chairman
7/2/26
Date

This research paper is approved in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY.**

EVANGELINE M. SEÑEDO, RMT, MATMRS
Dean, College of Medical Technology
7-10-26
Date

ABSTRACT

Despite the presence of bioactive phytochemicals reported in many medicinal plants, only limited studies have investigated the antibacterial potential of *Melanolepis multiglandulosa* (Reinw.ex Blume) Rchb. & Zoll) leaf extract against *Pseudomonas aeruginosa* ATCC 27853. This study evaluated the phytochemical composition and antibacterial activity of the plant using Gas Chromatography–Mass Spectrometry (GC–MS) and the Kirby–Bauer disc diffusion method. GC–MS analysis of the ethanolic leaf extract identified 48 compounds, of which 43.75% were reported to have antimicrobial activity, 27.08% anti-inflammatory activity, 18.75% antioxidant activity, and 8.33% antibacterial activity. However, the ethanolic leaf extract at concentrations of 25%, 50%, 75%, and 100% showed no zone of inhibition against *Pseudomonas aeruginosa* ATCC 27853 under the conditions employed in the study. This may be due to the natural resistance of the bacterium, limited extraction efficiency, poor diffusion of phytochemicals in the agar medium, and the use of Nutrient Agar instead of Mueller–Hinton Agar. Although no antibacterial activity was observed, the presence of bioactive compounds suggests that *Melanolepis multiglandulosa* still has potential medicinal value and supports the need for further studies using other bacterial isolates and improved testing methods.

Keywords: *antimicrobial, compounds, concentrations, nutrient agar, phytochemicals*

DEDICATION

*This research is wholeheartedly dedicated to our beloved **parents** and **families** for their unconditional love, endless sacrifices, understanding, and unwavering support throughout our academic journey.*

We also dedicate this work to all the people who became part of this study and generously shared their time, effort, guidance, and encouragement to help us successfully complete this research. Their kindness and support truly inspired us to persevere despite the challenges we encountered along the way.

Above all, we humbly dedicate this achievement to the Lord Almighty for granting us wisdom, strength, guidance, and countless blessings throughout this journey.

MARY JOSETH, NJ MARK, JOEL ANDRE, and **JHUNNABIE**

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MARY JOSETH, NJ MARK, JOEL ANDRE, and **JHUNNABIE**

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INTRODUCTION

Background of the study

Medicinal plants are important sources of natural compounds that help prevent and treat different kinds of diseases (Marelli, 2021). For many generations, people have used plants as remedies for common illnesses. In the Philippines, especially in Mindanao, traditional medicine remains a significant part of health practices, especially in rural areas where medicinal plants are easily available. One of these plants is *Melanolepis multiglandulosa*, which belongs to the family of *Euphorbiaceae* family and is locally recognized in the Philippines as alim or alom tree (Dapar, 2020). This plant is naturally found in several Asian and Pacific countries, including Southern Japan, Taiwan, Thailand, Malaysia, Indonesia, Papua New Guinea, the Solomon Islands, and the Mariana Islands (Apostol et al., 2016). Because of its availability and traditional uses, it has gained attention as a potential source of beneficial medicinal compounds. As interest in natural medicines continues to grow, many researchers are investigating medicinal plants as possible sources of new therapeutic agents.

In the Philippines, *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll is commonly used as a first-aid treatment for wounds, cuts, and skin infections. Traditional healers and local communities usually crush the fresh leaves or prepare them as a decoction before applying them directly to the affected area. This practice has been passed down

through generations and remains common in many communities today (Dapar et al., 2020). Aside from wound treatment the plant is also known for its anti-inflammatory, antibacterial, and wound-healing properties (Alhabri et al., 2023). Recent studies support these traditional uses and show that the leaves contain bioactive compounds such as flavonoids, tannins, saponins, and alkaloids, which are known to have antibacterial, anti-inflammatory, and antioxidant properties (Apostol et al., 2016; Lazo & Caburian, 2025).

Even though *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll is commonly used in traditional medicine, most research has focused on its roots. Recent studies by Dapar et al. (2020) and Lazo and Caburian (2025) found that the leaves also have strong antibacterial and wound-healing properties showing that they deserve more attention and further investigation. However, there is still limited information regarding the chemical composition of the leaf extract and the compounds responsible for its antibacterial activity. More studies are needed to validate its medicinal value using standardized laboratory methods.

This study aims to address these gaps by conducting Gas Chromatography–Mass Spectrometry (GC-MS) analysis and antibacterial testing of the ethanolic leaf extract of *Melanolepis multiglandulosa* against *Pseudomonas aeruginosa* ATCC 27853. By doing this, the study will provide scientific evidence to support its traditional use of the plant and help identify the compounds that may contribute to its antibacterial activity. The findings may serve as a basis for future studies and promote the development of local medicinal plants as natural, affordable, and effective sources of antibacterial agents in the Philippines.

Objectives

This study aims to determine the antibacterial activity of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll ethanolic leaf extract against *Pseudomonas aeruginosa* ATCC 27853. Specifically, it aimed to:

1. Identify the bioactive compounds present in the ethanolic leaf extract using Gas Chromatography–Mass Spectrometry (GC–MS) analysis; and
2. Evaluate the antibacterial activity of the ethanolic leaf extract at different concentrations (25%, 50%, 75%, and 100%) against *Pseudomonas aeruginosa* ATCC 27853 using Nutrient Agar, with Gentamicin as the positive control and distilled water as the negative control.

Significance of the Study

This study is important in the fields of science, medicine, ethnobotany, and community health. Scientifically, it provides new information about *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll leaves since most previous studies focused mainly on the roots. The GC–MS analysis identified the chemical compounds present in the leaves and may serve as baseline data for future studies. In medicine, the study evaluated whether the leaf extract has antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853, a bacterium known for its resistance to antibiotics which may

help in the search for natural sources of new antibacterial agents (Newman & Cragg, 2020). In ethnobotany, the study helps support the traditional use of the plant through scientific evidence. For the community, the findings may increase awareness of the potential medicinal value of local plants especially as affordable natural remedies in areas with limited healthcare access while also encouraging plant conservation and further research.

Scope and Delimitation

This study focused only on the leaves of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll while other parts such as the roots, bark, and stems were not included. GC–MS analysis was used to identify the chemical compounds but it can only detect volatile and heat-stable substances so some compounds may not have been identified. The identification of compounds also depended on the reference database used. The antibacterial test was conducted only against *Pseudomonas aeruginosa* ATCC 27853, so the effectiveness of the extract against other bacteria was not evaluated which limits the generalization of the findings. In addition, Nutrient Agar was used instead of Mueller–Hinton Agar (MHA) which may have affected the diffusion of the extract and the accuracy of the results. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests were also not performed so the exact antibacterial strength of the extract was not determined. Since the study was conducted in vitro the results may not fully represent what happens inside a living body where factors such as absorption,

digestion, and toxicity can affect the outcome. Despite these limitations, the study still provides useful initial information about the chemical compounds and possible antibacterial potential of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll leaf extract against *Pseudomonas aeruginosa* ATCC 27853.

MATERIALS AND METHODS

Research Design

This study employed an experimental laboratory research design combining qualitative, analytical, and microbiological methods to investigate the bioactive compounds and antibacterial activity of the *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll leaf extract. The research was divided into two major phases: GC–MS analysis, and In Vitro Antibacterial assay against *Pseudomonas aeruginosa* ATCC 27853 **(Figure 1).**

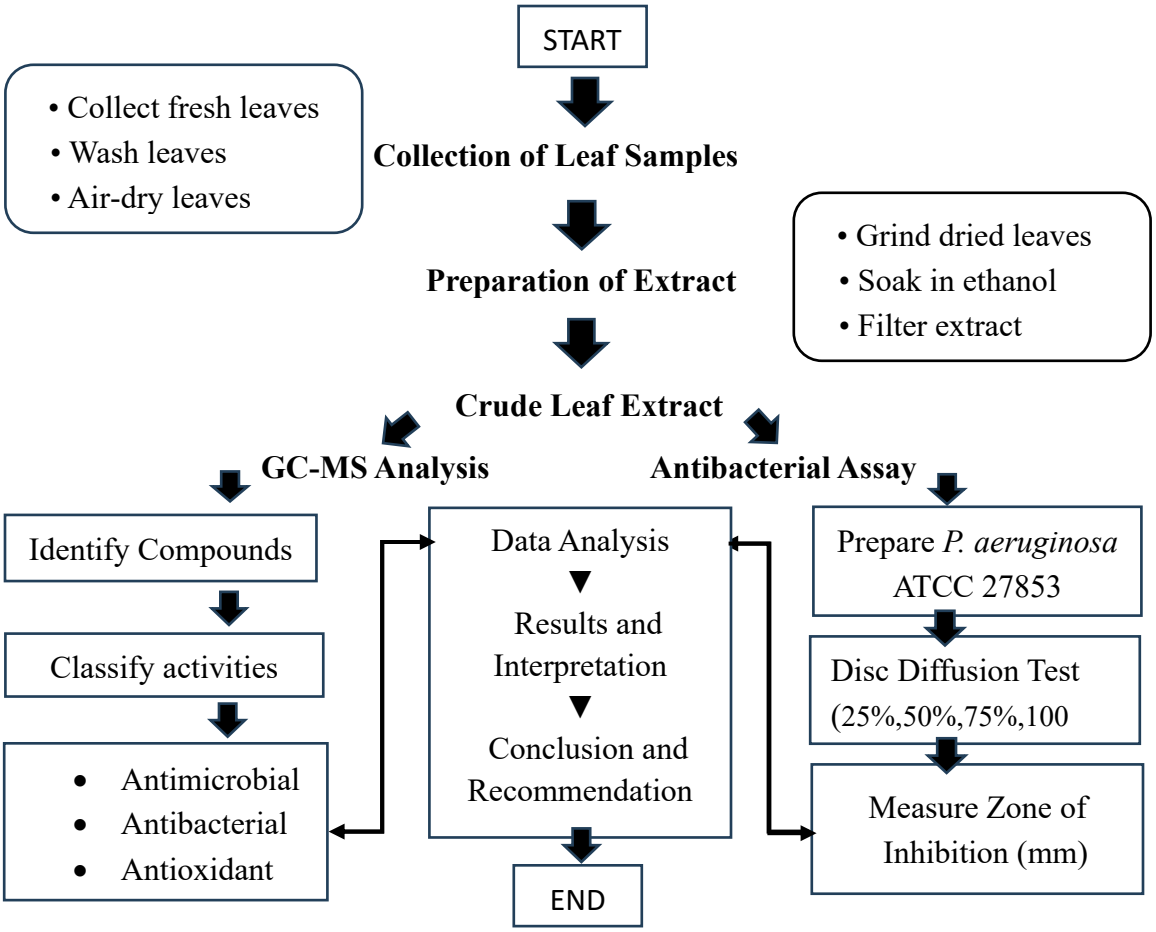

Figure 1. Experimental procedure of GC-MS and In vitro Antibacterial Assay

Research Setting

The study took place in Gumbil, Tudela, Misamis Occidental (**Figure 2.**), where the plant samples were collected. The extraction and antibacterial testing were conducted at the Natural Science Department of Misamis University, using clean and controlled laboratory equipment such as sterile work areas, autoclaves, and incubators. The rotary evaporation process was carried out at at Mindanao State University – Iligan Institute of Technology (MSU-IIT) to remove the solvent and concentrate the extract. For chemical analysis, the leaf samples were sent to Ateneo de Davao University Laboratory, where Gas Chromatography–Mass Spectrometry (GC–MS) was used to identify the compounds in the extract.

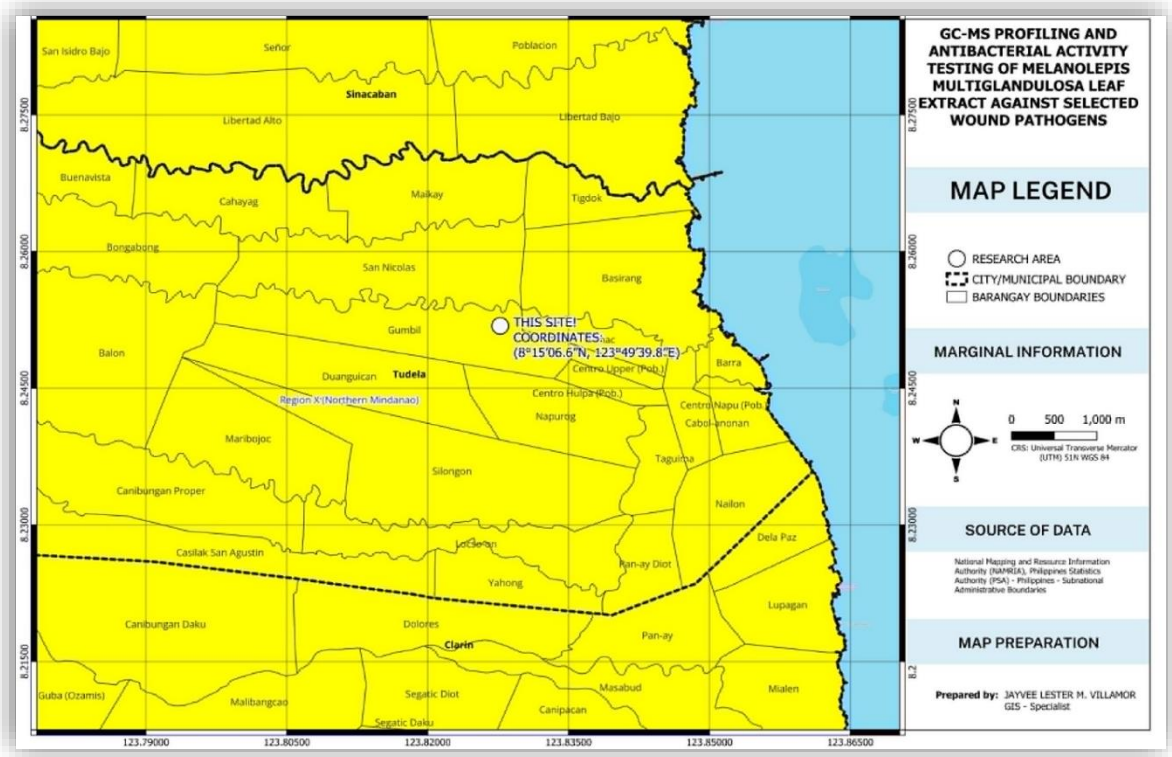

Figure 2. Map of collection site

Collection and Authentication of Plant Materials

Melanolepis multiglandulosa (Reinw. ex Blume) Rchb. & Zoll was initially identified by local residents of Gumbil, Tudela, Misamis Occidental, who are knowledgeable about its traditional use, and authenticated by Mr. Bobby Alaman of Misamis University. Fresh, healthy, mature leaves were collected early in the morning, washed with tap water, rinsed with distilled water, and air-dried in a shaded, well-ventilated area at room temperature. The dried leaves were then ground into a fine powder and stored in sterile, airtight amber containers at 4 °C until extraction.

Preparation of Sample

The dried powdered leaves of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll were extracted by maceration using absolute ethanol. Five hundred grams (500 g) of powdered leaves were soaked in 2 liters of ethanol for 5 days with occasional stirring. The mixture was then filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at the Mindanao State University–Iligan Institute of Technology (MSU-IIT). The solvent was evaporated at approximately 45 °C to obtain the crude ethanolic extract while preserving the integrity of heat-sensitive compounds (Dapar et al., 2021). The crude extract was transferred into sterile, airtight amber containers and stored at 4 °C until GC–MS analysis and antibacterial testing.

Extract Preparation

For antibacterial testing, the crude extract was prepared in four concentrations using distilled water: 25%, 50%, 75%, and 100% (pure/undiluted extract). For the 25% concentration, 6.25 mL of extract was mixed with 18.75 mL of distilled water; for 50%, 12.5 mL of extract was mixed with 12.5 mL of distilled water; and for 75%, 18.75 mL of extract was mixed with 6.25 mL of distilled water. The 100% solution remained as the pure undiluted ethanolic extract.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC–MS analysis of the *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll crude leaf extract was performed to identify its chemical constituents and determine potential bioactive compounds associated with its antibacterial activity (Dapar et al., 2021). A small portion of the extract was injected into the GC–MS instrument, where it was vaporized and separated in the chromatographic column, generating peaks corresponding to individual compounds. The detected peaks were identified by comparison with the National Institute of Standards and Technology (NIST) library, while the relative abundance of each compound was estimated from its peak area in the chromatogram.

In Vitro antibacterial assay

The in vitro antibacterial activity of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll leaf extract was tested using a validated strain of *Pseudomonas aeruginosa* (ATCC 27853), a bacterium commonly linked to wound infections and widely used in antimicrobial studies (Pang et al., 2019). The bacterial inoculum was standardized using the 0.5 McFarland standard, and Nutrient Agar was used for bacterial growth. The antibacterial activity was evaluated using the Kirby–Bauer disc diffusion method with gentamicin as the positive control and distilled water as the negative control. The antibacterial activity was determined by measuring the zone of inhibition around each disc. Positive and negative controls were included to validate the results, and all tests were conducted under aseptic conditions to ensure accuracy and prevent contamination.

Sterile filter paper discs were first autoclaved to ensure sterility and then impregnated with 10 µL of different concentrations of the Alim extract (25%, 50%, 75%, and 100%). After drying, the discs were aseptically placed on the inoculated agar plates and incubated at 37°C for 24 hours. The diameter of the zone of inhibition around each disc was then measured in millimeters (mm) using a vernier caliper to determine antibacterial activity (Dapar et al., 2021; Kebede et al., 2021).

Data Analysis

The data gathered from the study were analyzed using descriptive analysis. For the GC-MS analysis, the identified compounds in the ethanolic leaf extract of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll were recorded and classified according to their reported biological activities. For the antibacterial assay, the zone of inhibition produced by the different concentrations of the extract against *Pseudomonas aeruginosa* ATCC 27853 was observed and measured in millimeters (mm). The antibacterial activity of the extract was determined based on the presence or absence of a zone of inhibition.

Method of Data Collection and Processing

Ethanolic leaf extracts of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll were prepared at four concentrations: 25%, 50%, 75%, and 100%. The leaf extract was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) to identify its chemical compounds. For the antibacterial test, a standardized suspension of *Pseudomonas aeruginosa* ATCC 27853 adjusted to 0.5 McFarland standard was inoculated onto nutrient agar plates. The Kirby–Bauer disc diffusion method was used to evaluate the antibacterial activity of the extract. The compounds identified through GC-MS were listed and grouped according to their reported biological activities. The zones of inhibition around each disc were measured in millimeters (mm). The results were then compared with the positive control (Gentamicin) and negative control (distilled water) to determine the antibacterial activity of the extract.

RESULT AND DISCUSSION

Gas Chromatography – Mass Spectrometry Analysis

The result of the GC-MS analysis of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll leaf extract revealed the presence of 48 identified compounds, which is shown in **Table 1**.

Table 1. Result of GC-MS Analysis of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll

Compounds	Area (%)	Similarity Index (SI%)	Molecular Weight (g/mol)	Biological Properties	Organism Used
Undecane	0.19	97	156.31	Antimicrobial activity (Wang et. al., 2011)	Not Specified
Tridecane	0.09	97	184.36	Antimicrobial and Insecticidal activity(Wang et. al., 2011)	Not Specified
Hexadecane	0.07	95	226.44	Antimicrobial and Anti-oxidant (Geethalakshmi&Sarada.,2013)	*Staphylococcus aureus* (MTCC 29213), *Streptococcus faecalis* (MTCC0459), *Enterococcus faecalis* (MTCC 2729), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1035), *Salmonella typhi* (MTCC 98), *Proteus vulgaris* (MTCC 1771), *Bacillus subtilis* (MTCC 121), *Yersinia enterocolitica* (MTCC 840)

					and fungi such as *Candida albicans* (MTCC 183) and *Cryptococcus neoformans* (MTCC 1346).
Nonadecane	0.06	93	268.52	Antimicrobial (Ghavam et al. 2021)	*Aspergillus brasiliensis, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Candida albicans*
Pentadecane	0.06	97	212.41	Antimalarial, antimicrobial, and (Bruno et al., 2015)	*Leishmania infantum*
Hexadecane	0.07	93	226.44	Antioxidant and Antimicrobial (Geethalakshmi&Sarada.,2013)	*Staphylococcus aureus* (MTCC 29213), *Streptococcus faecalis* (MTCC0459), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1035), *Salmonella typhi* (MTCC 98), *Vibrio cholera* (MTCC 3906), *Proteus vulgaris* (MTCC 1771), *Bacillus subtilis* (MTCC 121), *Yersinia enterocolitica* (MTCC 840), *Candida albicans* (MTCC 183) and *Cryptococcus neoformans* (MTCC1346).
Octadecane	0.17	94	254.49	Limited Biological Reports	
Heptadecane	0.07	93	240.47	Anti-inflammatory Activity (Kim et al., 2013)	Not Specified
Pentadecane	0.15	97	212.41	Antimicrobial Activity (Bruno et al., 2015)	Promastigotes and Amastigotes of *Leishmania infantum* IPT1 strain.
Heneicosane	0.07	89	296.58	Antimicrobial activity (Biswas et al., 2017)	*Staphylococcus aureus, Sarcina lutea, Pseudomonas aeruginosa*, *Escherichia coli*
Heptadecane	0.12	96	240.47	Anti- inflammatory Activity (Kim et al.,2013)	Not Specified
Triacontane	0.11	87	422.81	Limited Biological Reports	
Docosane	0.16	96	310.6	Antibacterial activity (Nguyen et al., 2026)	*Erwinia amylovora* and *Ralstonia solanacearum*
Heneicosane	0.37	97	296.58	Antioxidant activity	Not Specified

				(Vanetha et al., 2020)	
Nonacosane (CAS) n-Nonacosane	0.84	97	408.79	Limited Biological Reports	
Hentriacontane	1.03	95	436.85	Anti-inflammatory (BenChem, 2026)	Not Specified
Docosane	1.94	96	310.6	Antibacterial activity (Nguyen et al., 2026)	*Erwinia amylovora* and *Ralstonia solanacearum*
Nonacosane	0.09	85	408.79	Limited Biological Reports	
Tetratetracontane	1.09	95	619.2	Antioxidant and antimicrobial (Rhetso et al., 2020)	*Staphylococcus aureus* and *Pseudomonas aeruginosa*.
Isopropyl Myristate	0.15	94	270.45	Penetration enhancer (Opadyke et al., 1976)	Not Specified
Ethyl 9-hexadecenoate	0.30	83	282.46	Anti-inflammatory activity (SmMolecule Inc.)	Not Specified
Ethyl 9-hexadecenoate	0.20	88	282.46	Anti-inflammatory activity (SmMolecule Inc.)	Not Specified
Hexadecanoic acid, ethyl ester	2.57	96	284.48	Anti-inflammatory and Antimicrobial activity (Ali Ghfil et al.,, 2025)	*S. aureus* and *Bacillus subtilis*
Lenoleic acid ethyl ester	1.38	95	308.5	Antioxidant, cardioprotective properties (Agunloye et al., 2025)	Not Specified
9,12,15-Octadecatrienoic acid, ethyl ester,(Z,Z,Z)-	3.01	88	306.48	Anti-oxidant and Antimicrobial (Tian et al., 2018)	Not Specified
Octadecanoic acid, ethyl ester	1.21	94	312.53	Anti-inflammatory, anticancer activities and antimicrobial (Agunloye et al., 2025)	Not Specified
Ethyl tridecanoate	0.09	76	242.4	Limited Biological Reports	

o-Menthan-8-ol	0.34	89	154.25	Antimicrobial and Anti-inflammatory (BenchChem Technical Support Team, 2026)	Not Specified
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.15	94	296.53	Antimicrobial (Cox-Georgian et al., 2019)	*E. coli* , *S. aureus* , and *Bacillus cereus*
1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-,(E,E)-	0.24	88	290.49	Anti-inflammatory and Antioxidant (Chan et al., 2016).	Not Specified
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-, (all-E)-	3.78	96	410.73	Antioxidant and antimicrobial (Wang, X., et al. 2024).	Not Specified
Terephthalic acid,di(2-ethylhexyl) ester	2.73	84	390.56	Antioxidant, and antimicrobial (El sayed et al., 2015)	*Cryptococcus neoformans*
n-Hexadecanoic acid	1.56	80	256.42	Anti-inflammatory and Antimicrobial (Mancini et al., 2015)	Not Specified
cis,cis,cis-7,10,13-Hexadecstrienal	0.81	89	234.38	Antimicrobial activity (Karar et al., 2022)	Not Specified
Propanoic acid,2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediylester	0.77	92	258.4	Limited Biological Reports	
1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	0.48	66	217.24	Antimicrobial (Arafat et al., 2025)	*Enterococcus durans*
1,2-Benzenedicarboxylic acid,bis(2-methylpropyl) ester	0.35	95	278.34	Contaminant	
1,2-Benzenedicarboxylic acid,bis(2-ethylhexyl) ester(CAS)Bis(2-ethylhexyl)Phthalate	0.12	94	390.56	Contaminant	

NEOPHYTADIENE	0.35	94	278.47	Anxiolytic-like and Antimicrobial (Rajeswaran & Rajan, 2025)	Not Specified
1,2-Diphenyltetramethyl disilane	0.35	65	270.46	Limited biological reports	
Dodecane, 4,6-dimethyl-	0.11	94	198.39	Limited Biological Reports	
3,5-dimethyldodecane	0.13	92	198.39	Limited Biological Reports	
9-Eicosyne	0.23	90	278.52	Antimicrobial (Rushdi et al., 2021)	*Aspergillus niger, Aspergillus flavus, Candida utilis, Fusarium solani,* and *Pythium parasiticum*
9-Octadecenamide,(Z)-	0.13	95	281.48	Anti-inflammatory activity (Isbelin et al., 2021)	Not Specified
9,12-Octadecanoic acid (Z,Z)-	0.10	86	280.45	Anti-inflammatory, Hypocholesterolemic, and anticancer activities (Isbelin et al., 2021)	Not Specified
2-Pentadecanone,6,10,14-trimethyl	0.09	92	268.48	Antibacterial (MedChem Express)	*S.aureus* and *Candida albicans*
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.08	83	276.37	Antibacterial Activity (Zaidi et al., 2021)	*Streptomyces* sp. EMB26
Dodecane, 4-cylohexyl	0.06	85	252.48	Limited biological reports	Dodecane, 4-cylohexyl

Based on the biological activities of the compounds shown in **Table 2**, 43.75% have antimicrobial properties, 27.08% have anti-inflammatory activity, 18.75% have antioxidant activity, and 8.33% have antibacterial activity. These results show that the leaf extract contains different active compounds that may have useful medicinal effects.

Table 2. Total percentage (%) of biological properties of the identified compounds

Biological Properties	Percentage (%)
Antimicrobial	43.75%
Anti-Inflammatory	27.08%
Antioxidant	18.75%
Antibacterial	8.33%

Several identified compounds, including 2,6,10,14,18,22-tetracosahexaene, 9,12,15-octadecatrienoic acid ethyl ester, terephthalic acid di(2-ethylhexyl) ester, hexadecanoic acid ethyl ester, linoleic acid ethyl ester, octadecanoic acid ethyl ester, *n*-hexadecanoic acid, neophytadiene, and 3,7,11,15-tetramethyl-2-hexadecen-1-ol, have been reported to exhibit antibacterial and antimicrobial activities (Agunloye et al., 2025; Cox-Georgian et al., 2019). These compounds may inhibit the growth of microorganisms by disrupting bacterial cells or interfering with essential cellular processes. For example, linoleic acid ethyl ester has been reported to exhibit antimicrobial activity against oral bacteria, including *Streptococcus mutans*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans* (Huang et al., 2010). These findings indicate the extract's potential antimicrobial and medicinal properties.

Furthermore, linoleic acid is known to block the bacterial enzyme enoyl-acyl carrier protein reductase (FabI), which is important for the production of fatty acids needed by bacteria to grow and survive (Zheng et al., 2005). When this enzyme is inhibited, bacteria cannot produce essential cell components, which can suppress their growth. Therefore, FabI is considered an important target for developing new antibacterial agents.

Fatty acids and their esters, such as hexadecanoic acid (palmitic acid), linoleic acid ethyl ester, and 9,12,15-octadecatrienoic acid ethyl ester, showed high peak areas in the chromatogram, indicating that they were present in larger amounts in the extract.

Previous studies have reported that these compounds may have anti-inflammatory, antioxidant, and antimicrobial activities (Agunloye et al., 2025). Their antimicrobial effect may be related to their ability to damage the bacterial cell membrane, increase membrane permeability, and interfere with important enzymes needed for bacterial survival (Desbois & Smith, 2010). These actions can weaken or inhibit bacterial growth.

Although more alkane hydrocarbons (e.g., undecane, tridecane, hexadecane, and nonadecane) were identified than fatty acid esters their cumulative percentage was lower because GC-MS abundance is based on peak area (Area %) rather than the number of detected compounds **(Figure 3)**. Many hydrocarbons were present only in small amounts whereas fewer fatty acid ester compounds showed larger peak areas resulting in greater quantitative abundance in the extract. The extract also contained terpenoids, fatty acids, aldehydes, ketones, and other minor compounds, which may contribute to its biological activities particularly its antimicrobial potential.

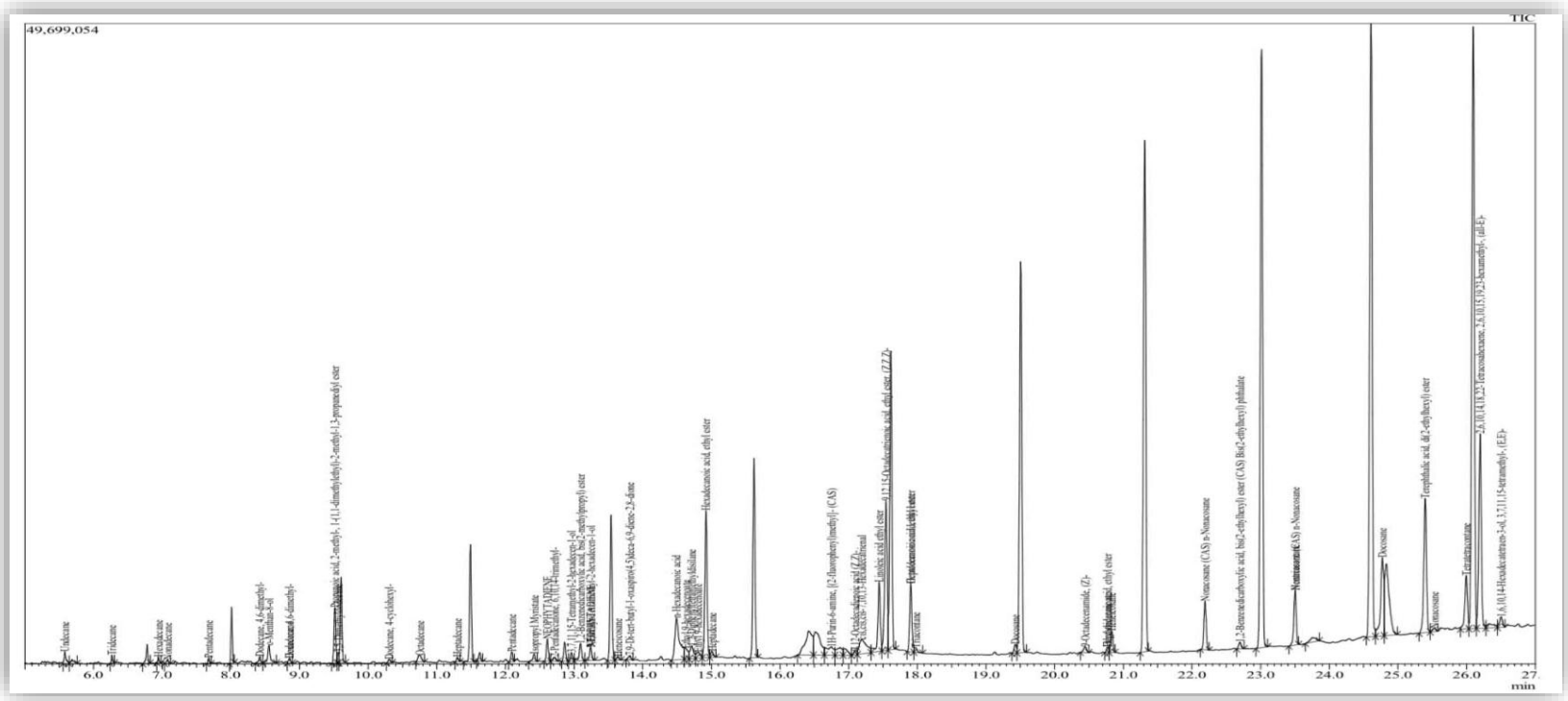

Figure 3. Peak Area of the Compounds

Furthermore, terpenoid-related compounds, including neophytadiene and phytol derivatives (e.g., 3,7,11,15-tetramethyl-2-hexadecen-1-ol), have been reported to exhibit antioxidant, anti-inflammatory, and antimicrobial activities (Cox-Georgian et al., 2019; Rajeswaran & Rajan, 2025).

These findings suggest that the leaf extract may possess therapeutic potential beyond its antibacterial activity.Rajeswaran & Rajan, 2025). The presence of compounds with multiple biological activities suggests that the extract may be useful for different medicinal applications. In traditional medicine, plants with diverse phytochemicals are often valued because they can provide several beneficial effects simultaneously. This supports the need for further studies on the pharmacological properties of the plant.

Long-chain hydrocarbons such as pentadecane and heptadecane were also detected. These compounds are commonly associated with plant cuticular waxes and may contribute to the plant's protective functions and extract stability (Wang et al., 2020). Some studies have also reported antimicrobial activity of pentadecane against *L. infantum* in vitro (Bruno et al., 2019) while heptadecane has been associated with anti-inflammatory effects (Kim et al., 2013). Although these hydrocarbons are not considered the main active antibacterial compounds, they may contribute to the overall biological activity of the extract. They may also interact with other phytochemicals, resulting in synergistic effect. Such interactions could improve the effectiveness of the crude extract compared to individual compounds alone.

Phthalate-related compounds, such as 1,2-benzenedicarboxylic acid esters and terephthalic acid derivatives, were also detected in the GC–MS analysis. However, these compounds may not originate from the plant. They are commonly recognized as contaminants originating from plastic materials used during sample preparation, storage, or laboratory analysis (Wang & Qian, 2021). Plastic containers, laboratory tools, solvents,

and even parts of the GC–MS instrument may release small amounts of phthalates during testing (Reid et al., 2007). Because of this, the presence of these compounds in the chromatogram should be interpreted carefully and may not represent true chemical components of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll.

The similarity index values for most identified compounds ranged from 90% to 97% indicating reliable compound identification. However, compounds with similarity scores below 80% may require further confirmation using additional analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy or authentic standards. Additional confirmation is important because compounds with low similarity scores may be incorrectly identified. Combining GC–MS with other analytical techniques can provide more accurate structural information. This approach would strengthen the validity of future phytochemical studies.

Overall, the GC-MS profiling of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll leaf extract revealed the presence of several phytochemical compounds previously reported to possess antimicrobial activities particularly fatty acid esters and terpenoid derivatives. These compounds may contribute to the antibacterial potential of the extract against *Pseudomonas aeruginosa* ATCC 27853. Further studies including compound isolation and additional in vitro and in vivo biological assays are recommended to further validate the antibacterial activity of the identified compounds. These findings provide baseline data for future studies and support the potential of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll as a plant-based antimicrobial source.

In vitro Antibacterial assay

Using *Pseudomonas aeruginosa* ATCC 27853 as the test bacterium was an appropriate scientific choice since it is an important opportunistic pathogen commonly found in healthcare-associated infections, wounds, respiratory infections, urinary tract infections, and immunosuppressed patients (Wilson et al., 2023). The bacterium is also widely used in antimicrobial testing because of its known resistance to many antibiotics (Pang et al., 2019). Testing the plant extract against a highly resistant bacterium helped determine whether the extract has antibacterial potential against difficult-to-treat pathogens. In addition, the use of a validated ATCC strain improved the reliability of the results. However, since only one bacterial isolate was tested the findings cannot be generalized to all microorganisms.

Although the leaf extract contained compounds previously reported to have antimicrobial properties the antibacterial test showed no visible zone of inhibition (ZOI) against *Pseudomonas aeruginosa* ATCC 27853 presented in **Table 3** and **Figure 4** at all concentrations tested (25%, 50%, 75%, and 100%). This is indicative that the extract did not show visible antibacterial activity against the test organism under the experimental conditions employed in the study. In comparison, the positive control exhibited a visible zone of inhibition confirming that the test organism remained viable and responsive to antibacterial agents while the negative control showed no inhibitory effect.

Table 3. Results for Inhibitory Zones against *Pseudomonas aeruginosa* ATCC 27853

Sample	Concentration (%)	Inhibitory Zone (mm)				
		Trial 1	Trial 2	Trial 3	Positive Control	Negative Control
Leaves	25%	0	0	0	21	0
	50%	0	0	0	22	0
	75%	0	0	0	24	0
	100%	0	0	0	21	0

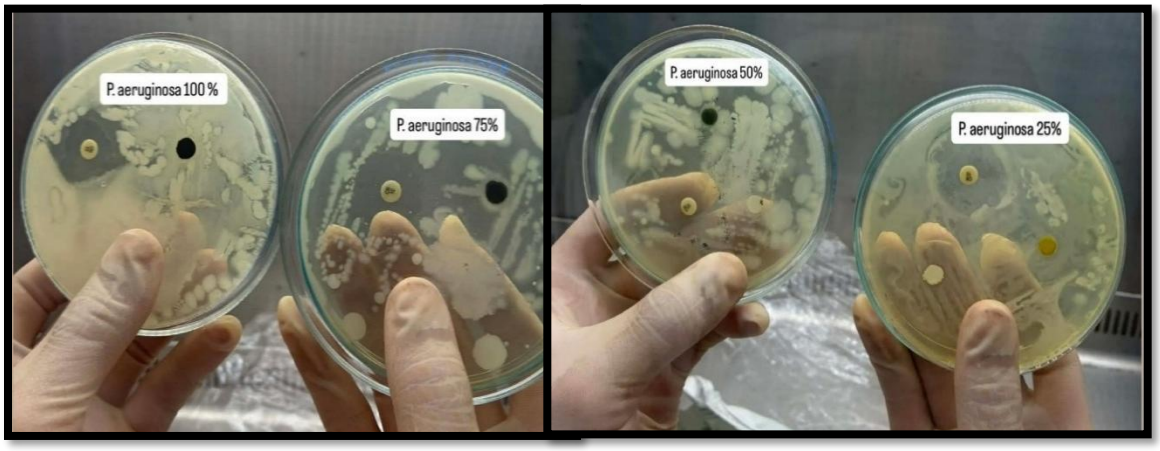

Figure 4. *Pseudomonas aeruginosa* ATCC 27853 in different concentrations

The antimicrobial effects of the extracts of the plants might differ based on the bacteria's strain, species, and cell wall composition (Pang et al., 2019). This suggests that the results of the present study cannot be generalized to other microorganisms without further investigation involving additional Gram-positive and Gram-negative bacteria.

The lack of antibacterial activity against *P. aeruginosa* (ATCC 27853) may primarily be associated with the intrinsic resistance mechanisms of the organism. This bacterium is recognized as one of the most resistant Gram-negative pathogens due to several structural

and physiological defense mechanisms (Elfadadny et al., 2024). One major factor is the presence of a low-permeability outer membrane that restricts the entry of many antimicrobial substances including plant-derived compounds (Pang et al., 2019). Moreover, *P. aeruginosa* (ATCC 27853) is equipped with efflux pumps that are highly efficient in pumping out toxic substances from the bacterial cells before their bactericidal activity takes effect (Elfadadny et al., 2024). The bacterium can produce enzymes that may break down or inactivate antimicrobial agents (Pang et al., 2019). Its ability to form biofilms also increases resistance by creating a protective layer that prevents antibacterial substances from entering the cells (Poole, 2011). These defense mechanisms may explain why no inhibition zone was observed at any concentration of the extract.

Aside from the intrinsic resistance characteristics of *P. aeruginosa* (ATCC 27853) several methodological and phytochemical factors may have influenced the outcome of the antibacterial assay (Balouiri et al., 2016). One important consideration is extraction efficiency. In this study we utilized ethanol as the extraction solvent. Ethanol was used as the extraction solvent because it can dissolve many plant compounds such as flavonoids, phenolics, tannins, alkaloids, and terpenoids (Azwanida, 2015; Dai & Mumper, 2010).

According to Azwanida (2015), the effectiveness of plant extraction largely depends on the polarity of the solvent, extraction conditions, and the chemical nature of the target compounds. Previous studies reported that solvent polarity and extraction conditions can affect the recovery of antimicrobial compounds (Ahamad et al., 2025). Although ethanol is considered an effective solvent some active compounds may not have been fully

extracted because of differences in polarity and extraction conditions (Ahamad et al., 2025; Pandey et al., 2025). Factors such as extraction time, temperature, solvent concentration, and preparation of plant material may also influence the amount of bioactive compounds in the extract (Dai & Mumper, 2010). Therefore, the absence of antibacterial activity may have been partly due to low amounts of active compounds in the extract.

The concentration, stability, availability, and interaction of phytochemicals may also affect biological activity (Dai & Mumper, 2010; Azwanida, 2015). It is possible that the active compounds were present only in small amounts that were not enough to inhibit a highly resistant bacterium such as *P. aeruginosa* ATCC 27853.

Another factor that may have affected the results is the use of the Kirby–Bauer disc diffusion method. Although this method is widely used for antibacterial testing, it may not always work well for crude plant extracts (Bubonja-Šonje et al., 2020). Some plant compounds have large molecules, poor water solubility, or different chemical properties that make it difficult for them to spread through the agar medium. Because of this, the extract may not produce a visible zone of inhibition even if antibacterial compounds are present. Therefore, the absence of a visible ZOI does not always mean that the extract has no antibacterial activity, but it may indicate poor diffusion of the compounds in the agar (Bubonja-Šonje et al., 2020).

The type of culture medium used may have also affected the results. Nutrient Agar is a general-purpose medium commonly used for growing bacteria because it contains nutrients such as peptone, beef extract, and sodium chloride (Tankeshwar, 2016). Some

studies have also used Nutrient Agar in antimicrobial testing involving *P. aeruginosa* (ATCC 27853) (Rahaman et al., 2024). However, Mueller–Hinton Agar (MHA) is the standard medium recommended for Kirby–Bauer antimicrobial testing because it allows better diffusion of antimicrobial compounds and more reliable measurement of inhibition zones (Rashid & Islam, 2024). Since this study used Nutrient Agar instead of MHA the movement of the plant extract in the agar may have been affected which could have contributed to the absence of visible inhibition. Therefore, the findings should be interpreted cautiously and the use of non-standard agar medium should be recognized as a limitation of the study.

Differences in bacterial structure may also explain why the results differed from previous studies. Lazo and Caburian (2025) reported antibacterial activity of the extract against *Staphylococcus aureus*. Unlike *Pseudomonas aeruginosa*, *Staphylococcus aureus* is a Gram-positive bacterium characterized by a thicker peptidoglycan layer but does not have the outer lipid membrane found in Gram-negative bacteria (Silhavy et al., 2010). Because of this, antimicrobial compounds can enter Gram-positive bacteria more easily making them generally more sensitive to plant extracts (Cowan, 1999). In contrast, the outer membrane of *Pseudomonas aeruginosa* acts as an extra barrier that reduces the effect of many antibacterial agents (Elfadadny et al., 2024). This difference in structure may explain why antibacterial activity was observed against *Staphylococcus aureus* in previous studies but not against *Pseudomonas aeruginosa* ATCC 27853 in the present study.

The findings of the present study indicate that the leaf extract showed no inhibitory effect against *P. aeruginosa* (ATCC 27853*)* under the experimental conditions employed. However, factors such as the natural resistance of the bacterium, extraction efficiency, poor diffusion of plant compounds in agar and the use of Nutrient Agar instead of Mueller–Hinton Agar may have affected the results. Further studies using different extraction methods, solvents, bacterial strains, and more sensitive antibacterial tests are recommended to better evaluate the antibacterial potential of the plant extract.

CONCLUSION AND RECOMMENDATION

This study identified the chemical compounds and evaluated the antibacterial activity of the ethanolic leaf extract of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll against *Pseudomonas aeruginosa* ATCC 27853. GC-MS analysis detected 48 compounds, including hydrocarbons, fatty acid esters, terpenoids, aldehydes, and alcohols, many of which have been reported to possess biological activities. However, the extract did not show antibacterial activity at any of the tested concentrations (25%, 50%, 75%, and 100%), as no zone of inhibition was observed. Despite this result, the presence of various bioactive compounds suggests that *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zollmay still have potential medicinal value and deserves further study.

Future studies should evaluate the antibacterial activity of *Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll against other bacterial species, including both Gram-positive and Gram-negative bacteria. The use of Mueller–Hinton Agar and standard antimicrobial testing procedures is recommended to improve the reliability of the results. Further research should also determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract. In addition, improved extraction methods and the isolation of specific bioactive compounds are recommended to further investigate the plant's antimicrobial and medicinal potential.

Declaration of the Use of AI and Online Tools

We, the undersigned researchers, hereby declare that we have used artificial intelligence (AI) tools as supplementary resources in the conduct and preparation of our research entitled: "GAS CHROMATOGRAPH–MASS SPECTROMETRY PROFILING, AND ANTIBACTERIAL ACTIVITY OF ALIM [*Melanolepis multiglandulosa* (Reinw. ex Blume) Rchb. & Zoll.] ETHANOLIC LEAF EXTRACT AGAINST *Pseudomonas aeruginosa*". The following AI tools were used to support various aspects of this study: ChatGPT (https://chatgpt.com/), QuillBot (https://quillbot.com/), and Google Scholar (https://scholar.google.com/). These AI tools were used to refine the research objectives, improve manuscript clarity and academic quality, identify relevant literature, and enhance overall structure and readability.

Representative prompts used during the preparation of the manuscript included:

- "Provide related studies on the phytochemical composition and antibacterial activity of *Melanolepis multiglandulosa*."
- "Paraphrase this paragraph for clarity while preserving its meaning."
- "Explain the principle of GC–MS in simple academic language."

AI tools were not used to generate data, conduct experiments, or replace scientific judgment. All AI-assisted content was reviewed, verified, and edited by the researchers, who assume full responsibility for the manuscript's accuracy, originality, and integrity.

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APPENDIX A. INFORMED CONSENT FORM

Misamis University

Ozamiz City

MISAMIS UNIVERSITY RESEARCH CENTER

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

MU-RC-042/13 November 2024

Thesis/Research Data Gathering Approval Form

Date: 01/14/26

Name of Authors: NJ Mark L. Sedenio, Joel Andre R. Varada, Jhonnabie F. Tabaro, Mary Joseth M. Sarmiento

College/Department: College of Medical Technology

Research Title: Gas Chromatography - Mass Spectrometry Profiling and Antibacterial Activity of Melanolepis multiglandulosa (Reinw. ex Blume) Rchb. & Zoll Leaf Extract Against *Pseudomonas aeruginosa*

Data Gathering Procedure:

a. Type of Research: (Kindly check)

☐ Quantitative ☐ Qualitative ☑ Experimental

☐ Approved Research Title & Problem/Objectives ☐ Approved Data Gathering Procedure ☐ Approved Data Gathering Instrument/Data Sheets

b. Respondents/Participants/Subject/Samples to be Collected: Leaf of Melanolepis multiglandulosa

c. Procedure:

☐ Survey ☐ Interview ☑ Experimentation ☐ Laboratory Testing

d. Place of Implementation/Study Area: ______

*If to be conducted **outside the school campus**, indicate the nature of transportation to be used:*

☐ Own private vehicle ☑ public utility vehicle ☐ rental ☐ Others, please indicate ______

e. Date/s of Implementation/Sampling: ______

Attachments:

☐ Approved Letter of Consent from the designated authority in the area/place were the study is to be conducted

☑ Signed Parents' Consent

The thesis proposal mentioned above is hereby recommended for the commencement of data gathering implementation.

Recommended by:

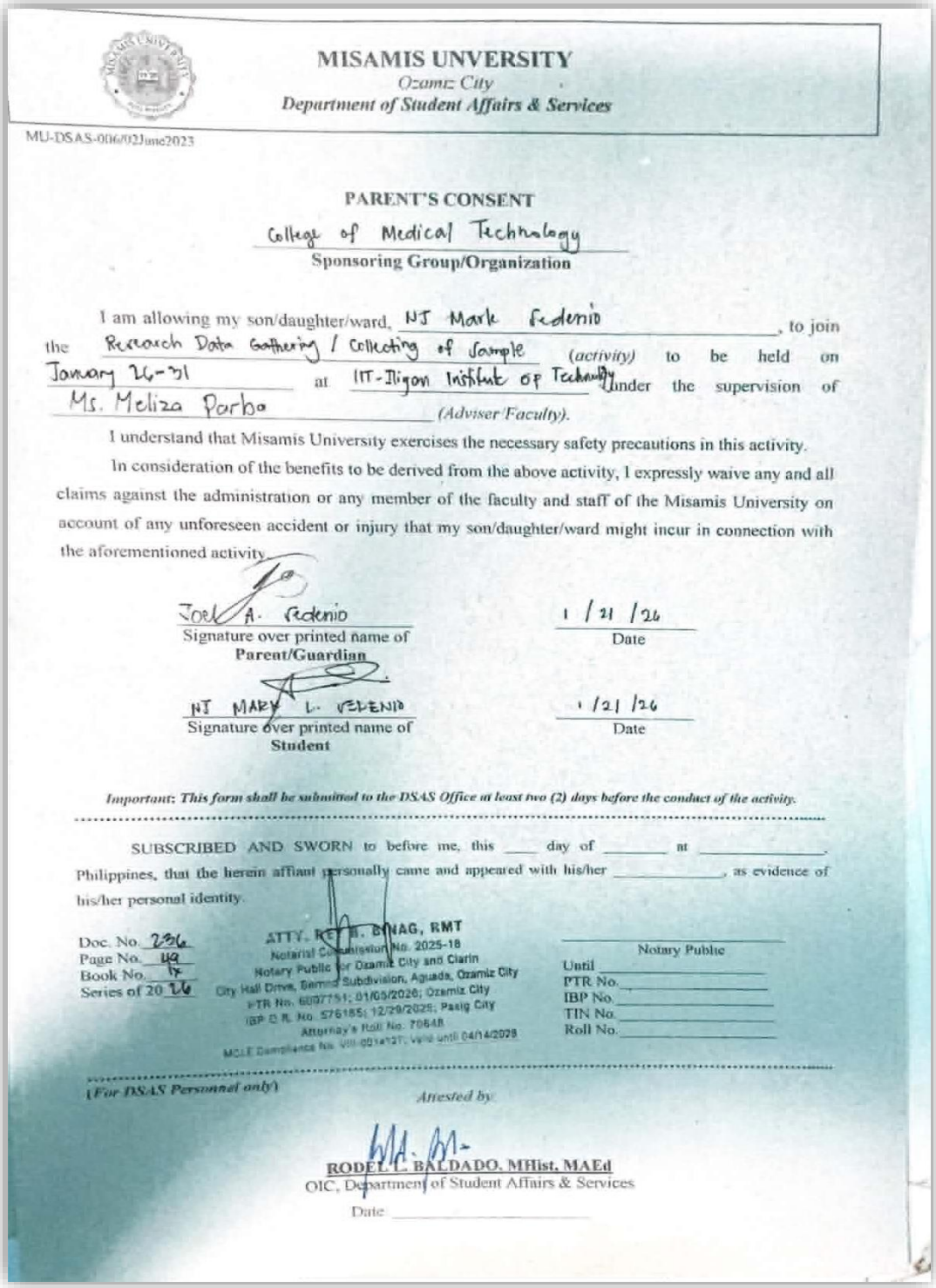
MELIZA PARBA, PhD
Thesis/Research Adviser

DR. NELFA D. CANINI
Thesis/Research Instructor

Approved by:

MS. EVANGELINE M. VEÑEDO, RMT, MATMRS
College Dean

APPENDIX B. APPROVED LETTERS OF CONSENT



MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

College of Medical Technology
Sponsoring Group/Organization

I am allowing my son/daughter/ward, NJ Mark Rodenio, to join the Research Data Gathering / Collecting of Sample *(activity)* to be held on January 26-31 at IIT-Iligan Institute of Technology under the supervision of Ms. Meliza Parba *(Adviser/Faculty)*.

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

Joel A. Rodenio — 1/21/26
Signature over printed name of **Parent/Guardian** — Date

NJ MARK L. RODENIO — 1/21/26
Signature over printed name of **Student** — Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this ____ day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 236
Page No. 49
Book No. 17
Series of 2026

ATTY. RET A. BINAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Barmac Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576185; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-0014127; Valid until 04/14/2028

Notary Public
Until
PTR No.
IBP No.
TIN No.
Roll No.

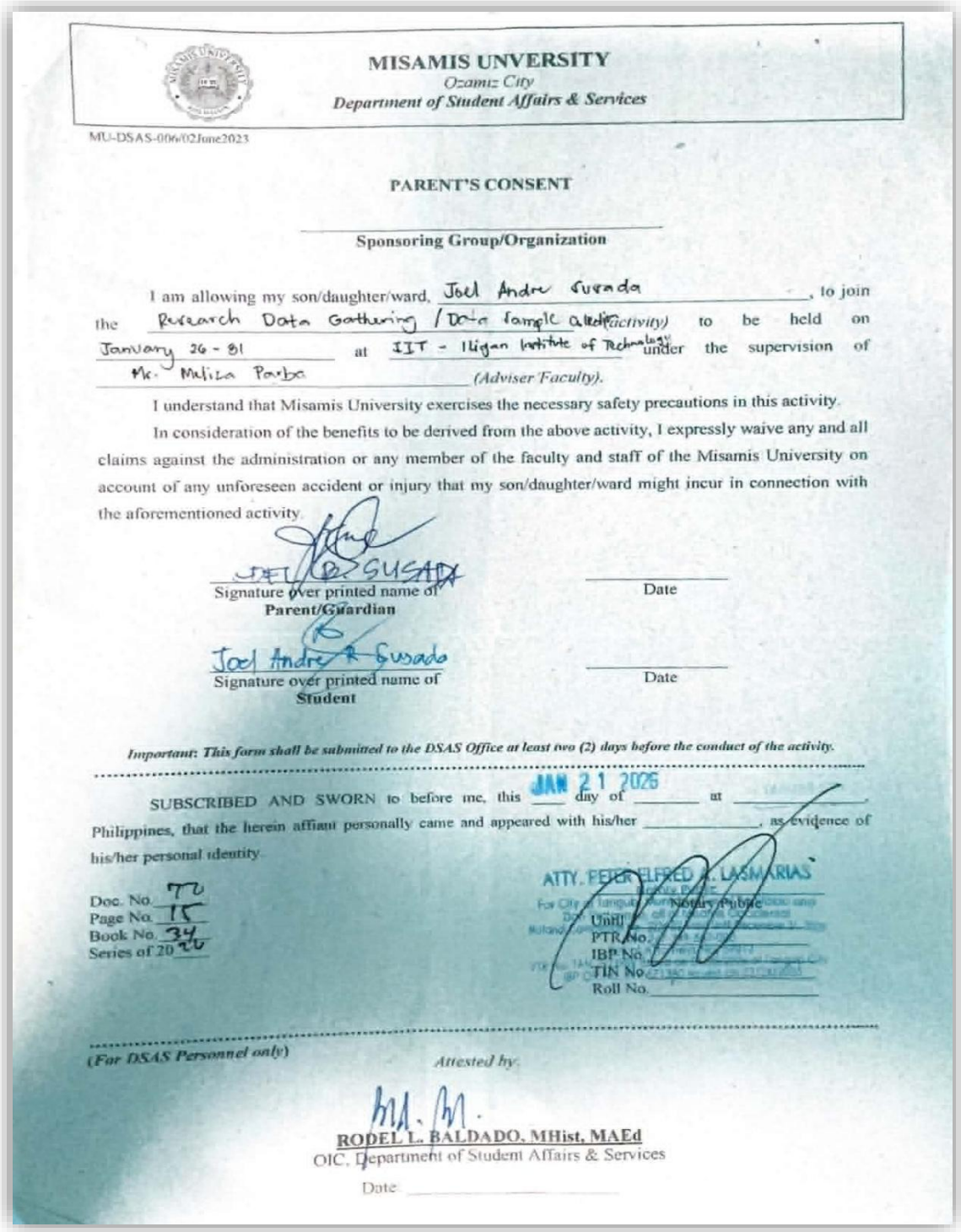
(For DSAS Personnel only)

Attested by:

RODEL C. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date

APPENDIX B. Cont'd



MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Joel Andre Susada, to join the Research Data Gathering / Data Sample Collection *(activity)* to be held on January 26 - 31 at IIT - Iligan Institute of Technology under the supervision of Mr. Melita Parba *(Adviser/Faculty)*.

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

JOEL D. SUSADA
Signature over printed name of **Parent/Guardian**

Date

Joel Andre S. Susada
Signature over printed name of **Student**

Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this JAN 21 2026 day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 77
Page No. 15
Book No. 34
Series of 2026

ATTY. PETER ALFRED A. LASMARIAS
Notary Public
Until
PTR No.
IBP No.
TIN No.
Roll No.

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date

APPENDIX B. Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

College of Medical Technology
Sponsoring Group/Organization

I am allowing my son/daughter/ward, Mary Joseth M. Sarmiento, to join the Research Data Gathering / Data Collection *(activity)* to be held on January 26-31 at IIT-Iligan Institute of Technology under the supervision of Ms. Meliza Parba *(Adviser Faculty)*.

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

HERLYN JOY M. SARMIENTO
Signature over printed name of **Parent/Guardian**

01-14-26
Date

Mary Joseth M. Sarmiento
Signature over printed name of **Student**

01-14-26
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this ____ day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 237
Page No. 49
Book No. IX
Series of 2026

ATTY. REY J. [illegible]NAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576105; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-0014127; Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

APPENDIX B. Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

College of Medical Technology
Sponsoring Group/Organization

I am allowing my son/daughter/ward, Jhunnabie E. Tabamo, to join the Research Data Gathering / Collecting of Sample *(activity)* to be held on January 26-31 at IIT-Iligan Institute of Technology under the supervision of Ms. Meliza Parba *(Adviser/Faculty)*.

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

MA. FLOXDELIANA E. TABAMO
Signature over printed name of
Parent/Guardian

01-16-2026
Date

JHUNNABIE E. TABAMO
Signature over printed name of
Student

01-16-2026
Date

Important*: *This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this ___ day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 238
Page No. 49
Book No. IX
Series of 2026

ATTY. [illegible] G, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576160; 12/20/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No: VIII-0014127; Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date _____

APPENDIX B. Cont'd

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV

ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

Date: March 2, 2026

Dr. Haydee D. Villanueva
Dean, College of Arts and Sciences
Misamis University

Subject: *Request for Permission to Use Laboratory Rooms, Equipment, and Reagents for Research Data Gathering*

Dear Dr. Villanueva,

We respectfully write this letter to request permission from your good office to allow us to use the laboratory rooms, laboratory equipment, and reagents of the Natural Sciences Department of the College of Arts and Sciences for our research data gathering **from January to April**.

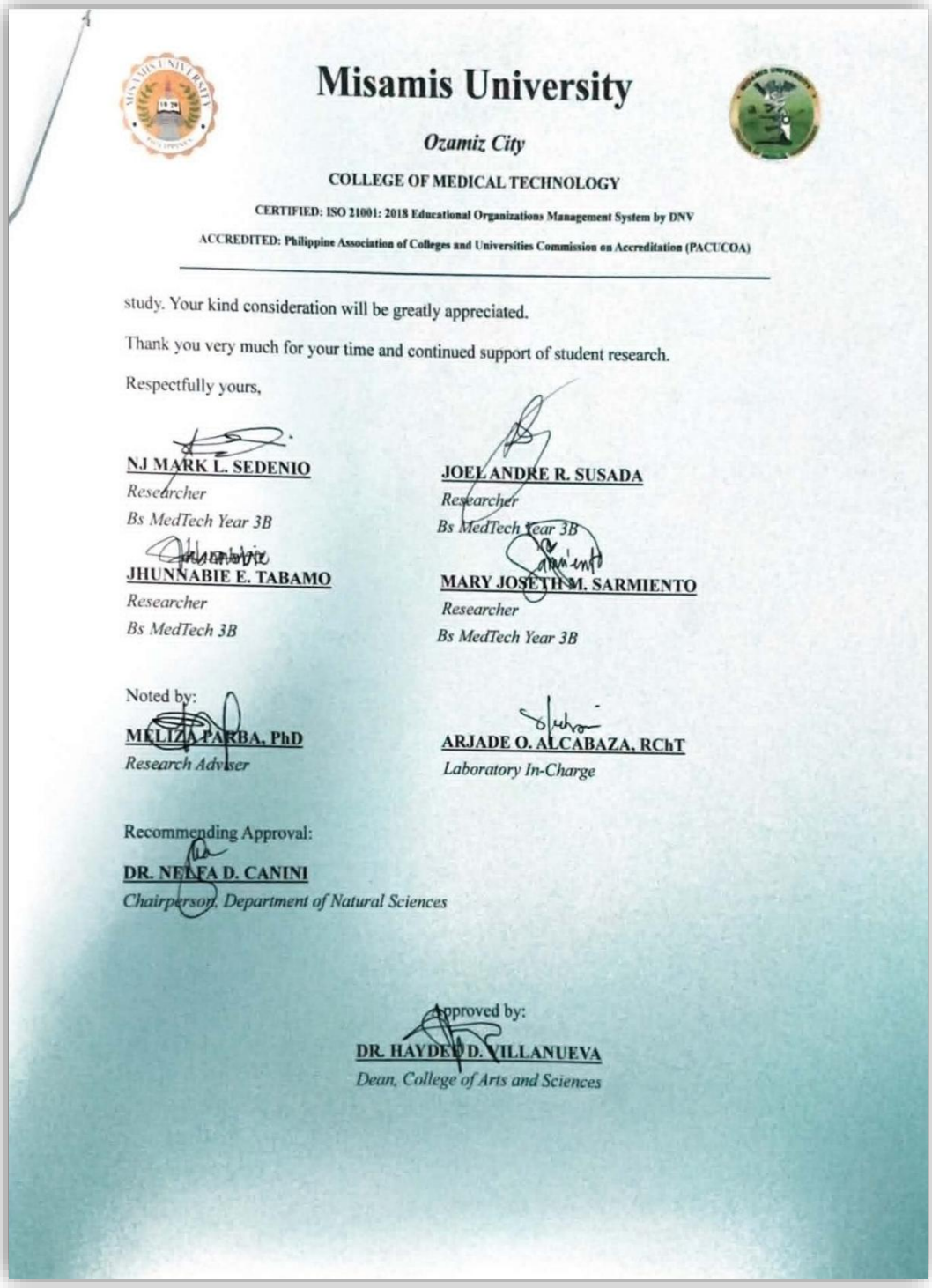
Our research study is entitled **"GC-MS Profiling and Antibacterial Activity of *Melanolepis multiglandulosa* Leaf Extract against** *Pseudomonas aeruginosa*." This study aims to identify the chemical components of the plant extract through Liquid Chromatography–Mass Spectrometry (LC-MS) and to evaluate its antibacterial activity against selected wound-causing pathogens. Access to the laboratory facilities is essential for conducting procedures such as sample preparation, extraction, analysis, microbial culture, and antibacterial testing.

We assure your good office that all laboratory activities will be conducted under proper supervision and in full compliance with laboratory safety rules, biosafety guidelines, and institutional policies. Proper handling, use, and disposal of reagents, chemicals, and biological materials will be strictly observed. We also commit to using the facilities responsibly and ensuring that all equipment and laboratory areas are properly cleaned and returned in good condition after use.

Furthermore, we will coordinate our laboratory schedule with the department to avoid disruption of regular classes and laboratory activities. The facilities and materials will be used solely for academic and research purposes.

In this regard, we humbly request your approval and support for the use of the Natural Sciences Department laboratory facilities from **January to April** for the successful completion of our research

APPENDIX B. Cont'd



Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV

ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

study. Your kind consideration will be greatly appreciated.

Thank you very much for your time and continued support of student research.

Respectfully yours,

NJ MARK L. SEDENIO
Researcher
Bs MedTech Year 3B

JOEL ANDRE R. SUSADA
Researcher
Bs MedTech Year 3B

JHUNNABIE E. TABAMO
Researcher
Bs MedTech 3B

MARY JOSETH M. SARMIENTO
Researcher
Bs MedTech Year 3B

Noted by:

MELIZA PARBA, PhD
Research Adviser

ARJADE O. ALCABAZA, RChT
Laboratory In-Charge

Recommending Approval:

DR. NELFA D. CANINI
Chairperson, Department of Natural Sciences

Approved by:

DR. HAYDEE D. VILLANUEVA
Dean, College of Arts and Sciences

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV

ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

LETTER OF INTENT

March 3, 2026

ENJELYN C. GOMEZ
Laboratory-in-Charge, Department of Chemistry
Mindanao State University – Iligan Institute of Technology
Iligan City, Lanao del Norte

Dear Ma'am Gomez,

Greetings of peace!

We are third-year Bachelor of Science in Medical Technology students from Misamis University currently conducting our research study entitled **"GC-MS Profiling and Antibacterial Activity of *Melanolepis multiglandulosa* Leaf Extract Against** *Pseudomonas aeruginosa*"

This study is being conducted in partial fulfillment of the academic requirements for the said degree.

In line with this, we respectfully seek your permission to conduct part of our research experimentation within your institution, specifically at the Department of Chemistry. We would like to humbly request your assistance in allowing us to use your rotary evaporator for the concentration of our plant extract.

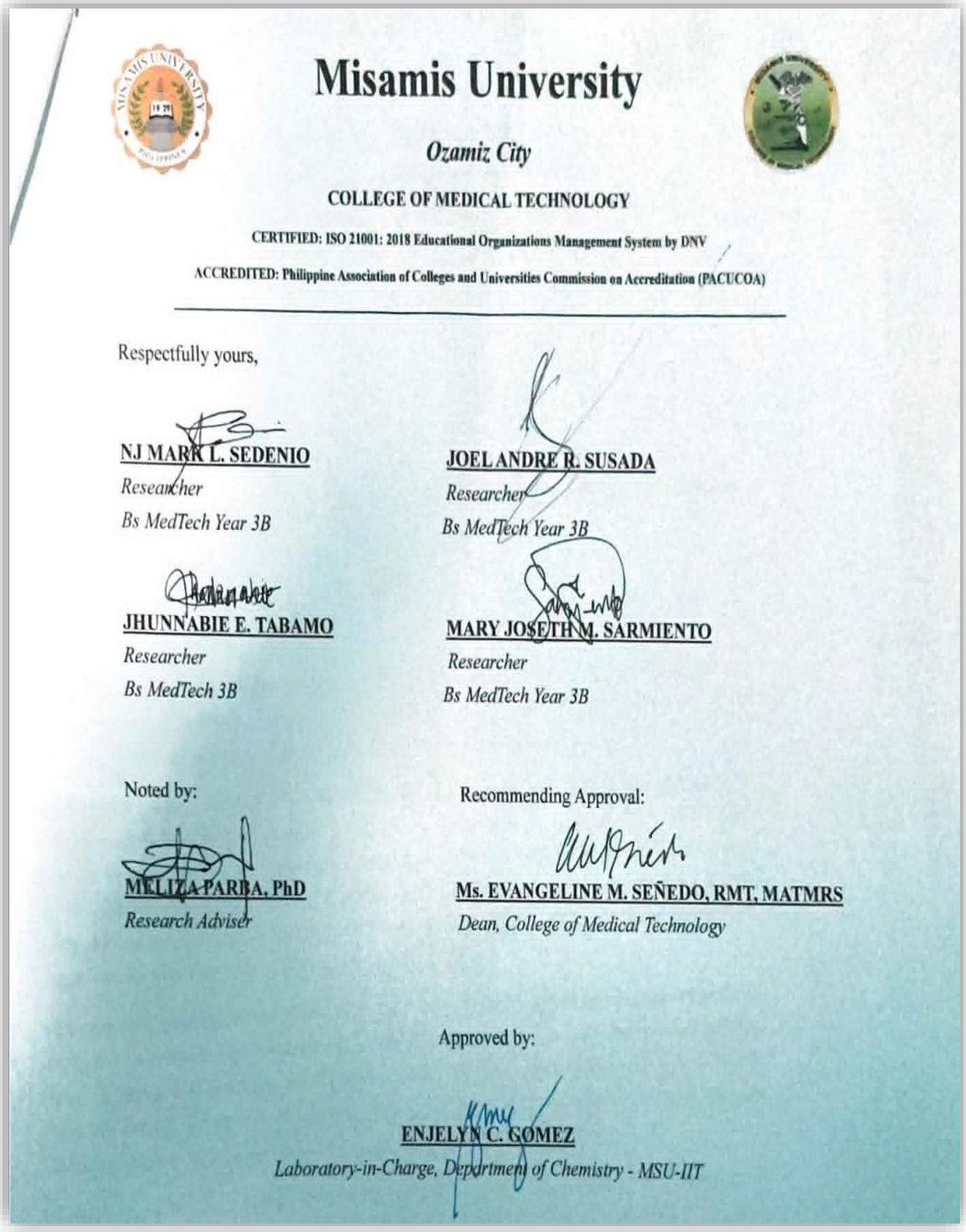
We would like to inform you that we have already completed the ethanolic extraction of *Melanolepis multiglandulosa* leaves, and our extract is now prepared and ready for rotary evaporation. Access to your laboratory equipment, particularly the rotary evaporator, would greatly help us in ensuring accurate and efficient concentration of our sample prior to GC-MS profiling and antibacterial testing.

We assure you that we are willing to comply with all laboratory policies, safety protocols, requirements, and fees set by your institution. We will strictly follow proper laboratory procedures and handle all materials responsibly.

We sincerely hope for your kind consideration and favorable response. Your support will greatly contribute to the completion and success of our research study.

Thank you very much for your time and assistance.

APPENDIX B. Cont'd



Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV

ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

Respectfully yours,

NJ MARK L. SEDENIO
Researcher
Bs MedTech Year 3B

JOEL ANDRE R. SUSADA
Researcher
Bs MedTech Year 3B

JHUNNABIE E. TABAMO
Researcher
Bs MedTech 3B

MARY JOSETH M. SARMIENTO
Researcher
Bs MedTech Year 3B

Noted by:

MELIZA PARBA, PhD
Research Adviser

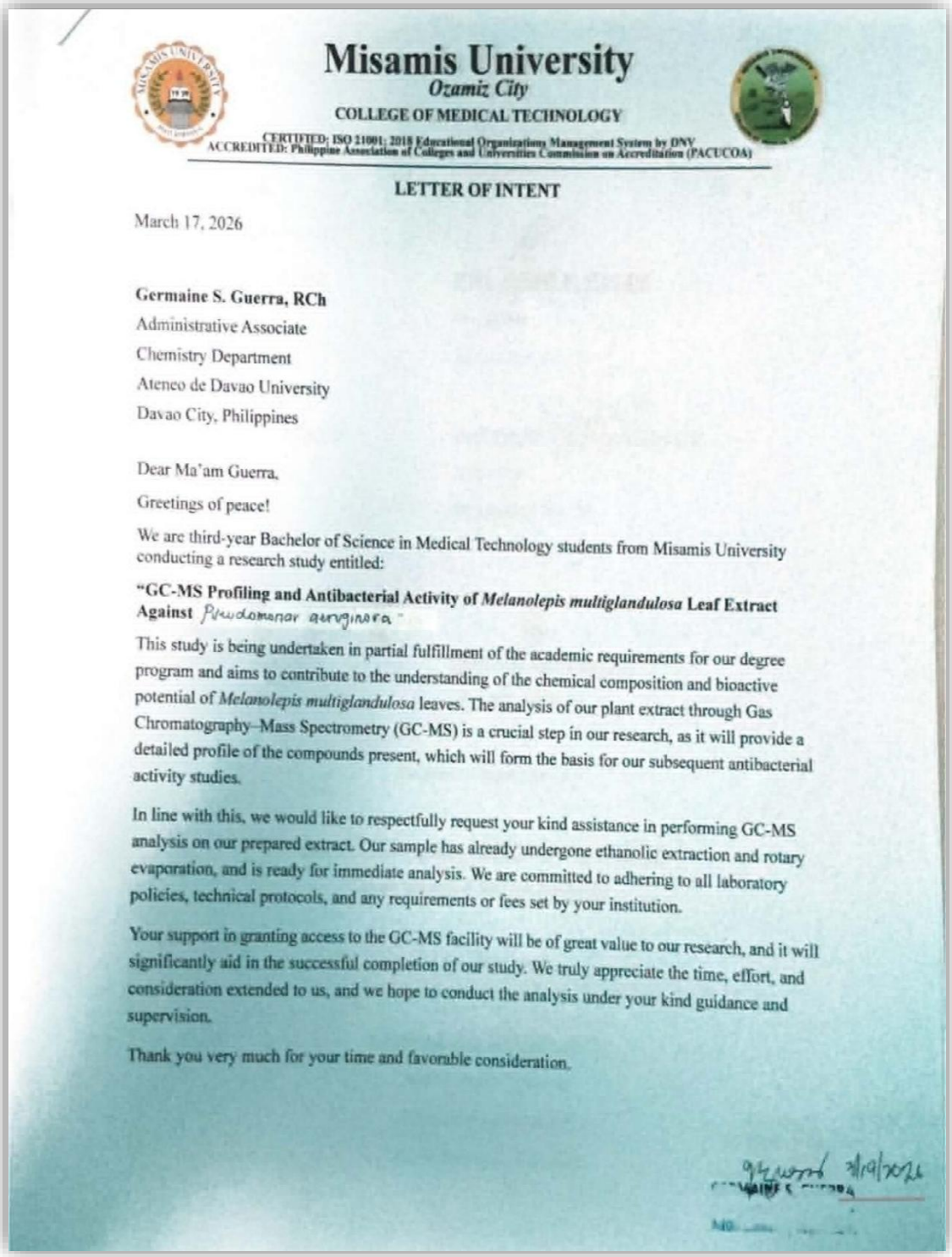
Recommending Approval:

Ms. EVANGELINE M. SEÑEDO, RMT, MATMRS
Dean, College of Medical Technology

Approved by:

ENJELYN C. GOMEZ
Laboratory-in-Charge, Department of Chemistry - MSU-IIT

APPENDIX B. Cont'd



Misamis University
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY
CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

LETTER OF INTENT

March 17, 2026

Germaine S. Guerra, RCh
Administrative Associate
Chemistry Department
Ateneo de Davao University
Davao City, Philippines

Dear Ma'am Guerra,

Greetings of peace!

We are third-year Bachelor of Science in Medical Technology students from Misamis University conducting a research study entitled:

"GC-MS Profiling and Antibacterial Activity of *Melanolepis multiglandulosa* Leaf Extract Against *Pseudomonas aeruginosa*"

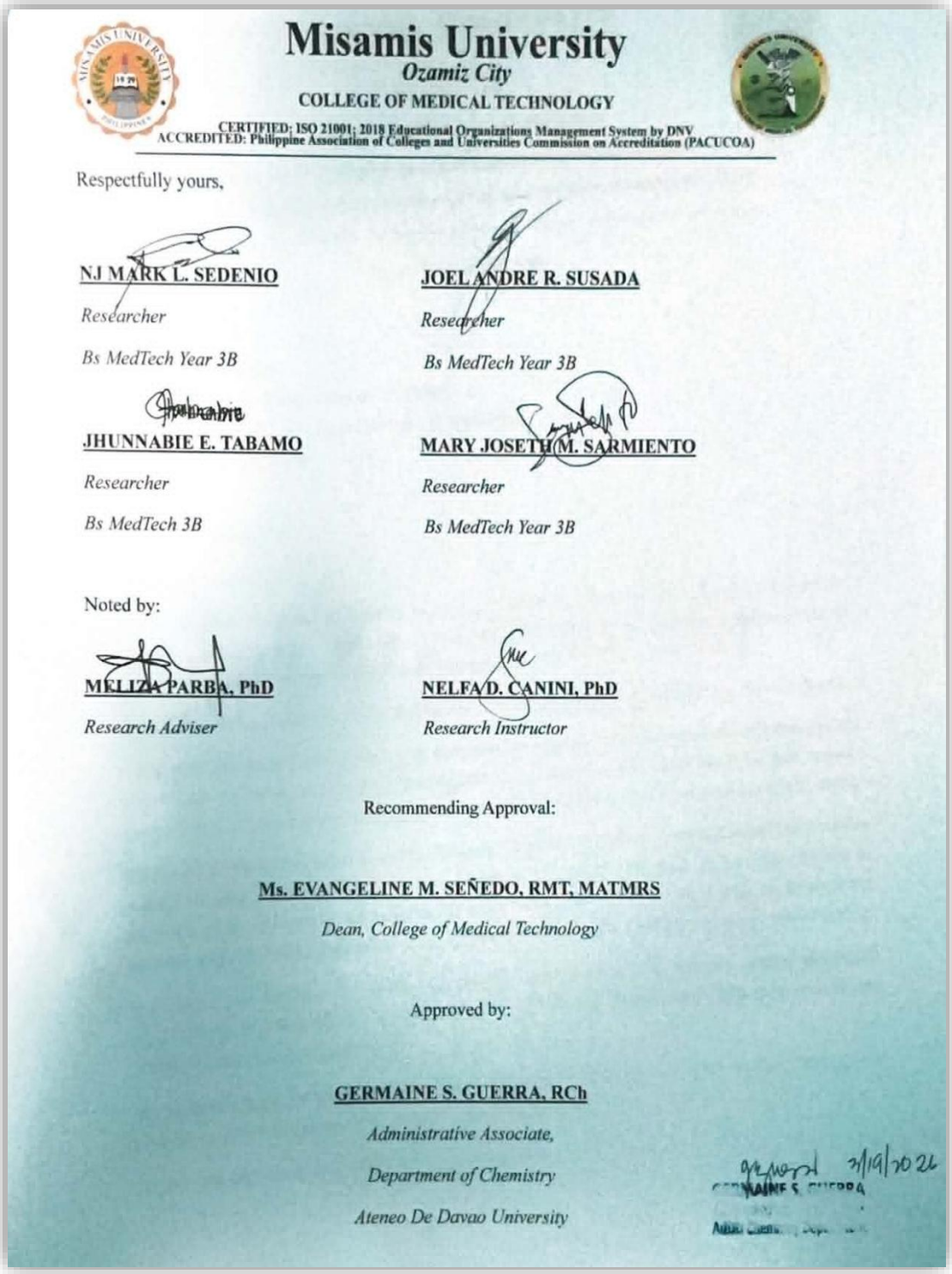
This study is being undertaken in partial fulfillment of the academic requirements for our degree program and aims to contribute to the understanding of the chemical composition and bioactive potential of *Melanolepis multiglandulosa* leaves. The analysis of our plant extract through Gas Chromatography–Mass Spectrometry (GC-MS) is a crucial step in our research, as it will provide a detailed profile of the compounds present, which will form the basis for our subsequent antibacterial activity studies.

In line with this, we would like to respectfully request your kind assistance in performing GC-MS analysis on our prepared extract. Our sample has already undergone ethanolic extraction and rotary evaporation, and is ready for immediate analysis. We are committed to adhering to all laboratory policies, technical protocols, and any requirements or fees set by your institution.

Your support in granting access to the GC-MS facility will be of great value to our research, and it will significantly aid in the successful completion of our study. We truly appreciate the time, effort, and consideration extended to us, and we hope to conduct the analysis under your kind guidance and supervision.

Thank you very much for your time and favorable consideration.

APPENDIX B. Cont'd



Misamis University
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY
CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

Respectfully yours,

NJ MARK L. SEDENIO
Researcher
Bs MedTech Year 3B

JOEL ANDRE R. SUSADA
Researcher
Bs MedTech Year 3B

JHUNNABIE E. TABAMO
Researcher
Bs MedTech 3B

MARY JOSETH M. SARMIENTO
Researcher
Bs MedTech Year 3B

Noted by:

MELIZA PARBA, PhD
Research Adviser

NELFA D. CANINI, PhD
Research Instructor

Recommending Approval:

Ms. EVANGELINE M. SEÑEDO, RMT, MATMRS
Dean, College of Medical Technology

Approved by:

GERMAINE S. GUERRA, RCh
Administrative Associate,
Department of Chemistry
Ateneo De Davao University

3/19/2021

APPENDIX C. PERMIT

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV

ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

Date: January 16, 2026

Hon. Eva B. Oaminal

Barangay Captain

Barangay Gumbil

Tudela, Misamis Occidental

Subject: *Request for Permission to Collect Plant Samples for Research Study*

Dear Honorable Barangay Captain Oaminal,

We respectfully write this letter to request permission from your good office to allow us to collect plant samples of ***Melanolepis multiglandulosa***, locally known as **Alim**, in Barangay Gumbil, Tudela, Misamis Occidental for our research study.

Our research is entitled "**GC-MS Profiling and Antibacterial Activity of *Melanolepis multiglandulosa* Leaf Extract against** *Pseudomonas aeruginosa* ." The purpose of this study is purely academic and scientific. The collected plant samples will be used only for laboratory analysis related to our research.

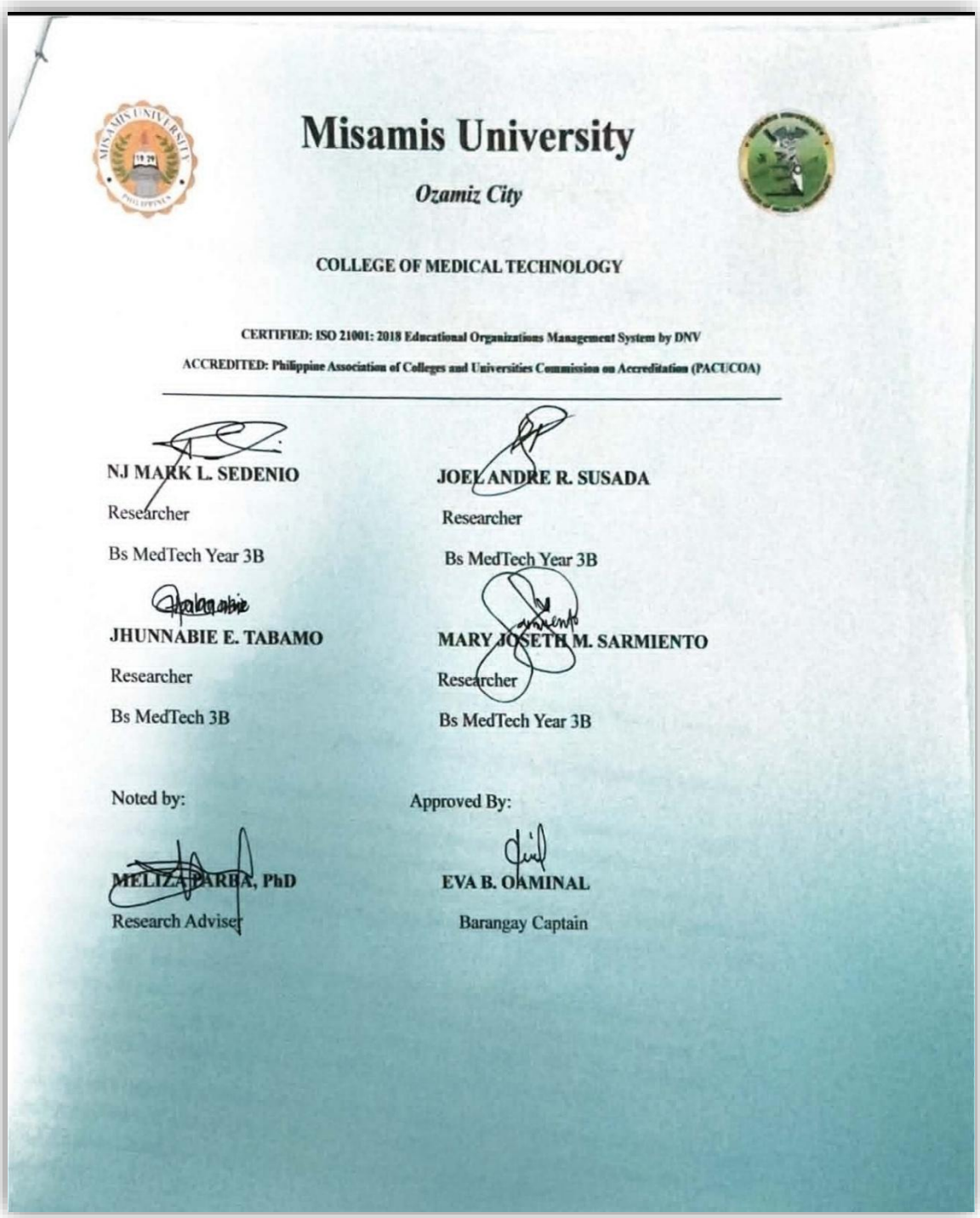
We assure your good office that the collection will be done responsibly and ethically, with minimal impact on the environment. Only the necessary plant parts will be gathered, and all barangay rules and environmental guidelines will be strictly followed. No damage will be caused to the community or its natural resources.

In this regard, we humbly request your approval and support for the conduct of this research activity in your barangay. Your kind permission will greatly help in the successful completion of our study.

Thank you very much for your time and kind consideration.

Respectfully yours,

APPENDIX C. Cont'd



Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV

ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

NJ MARK L. SEDENIO
Researcher
Bs MedTech Year 3B

JOEL ANDRE R. SUSADA
Researcher
Bs MedTech Year 3B

JHUNNABIE E. TABAMO
Researcher
Bs MedTech 3B

MARY JOSETH M. SARMIENTO
Researcher
Bs MedTech Year 3B

Noted by:

MELIZA BARBA, PhD
Research Adviser

Approved By:

EVA B. OAMINAL
Barangay Captain

APPENDIX D. *Melanolepis multiglandulosa* Leaf

Appendix D.1. Leaf sample

APPENDIX E. DOCUMENTATION

Appendix E. 1. Washing of leaf samples

APPENDIX E Cont'd

Appendix E.2. Drying and grinding of leaf samples

APPENDIX E Cont'd

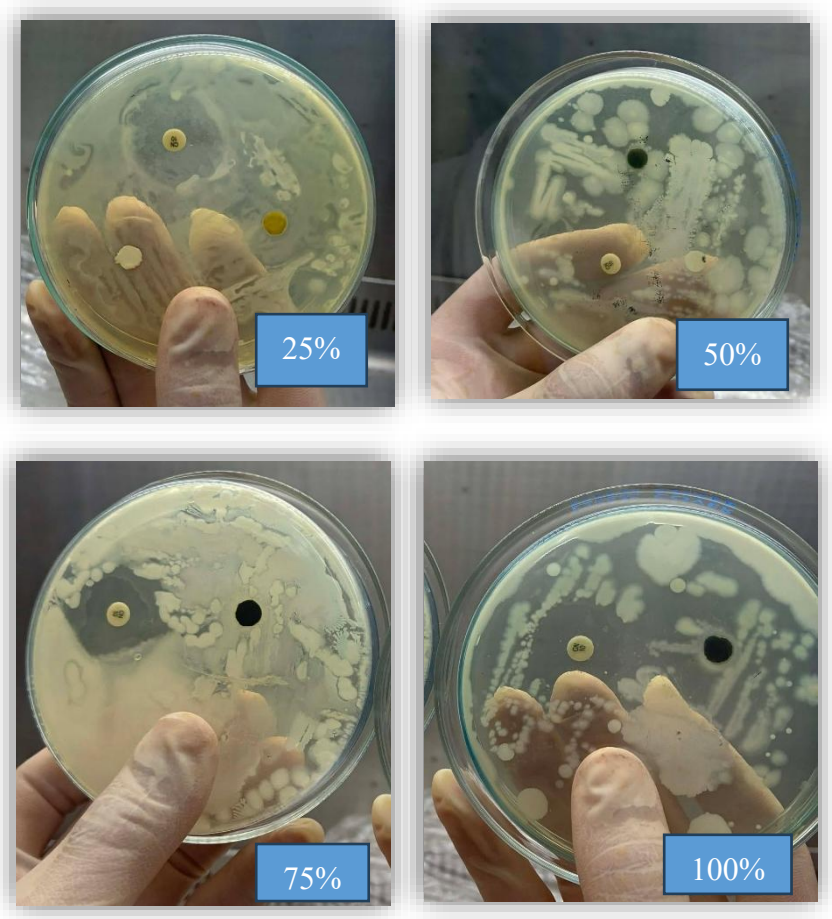
Appendix E.3. Leaf sample extraction

Appendix E.4. Filtration

APPENDIX E Cont'd

Appendix E.5. Antibacterial Testing

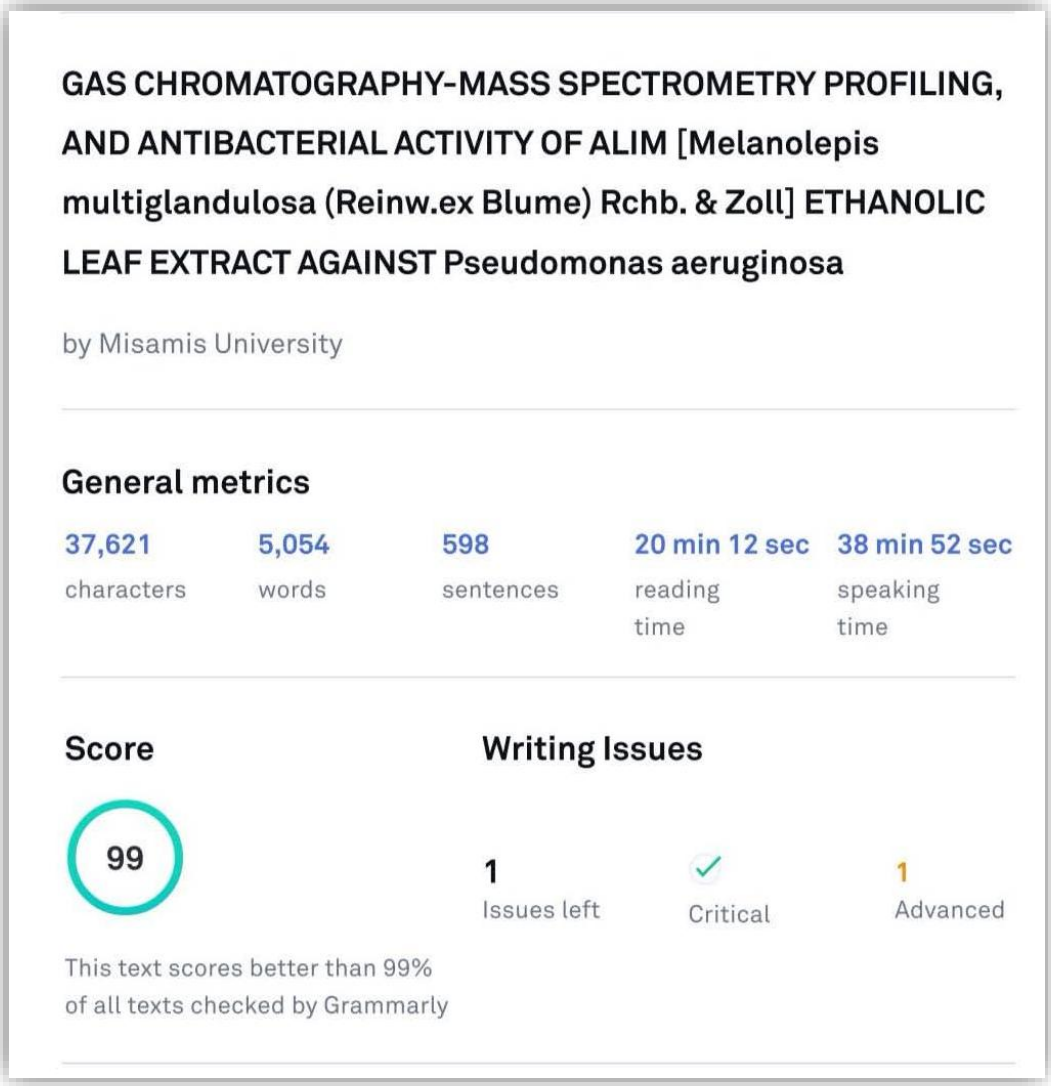
APPENDIX F. ANTIBACTERIAL RESULT



***Pseudomonas aeruginosa* ATCC 27853**

Appendix F.1. No Zone of Inhibition Result

APPENDIX G. GRAMMARLY AND TURNITIN RESULTS



GAS CHROMATOGRAPHY-MASS SPECTROMETRY PROFILING, AND ANTIBACTERIAL ACTIVITY OF ALIM [Melanolepis multiglandulosa (Reinw.ex Blume) Rchb. & Zoll] ETHANOLIC LEAF EXTRACT AGAINST Pseudomonas aeruginosa

by Misamis University

General metrics

37,621	5,054	598	20 min 12 sec	38 min 52 sec
characters	words	sentences	reading time	speaking time

Score

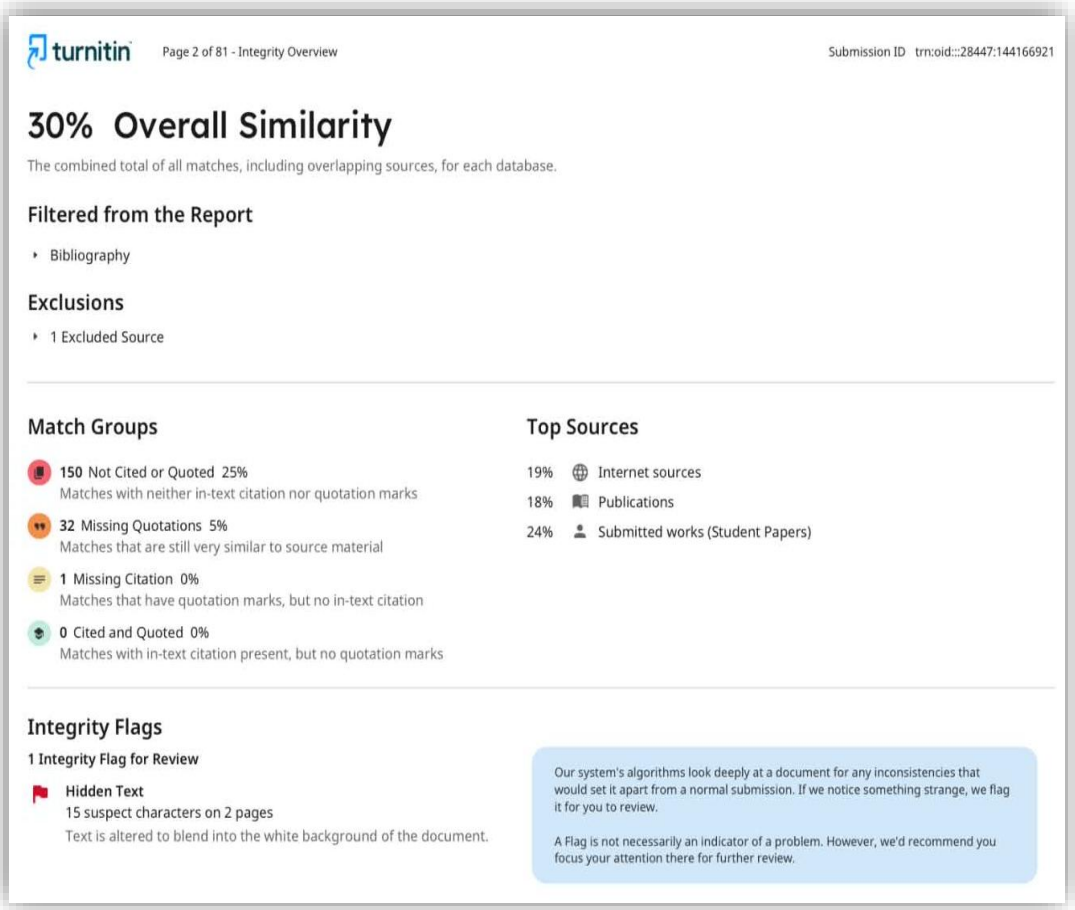
99

This text scores better than 99% of all texts checked by Grammarly

Writing Issues

1	✓	1
Issues left	Critical	Advanced

APPENDIX H. TURNITIN RESULTS



turnitin Page 2 of 81 - Integrity Overview Submission ID trn:oid:::28447:144166921

30% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography

Exclusions

- 1 Excluded Source

Match Groups

150 Not Cited or Quoted 25%
Matches with neither in-text citation nor quotation marks

32 Missing Quotations 5%
Matches that are still very similar to source material

1 Missing Citation 0%
Matches that have quotation marks, but no in-text citation

0 Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

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CURRICULUM VITAE

Name: NJ MARK L. SEDENIO

Age: 21

Birthdate: April 04, 2005

Civil Status: Single

Nationality: Filipino

Home Address: P-3 Gumbil, Tudela, Misamis Occidental

Current Address: P-3 Gumbil, Tudela, Misamis Occidental

Contact Number: 09978610144

E-mail Address: njmarksedenio@gmail.com

Educational Background

Tertiary Education:	Misamis University H.T. Feliciano St. Aguada, Ozamiz City 2026- Present
	Misamis University H.T. Feliciano St. Aguada, Ozamiz City 2025-2026
Secondary Education:	Clarin National High School Poblacion 3, Clarin, Misamis Occidental 2022-2023
Primary Education:	Gumbil Elementary School P-3 Gumbil, Tudela, Misamis Occidental 2016-2017

CURRICULUM VITAE

Name: JOEL ANDRE R. SUSADA

Age: 21

Birthdate: April 09, 2005

Civil Status: Single

Nationality: Filipino

Home Address: P-1 Silanga Tangub City, Misamis Occidental

Current Address: P-1 Silanga Tangub City, Misamis Occidental

Contact Number:09505266553

E-mail Address: andoyaxsusada@gmail.com

Educational Background

Tertiary Education: :	Misamis University H.T. Feliciano St. Aguada, Ozamiz City 2026- Present
	Misamis University H.T. Feliciano St. Aguada, Ozamiz City 2025-2026
Secondary Education:	St. Michael's High School Brgy. 1 Tangub City 2022-2023
Primary Education:	St. Michael's High School Brgy. 1 Tangub City 2016-2017

CURRICULUM VITAE

Name: Mary Joseth M. Sarmiento

Age: 22

Birthdate: October 28, 2003

Civil Status: Single

Nationality: Filipino

Home Address: Poblacion 1, Purok 4, Clarin Misamis Occidental

Current Address: Poblacion 1, Purok 4, Clarin Misamis Occidental

Contact Number: 09682824616

Gmail account: maryjosethsarmiento@gmail.com

Educational Background

Tertiary Education: :	Misamis University H.T. Feliciano St. Aguada, Ozamiz City 2026- Present
	Misamis University H.T. Feliciano St. Aguada, Ozamiz City 2025-2026
Secondary Education:	Clarin National High School Poblacion 3, Clarin, Misamis Occidental 2022-2023
Primary Education:	Antero Uy Roa Memorial School Poblacion 1, Clarin, Misamis Occidental 2016-2017

CURRICULUM VITAE

Name: Jhunnabie E. Tabamo

Age: 21

Birthdate: September 30, 2004

Civil Status: Single

Nationality: Filipino

Home Address: Purok Santal 1, Poblacion, Sinacaban, Misamis Occidental

Current Address: Purok Santal 1, Poblacion, Sinacaban, Misamis Occidental

Contact Number: 09468552833

Gmail account: jhunnabietabamo@gmail.com

Educational Background

Tertiary Education:	Misamis University H.T. Feliciano St. Aguada, Ozamiz City 2026- Present
	Misamis University H.T. Feliciano St. Aguada, Ozamiz City 2025-2026
Secondary Education:	Clarin National High School Poblacion 3, Clarin, Misamis Occidental 2022-2023
Primary Education:	Sinacaban Central School Sinacaban, Misamis Occidental 2016-2017