International Journal of Current Research and Applied Studies, (IJCRAS)

ISSN: 2583-6781

available at https://ijcras.com/

Volume 4 Issue 6 Nov-Dec 2025, Page 28-40

To cite this article: Maria T.L. Ruma, Rony S. Maubo, Mangadas Lumban Gaol and Reny Dule Muhu (2025). ANALYSIS TYPES AND ANTIBACTERIAL ACTIVITY OF SECONDARY METABOLITES OF FLOWERS OF *Martynia annua L*., A LOCAL PLANT OF TIMOR ISLAND, International Journal of Current Research and Applied Studies (IJCRAS) 4 (6): Article No. 136, Sub Id 243

ANALYSIS TYPES AND ANTIBACTERIAL ACTIVITY OF SECONDARY METABOLITES OF FLOWERS OF *Martynia annua L*., A LOCAL PLANT OF TIMOR ISLAND

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DOI : https://doi.org/10.61646/IJCRAS.vol.4.issue6.136

ABSTRACT

Martynia annua L. is a local herb often used by the people of Timor Island as a traditional medicine. This study aims to determine the types of secondary metabolite compounds and antibacterial activity of M. annua flower extract against the growth of Escherichia coli Mig. Extraction was carried out by the maceration method using 96% ethanol. Antibacterial tests were carried out by the disc diffusion method, with treatment concentrations of 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm, tetracycline positive control, and negative control using distilled water. Data were analyzed using analysis of variance. The results showed that M. annua flower extract contains alkaloids, flavonoids, steroids, and saponins. Antibacterial activity tests showed that M. annua flower extract inhibited the growth of E. coli, with an effective concentration of 400 ppm, an average inhibition zone of 10.36 mm, and was classified as strong. Thus, this plant has potential for future use, particularly in relation to the development of medicinal plants. However, further research is needed to utilize and commercialize this plant product.

Keywords: Martynia annua, Escherichia coli, secondary metabolites, antibacterial activity

INTRODUCTION

In December 2021, the government of Sierra Leone introduced a new secondary school curriculum. One of the major changes was the addition of a new subject area called "Mathematics & Numeracies" (Global Partnership for Education, 2022; Politico SL, 2021). This new curriculum is designed to help students develop better thinking, problem-solving, and number skills. It also gives teachers more flexibility in how they teach. However, it requires more teaching materials, better training, and stronger support for teachers.

Many schools, especially in rural areas, face serious challenges in meeting these new demands. In the Eastern Region of Sierra Leone, schools often do not have enough trained teachers. Many buildings are in poor condition, and schools do not have enough books or teaching materials (Night Watch Newspaper, 2023). These problems make it harder for teachers to deliver the new curriculum properly.
This study looks at the challenges teachers face when trying to teach the new mathematics curriculum. It focuses on senior secondary schools in both rural and urban areas of the Eastern Region. Rural schools from the Kailahun district and urban schools from Kenema city and Koidu town were included. The study focuses on four main areas: teacher qualifications, availability of teaching materials and school infrastructure, teacher training and support, and how teachers assess students.

The goal of this research is to help policymakers understand these challenges so they can take action to improve the quality of math education in all parts of the country.

Indonesia is a tropical country rich in plant diversity. Many plants contain secondary metabolites that have been used in traditional medicine for generations. Secondary metabolites are compounds found in plant parts and are useful as ingredients in traditional medicines for health, such as antioxidants, antibacterials, anti-inflammatory, and anticancer properties (Purwanto 2022). Herbs are substances or products derived from plants that have medicinal properties, flavor, or aroma, used to maintain health, prevent, or treat disease.

One popular plant known to contain secondary metabolites in Timor Island is the devil's claw (*Martynia annua* L.). In East Nusa Tenggara, it is known by various local names, such as Haukorbaos (Batuna), Kuaklulu (Soe), and Kai ku'u (Sabu). This diversity of local names reflects the long-standing familiarity of this plant among local communities, including in traditional medicinal practices (Ukratalo 2025). Martynia is a monotypic genus in the Martyniaceae family, consisting of a single species, *M. annua*, commonly known as cat's claw, tiger's claw, and ice plant. or devil's claw (Fontana et al. 1962). This plant is native to Mexico, Central America, and the Caribbean and has been introduced throughout the tropics.

M. annua (family Martyniaccae) is used to treat epilepsy and is applied topically to tuberculosis of the cervical lymph nodes of camels. Leaf juice is used as a gargle for sore throats, the fruit for inflammatory diseases, and the leaf paste is applied topically to wounds in livestock (Khare 2007). Root extracts have shown fungicidal activity against *Acaulospora scrobiculata* and *Sclerocystis sinuosa* (Kamble et al. 2012). Phytochemical studies on *M. annua* have revealed the presence of glycosides, flavonoids, tannins, carbohydrates, phenols, and anthocyanins in the leaves (Lodhi and Singhai 2013). The seeds are reported to contain linoleic acid, arachidic acid, pelargonidin-3-5-diglucoside, palmitic acid, apigenin, and apigenin-7-O-glucuronide (Gunasegaran and Vidya 1992). The flowers contain cyanidin-3-galactoside, while p-hydroxybenzoic acid and sinapic acid are found in the leaves and gentisic acid is found in the fruit (Mali et al. 2002).

Herbs derived from plant organs containing secondary metabolites have antimicrobial potential (Ximenis et al. 2022). Herbs derived from various plant organs (leaves, rhizomes, stems) are rich in secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and terpenoids, which function as natural defenses and possess strong antimicrobial potential, all of which offer active compounds against microorganisms such as bacteria. Secondary metabolites with antimicrobial potential can be found in various plant parts (organs), such as leaves, stems, roots, flowers, fruits, and seeds. Antimicrobials are substances that can inhibit the growth of microorganisms such as bacteria, viruses, and fungi. Therefore, the secondary metabolites found in *M. annua* flowers have antibacterial potential, particularly in inhibiting bacterial growth.

Escherichia coli Mig is a common bacterium found in the digestive tract of humans and animals. Most species live normally in the body without causing disease and are even beneficial in the digestive process. However, certain species are pathogenic and cause diseases, such as gastrointestinal and urinary tract infections (Ximenis et al. 2022). Common symptoms of *E. coli* infection include sudden diarrhea, abdominal cramps or pain, nausea, vomiting, loss of appetite, fatigue, and fever. More serious symptoms include bloody urine, decreased urine output, pale skin, bruising, and dehydration (Fadhillah et al. 2019).

Given the high incidence of pathogenic *E. coli* infections, effective natural antibacterial alternatives are needed. One strategy that can be developed is the use of plants containing secondary metabolites, such as *M. annua*, which are useful as traditional medicines. These plants are inexpensive, readily available, and readily available in the local environment. Therefore, this study was conducted to determine the secondary metabolite compounds and antibacterial activity of *M. annua* flower extracts from Timor on the growth of *E. coli*.

MATERIALS AND METHODS

This research was conducted from March to November 2025. *M. annua* flower samples were taken from Lasiana, Kelapa Lima District, Kupang City Timor Island. Secondary metabolite compound analysis was conducted in the Biology Laboratory of the Faculty of Science and Engineering, Nusa Cendana

University, and antibacterial activity testing for *E. coli* was conducted in the Biology Laboratory of the Faculty of Teacher Training and Education, Nusa Cendana University.

The equipment used in this study included a blender, 60-mesh sieve, pH meter, macerator, spatula, colony counter, rotary evaporator, analytical balance, glass funnel, autoclave, volumetric flask, graduated cylinder, Erlenmeyer flask, beaker, test tube, stirring rod, petri dish, dropper, volumetric pipette, loop needle, sprig, Bunsen burner, tweezers, laminar air flow, water bath, tube clamp, and camera. The materials used were *M. annua* flowers, pure *E. coli* culture, 96% ethanol, distilled water, tetracycline, Nutrient Broth (NB), Nutrient Agar (NA), Mueller Hinton Agar (MHA), aluminum foil, clean wrap, H_2SO_4, 1% FeCl, HCl, reagents (Dragendorff, Mayer, Wagner), glacial acetic acid, Whatman No. 42 filter paper, rubber, 6 mm diameter paper discs, and label paper.

The research design used was a Completely Randomized Design (CRD) with seven treatments: extract concentrations of 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm, positive control using tetracycline, and a negative control using distilled water. Three replications resulted in 21 experimental units.

Phytochemical Screening

1. Flavonoid test. About 0.5 grams of dry *M. annua* flower extract into a test tube, then add 5 mL of ethanol. The mixture is heated for approximately 5 minutes, then add 10 drops of concentrated HCl and 0.2 grams of magnesium powder. A color change to reddish-black, yellow, or orange indicates the presence of flavonoids (Sulistyarini et al. 2023).
2. Tannin test. About 0.5 grams of dry *M. annua* flower extract into 10 mL of hot water and add 1% $FeCl_3$. A color change to blackish-green indicates the presence of tannins (Sulistyarini et al. 2023).
3. Saponin test. About 0.5 grams of dry *M. annua* flower extract was weighed into a test tube, and 10 mL of heated distilled water was added. The mixture was homogenized by vigorous shaking for approximately 1 minute. The solution was allowed to stand for 10 minutes and the formation of foam was observed, indicating a positive saponin result (Sulistyarini et al. 2023).
4. Steroid and Triterpenoid test. About 0.5 grams of dry *M. annua* flower extract was placed into a test tube. Glacial acetic acid was added until the herb was submerged, and the mixture was allowed to stand for 15 minutes. Six drops of the sample solution were placed into the test tube, followed by 2-3 drops of H_2SO_4. The presence of triterpenoids is indicated by a brownish or violet color, while the presence of steroids is indicated by a blue-green color (Khafid et al. 2023).
5. Alkaloid test. About 0.5 g of dry extract of *M. annua* flowers was weighed and then dissolved in 10 mL of distilled water. The solution was dripped with 6 mL of 2 NHCl, then heated over a water bath for 2 minutes. The solution was divided into three parts of 3 mL each. Each part of the solution was then dripped with a different reagent: The first was dripped with Dragendorff's reagent, the second was with Mayer's reagent, and the third was with Wagner's reagent. Positive alkaloid results were indicated by the formation of an orange precipitate in the solution with Dragendorff's reagent, a white

precipitate upon the addition of Mayer's reagent, and a brown precipitate upon the addition of Wagner's reagent (Ananta et al. 2024).

Antibacterial Activity Test

The antibacterial activity test was conducted as follows (Karyawati et al. 2023):

1. Prepare solidified MHA media, then take 0.1 mL of bacterial suspension and spread it into the plates with a sterile spreader. The MHA media containing the bacterial suspension was incubated for 15 minutes.
2. Dip paper discs were soaked in the positive control solution, negative control solution, and test solution for 30 minutes. The discs were then placed on the surface of the media using sterile tweezers.
3. The antibacterial activity test for each concentration and control was repeated three times.
4. The media were placed in an incubator for incubation at 37°C for 24 hours.
5. The zones of inhibition formed were measured using an automatic colony counter scan 500 connected to a computer. Observed under a light to see the zones of inhibition, then the diameter of the circle was adjusted according to the zone of inhibition formed on each disc. The colony counter will automatically calculate the diameter of the inhibition zone formed.
6. The diameter of the inhibition zone formed will be compared with the inhibition response classification (Table 1).

Table 1. Standard measurement of inhibition zone diameter (Winastri et al. 2020).

Inhibition Zone Diameter	Inhibitory Force
≥ 21 mm	Very strong
11-20 mm	Strong
6-10 mm	Moderate
< 5 mm	Weak

Data Analysis

Data were analyzed descriptively and tabulated in tables and figures. Antibacterial activity was determined using a one-way ANOVA followed by a Tukey test.

RESULTS AND DISCUSSION

Secondary Metabolite Compounds in M. annua Flower Extract

Based on research results, it was found that extract of *M. annua* flower contained alkaloids, flavonoids, saponins, and steroids, which were interpreted as positive (+), while tannin were not found, which was interpreted as negative (-) (Table 2).

Table 2. Analysis of secondary metabolite compounds *M. annua* flowers.

Secondary Metabolite Compounds	Reagents	Flower Extract	
		Observation Results	Interpretation
Alkaloids	**Dragendorff**	Orange Precipitation	+
	Mayer	White Precipitation	+
	Wagner	Brown Precipitation	+
Flavonoids	**Concentrated HCL + Mg**	Color Change (reddish-black)	+
Tannin	**$FeCl_3$ 1%**	Color Change (blackish-green)	-
Saponin	**Aquades**	Foam stable for 10 minutes	+
Steroids	**CH_3COOH & H_2SO_4**	Blue-green	+

The qualitative test results for alkaloid compounds were declared positive because the *M. annua* flower extract showed the formation of an orange precipitate in the solution with Dragendorff's reagent, a white precipitate upon the addition of Mayer's reagent, and a brown precipitate upon the addition of Wagner's reagent. These test results are in accordance with the research of Ananta et al. (2024) which stated that positive alkaloid results are characterized by the formation of an orange precipitate in the solution with Dragendorff's reagent, a white precipitate upon the addition of Mayer's reagent, and a brown precipitate upon the addition of Wagner's reagent. In the health sector, alkaloids act as nervous system stimulants, reduce pain, as antimicrobials, regulate blood pressure, treat heart disease, and as sedatives. In addition, alkaloids are also used to fight microbial infections and help stabilize blood pressure (Ananta et al. 2024). The light brown to orange precipitate using Dragendorff's reagent is formed due to the interaction between Bi3+ ions from bismuth nitrate and potassium iodide, which then produces potassium tetraidiobimumate. The white precipitate formed using Mayer's reagent is due to the interaction of the nitrogen atoms of the alkaloids with the K+ ions in potassium tetraiodomerkurt(II), forming a potassium-alkaloid complex. The brown precipitate formed using Wagner's reagent occurs because the free electrons of the alkaloid nitrogen bind to the K+ ions in the reagent, forming a potassium-alkaloid complex (Ferdinan et al. 2021). One of the main benefits of alkaloids is as antibacterials; their mechanism of action is thought to be by disrupting the peptidoglycan component of bacterial cell walls, thus preventing the cell wall from forming properly and ultimately leading to bacterial cell death (Robinson 1995).

The flavonoid compound test in *M. annua* flower extract was declared positive because after the addition of magnesium and concentrated HCl, the test sample changed color to reddish-black. These compound test results align with research by Sulistyarini (et al. 2023), who stated that a color change to reddish-black, yellow, or orange indicates the presence of flavonoid compounds. In general, flavonoids in plants are bound to sugars in the form of glycosides and aglycones. Flavonoids have antiviral, antimicrobial, blood pressure-lowering, liver dysfunction treatment, and antioxidant properties (Ananta et al. 2024).

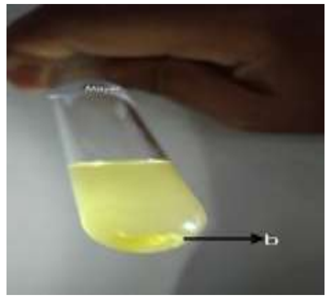
The tannin test in *M. annua* flower extract was negative because after the test sample was mixed with the F_eCl_3 reagent, the resulting color changed to blackish-brown. A color change to blackish-green indicates the presence of tannins (Sulistyarini et al. 2023). Extracts containing polyphenols will form complex compounds with coordinated bonds, indicated by a color change to blue-black or brownish-green (Cahyaningsih et al. 2021). Tannins are found in various plants, including leaves, stems, roots, fruits, and bark. These compounds are stored in cell vacuoles in the tissue (Hassanpour et al. 2011).

The saponin test results for *M. annua* flower extract were positive due to the formation of stable foam after the solution was homogenized for approximately 1 minute and allowed to stand for 10 minutes. This test aligns with research by Sulistyarini et al. (2023), who stated that the solution was allowed to stand for 10 minutes and observed for the formation of foam, indicating a positive saponin result. The formation of foam is due to the presence of glycosides, which have the ability to form bubbles in water, which form glucose and other compounds. The glycoside bonds in saponins cause these compounds to tend to be polar. Saponins also have soap-like properties, with two polar sides and one non-polar side (Ravelliani et al. 2021). Saponins are beneficial in the health sector, including reducing blood cholesterol levels, possessing high antioxidant activity, and functioning as anti-stress and anti-aging compounds (Ruma et al. 2024).

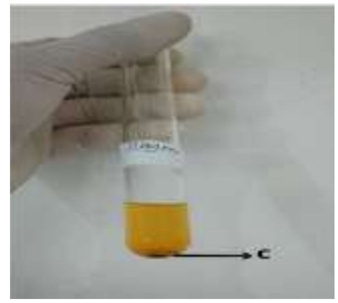
The test results of the steroid compound of *M. annua* flower extract were declared positive because the color of the filtrate changed to green after the addition of 3 (three) drops of acetic acid and 3 (three) drops of H_2SO_4. The presence of steroids is indicated by the presence of a blue-green color (Khafid et al. 2023). The color change to green-blue is based on the ability of steroid compounds to form color by H_2SO_4 in acetic acid solvent (Merliana & Saleh 2011). The color change is caused by differences in groups on the C-4 atom. In the medical field, steroids are used as ingredients in the manufacture of drugs, contraceptives, preventing inflammation and treating rheumatism (Ananta et al. 2024). The results of the qualitative test of secondary metabolite compounds of *M. annua* flower extract are in Figure 1.

Alkaloid Test with 3 reagents

Dragendorff's reagent



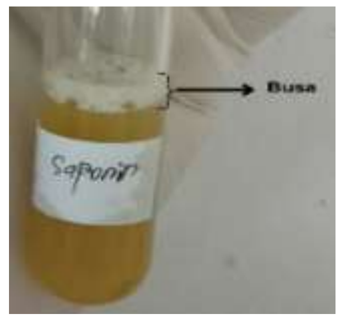
Mayer's reagent



Wagner reagent

Flavonoid

Tannin



Saponin

Steroid

Figure 1. Qualitative test of secondary metabolite compounds of *M. annua* flower

Activity test of secondary metabolites of *M. annua* flower extract on the growth of *E. coli* bacteria

The results showed that flower extract of *M. annua* can inhibit the growth of *E. coli*, as shown by the formation of a clear zone (zone of inhibition) around the paper disc (Table 3).

Table 3. Activity of secondary metabolites of *M. annua* flower extract on the growth of *E. coli*

Extract Concentrations	Inhibition Zone Diameter (mm)			$\overline{x}$ ± SD	Category
	R1	R2	R3		
K-(aquades)	0,0	0,0	0,0	0,0 ± 0[a]	Weak
K1 (200 ppm)	9,3	9,6	8,9	9,26 ± 0,35[b]	Moderate
K2 (400 ppm)	10,7	10,4	10,0	10,36 ± 0,35[c]	Strong
K3 (600 ppm)	11,9	11,1	11,5	11,5 ± 0,40[d]	Strong
K4 (800 ppm)	12,6	12,2	12,6	12,46 ± 0,20[e]	Strong
K5 (1000 ppm)	15,2	15,9	15,6	15,56 ± 0,35[f]	Strong
K+	16,7	16,3	17,0	16,6 ± 0,35[g]	Strong

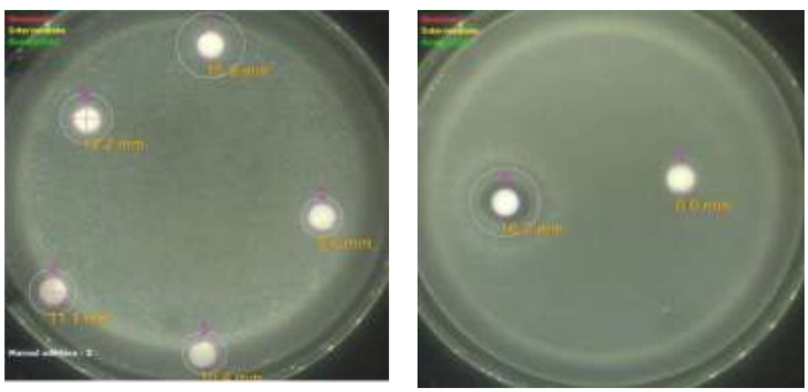
Description: The inhibition zone diameter categories according to Winastri et al. (2020): ≥ 21 mm is categorized as Very strong, 11-20 mm as Strong, 6-10 mm as Moderate, and <5 mm as Weak. Different superscripts indicate significant differences. R = replications.

Based on the data in Table 3, the positive control had the largest inhibition zone with an average value of 16.6 ± 0.35, categorized as strong. The 1000 ppm concentration (K5) had the highest average inhibition zone diameter of 15.56 mm, categorized as strong, while the 200-ppm concentration (K1) had the lowest average inhibition zone diameter of 9.26 mm, categorized as moderate. This is suspected because the 1000 ppm concentration contains more bioactive compounds that act as antibacterial agents than the 200-ppm concentration. The results of this study are consistent with the research of Ximenis et al. (2022) stated that increasing the extract concentration will increase the antibacterial compounds, thereby increasing the inhibitory effect on bacterial growth. The higher the extract concentration, the larger the resulting zone of inhibition. Increasing the extract concentration is directly proportional to the increase in the diameter of the zone of inhibition (Rehe et al. 2023).

Tetracycline was used as a positive control because it is commonly used in antibacterial activity studies due to its effectiveness in inhibiting the growth of both gram-positive and gram-negative bacteria. Tetracycline is a bacteriostatic antibiotic that works by inhibiting bacterial growth (Komalasari et al. 2021). Tetracycline can be used against aerobic and anaerobic bacteria, as well as gram-positive and gram-negative bacteria. Furthermore, tetracycline can also fight several types of germs such as Spirochaeta, Mycoplasma, Rickettsia, Chlamydia, Legionella, and some protozoa. Based on its mechanism of action, tetracycline belongs to the group of antibiotics that stop bacterial growth, rather than directly killing it. This drug enters bacterial cells through two pathways: passive diffusion and active transport. Once inside the cell, tetracycline binds to ribosomal subunits and prevents the tRNA complex that carries amino acids from entering the binding site. As a result, protein formation is halted, and the bacteria are unable to metabolize.

Based on the ANOVA test results, it was found that the concentration of *M. annua* flower extract affected the growth of *E. coli* ($p < 0.05$). The 1000 ppm *M. annua* flower extract concentration showed the highest average inhibition zone diameter and was categorized as strong. However, the 400 ppm concentration was optimal in inhibiting *E. coli* growth. This is because the 400 ppm concentration was categorized as strong, similar to the highest 1000 ppm treatment, although it had a lower average value but was significantly different from the other treatments.

The inhibitory activity of *M. annua* flower extract against *E. coli* bacteria is thought to originate from the presence of active compounds within the extract. The yield value is related to the amount of bioactive compounds contained in the plant. A yield value of <10% is considered poor (Amalo et al. 2024). The yield of *M. annua* flower extract obtained in this study was 12.03%. The higher the yield, the greater the amount of substances contained within. Extraction results are influenced by several factors, including time, solvent, temperature, and sample size. Determining the yield aims to determine the levels of secondary metabolites extracted by the solvent, although it cannot indicate the specific types of compounds contained (Adriana et al. 2024). The results of the antibacterial activity test of *M. annua* flower extract on the growth of *E. coli* bacteria are shown in Figure 2.



M. annua extract concentration | Aquadest and tetracycline

Figure 2. Clear zone formed by the antibacterial activity of *M. annua* flower extract

M. annua flower extract contains antibacterial compounds capable of inhibiting the growth of *E. coli*. The antibacterial test results of *M. annua* flower extract on the growth of *E. coli* bacteria were observed by the formation of a zone of inhibition or clear zone using an automatic colony counter scan 500 connected to a computer. The secondary metabolite compounds contained in *M. annua* flower extract can inhibit bacterial growth. Alkaloids, for example, can inhibit the formation of peptidoglycan, the main component of bacterial cell walls, thus causing the cell wall to form improperly and leading to cell death. Alkaloids can interact with bacterial cell membranes, causing changes in permeability, resulting in leakage of cell contents and cell death. The mechanism of action of flavonoids is bacteriolytic, inhibiting protein synthesis, DNA synthesis, and RNA synthesis, forming complexes with extracellular and soluble proteins, thus damaging the bacterial cell membrane, followed by the release of intracellular compounds (Nababan et al. 2020).

The saponins contained in *M. annua* flower extract function as antibacterial agents by reducing surface tension on the cell wall and disrupting membrane permeability. This mechanism is related to the detergent-like properties of saponins, which possess surface activity. These compounds can penetrate vulnerable bacterial cell walls and interact with the cytoplasmic membrane. This interaction disrupts membrane stability, resulting in the loss of bacterial function and ultimately death (Ximenis et al. 2022).

Based on the research results, it can be concluded that the extract of local Timor *M. annua* flowers contains bioactive compounds: alkaloids, flavonoids, steroids, and saponins. *M. annua* flower extract is also able to inhibit *E. coli* bacteria, with an effective concentration inhibiting the growth of *E. coli* being 400 ppm with an average inhibition zone value of 10.36 mm and is classified as a strong category. Further research can be conducted on the analysis of the main bioactive compounds in local Timor *M. annua* flowers that play a role in inhibiting the growth of *E. coli*; and a test of the inhibitory power of *M. annua* flowers against other gram-negative bacteria, as well as concentrations of less than 400 ppm and more than 1000 ppm using pure extracts.

ACKNOWLEDGEMENTS
This research was funded by a grant from Department of Biology Faculty of Science and Engineering Nusa Cendana University for Fiscal Year 2025. The researcher expresses their gratitude to the Head of Department for financing this research and providing other supporting facilities for conducting research.

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