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Effectiveness test of African leaf ethanol extract against *Salmonella typhi* bacteria

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Abstract

Salmonella typhi is a gram-negative bacterium that is a pathogen that causes dangerous infectious diseases. The bacteria resist almost all antibiotics, such as penicillin, chloramphenicol, and methicillin. The problem of resistance has become a global problem, so new antibacterial materials are needed that can inhibit the growth of *Salmonella typhi*. One of the plants originating from the continent of Africa is used as a medicine to treat diarrhea, diabetes, and malaria. Where to previous studies, African leaves contain flavonoids and tannins, which can inhibit the growth of pathogenic bacteria. This study aims to determine the antibacterial activity of African leaf extract on the growth of *Salmonella typhi*. This research is an experimental study with various treatments using African leaf extract concentrations of 10%, 20%, 40%, 60%, 80%, and 100%. Antibacterial activity test based on the presence of inhibition zones formed. The results showed differences in the inhibition zone between extract concentration treatments on the growth of *Salmonella typhi* bacteria. At a concentration of 100, the formation of an inhibition zone with the largest diameter of 73.9% could be seen, and at a concentration of 20%, only an inhibition zone with a diameter of 23.7%. The antibacterial activity of African leaves can inhibit the growth of *Salmonella typhi* bacteria because an inhibition zone is formed.

Keywords: Antibacterial; Inhibition; Flavonoids; extracts

1. Introduction

Indonesia is the largest archipelagic country in the world because it has an area of 45,000 km^2 and consists of 17,500 islands. It causes Indonesia to have the second-highest biodiversity in the world after Brazil. Additionally supporting this biodiversity is Indonesia's equator-passing location, which brings rain to almost the entire country. This situation causes extraordinary biodiversity, where 17% of plant species from all species on the earth's surface are found in Indonesia [1]. In Indonesia, about 30,000 species of 40,000 plants exist on earth. About 300 species found in Indonesia have been used as traditional medicines, passed down from generation to generation, so they are called medicinal plants. Medicinal plants are ingredients in the form of a plant, animal, mineral ingredients, galenic preparations, or mixtures of these materials which have been used for generations for treatment and can be applied according to the norms in force in society. As much as 74% of traditional medicine's raw materials are wild plants in the forest [2; 3; 4].

Plants can be used as medicine because they contain secondary metabolites. Secondary metabolite compounds are chemical compounds that have the ability to bioactivity and function as plant protectors. Fifteen thousand secondary metabolites have been identified, and every year 4,000 new secondary metabolites are found. Several secondary metabolites include flavonoids, saponins, tannins, alkaloids, terpenoids, glycosides, and sterols, giving rise to a bitter taste [5]. The use of plants as medicine has recently become increasingly popular in society. The more expensive medicines make people look for other alternatives for treatment, namely by utilizing medicinal plants. In addition to getting drugs at affordable prices and reducing the number of antibiotic resistance, people can choose medicinal plants around them, which have become a tradition or habit [6]. One of the plants often used as a medicinal plant is an African leaf (Vernonia amygdalina). This plant is often used as a vegetable and is believed to be used as antirheumatic,

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antidiarrheal, and antihypertensive and lowers blood sugar levels in African countries and countries around the Middle East. According to research that has been done—including research done in Nigeria—this plant has antibacterial activity against the Porphory gingivalis bacteria in the teeth. Local people for generations have believed that African leaves can be used as an anti-diabetic by boiling young African leaves and then drinking them. These leaves are also used to treat fever and are made infusion to further reduce the bitter taste found in African leaves [7; 8]. Infection is a big threat to humans. Infection is caused by infectious agents that attack humans directly or indirectly. The infectious agent that often causes infection in humans is the bacterium *Salmonella typhi*. These bacteria enter the human body through contaminated food and drink. *Salmonella typhi* is capable of causing a large number of infections in humans, including typhoid fever (enteric fever), focal systemic infections, septicemia, and gastroenteritis which varies in the form of diarrhea and dysentery [9].

Typhoid fever is a very important public health problem whose causes are closely related to urbanization, overcrowding, environmental health, poor water and sanitation sources, and low standardization of food processing hygiene. The spread of this disease is almost often through contaminated food and drink. According to WHO data, the incidence of typhoid fever in Indonesia is usually in children aged 2-5 years, with an incidence rate of 147.7 per 100,000 people, and is the highest incidence of typhoid fever after Bangladesh and Pakistan. In 2014 the National Basic Health Research reported that the prevalence of typhoid fever in Central Java was 1.61%, spread across all districts with a different prevalence in each place. The prevalence of typhoid fever ranges from 0.8% in infants to 1.9% in people aged 5 to 19 years [10; 11; 12]. Beginning in 1972 in the United States, Mexico, and Great Britain, *Salmonella typhi* was found resistant to several antibiotics, the first-line therapy for typhoid fever infection. These antibiotics are chloramphenicol, ampicillin, tetracycline, sulphonamides, streptomycin, and trimethoprim-sulfamethoxazole. Bacterial resistance to antibiotics results in a significant increase in mortality and treatment costs. Meanwhile, the discovery of new antibiotics is decreasing [13; 14].

Based on the above background, the authors are interested in researching the concentration of African leaf extract, which is effective for inhibiting *Salmonella typhi* bacteria using the Kirby Bauer diffusion method as an alternative therapy to help conventional treatments are increasingly experiencing resistance. The formulation of the research problem is "does the ethanol extract of African leaves has effectiveness in inhibiting the growth of *Salmonella typhi* colonies?" The research aims to prove the ability of African leaf extract to inhibit the growth of *Salmonella typhi* bacteria.

2. Literature Review

This plant grows in tropical and subtropical countries, including Indonesia. African plants come from the Asteraceae tribe, which also grows in tropical areas such as Southeast Asia, Malaysia, China, and India. This plant can easily be found in gardens or as a hedge plant [15]. African leaf is a small tree plant with a height of 2 -10 m with elliptical leaves with a diameter of about 6 mm. African leaves have dark green leaves with a characteristic odor and bitter taste, and the bark is gray or brown, rather rough, extending longitudinally [16]. Nigeria (ewuro); Cameroon (muop); Tanzania (tuntwono); Uganda (Mululuza); China (Ikaruga); English (bitter leaf); Indonesia (African leaf, bitter leaf); Kalimantan (bismillah leaf); Malaysia (bismillah leaf, South Africa leaf); Rwanda (Umubilizi); French (Ndole).

Anthraquinones, koumarins, xanthones, lutein, lignans, sesquiterpenes, vitamins A, C, E, and vitamins B1 and B2 are chemical compounds found in African leaves. The lutein content in African leaves acts as an antidiabetic and antioxidant, which can also act as an immunostimulant. An antioxidant is one of the functions of anthraquinones, which are phenol derivatives. Anthraquinones have antibacterial properties that can inhibit the enzymes arachidonic acid, kaemferol, and antharaquinones play a role in inhibiting COX activity in the inflammatory process. Research on the content of antharaquinones compounds from African leaves is known to function as an antibacterial transmitted through food such as *Staphyloccocus aureus, Pseudomonas aureoginosa, E.coli, Salmonella Enteric, and K. Pneumonia. Chibuzor and Eberchi* conducted a study and reported that the phbatannin compound in African leaves acts as an antidiarrheal by inhibiting the peristaltic activity of the small intestine, thereby inducing mucosal permeability and electrolyte changes, thereby maintaining peristaltic function. Lutein plays a role in inducing pharmacological action having a beneficial effect by lowering blood sugar levels. African easy leaves have terpenoids that reduce blood sugar levels by inhibiting the processes of glycconeogenesis, glycogenolysis, and saponins, which reduce hyperglycemia related to oxidative stress [17].

African leaves (Vernonia amygladina) have been traditionally used in Africa as a traditional vegetable consumed by squeezing to remove the bitter taste. African leaf plants have a flavonoid component that acts as an antibacterial. Flavonoids are one of several secondary metabolite compounds that exist in this plant. So far, flavonoids have antioxidative properties and several compounds, including quercetin, kaemferol, myricetin, apigenin, luteolin, vitexin, and isotexin, which are usually found in cereals, vegetables, fruit, and their processed products which act as

antioxidants. The things above strengthen the notion that flavonoids are antioxidants. Flavonoid compounds in African leaves can inhibit DNA and RNA nucleic synthesis, cytoplasmic membrane function, and bacterial energy metabolism [18]. Nowadays, alternative medicine is one of the options for treating antibiotic resistance, which is a health problem in the world, especially in Indonesia. African leaves have many benefits, such as antibacterial, antifungal, antimalarial, anticancer, antiallergic, antidiabetic, and antioxidant. This effect is contained in the natural chemical content of these plants [19].

Antioxidant activity test was carried out by scavenging free radicals using in vivo method, using streptozotocin-induced diabetic rats. Testing the activity of African leaf extract using the in vivo method gives the following. African leaf extract in rats showed a significant increase in superoxide, dismutase, catalase, glutathione, and malondialdehyde. Besides that, the extract given to rats resulted in a decrease in lipids when compared to controls. The chemopreventive effects of other African leaves are associated with the ability to scavenge free radicals, interfere with the binding of DNA from several transcription factors, and induce detoxification [21]. There was also research conducted by Dwisera Dwisamolla and Mega Linda in 2014 using the DPPH method. This study aimed to determine the antioxidant activity of the ethanol extract of South African leaves (Vernonia amygdalina Del) compared with vitamin C. The antioxidant activity was measured using the DPPH method on the ethanol extract of African leaves with concentrations (of 1000, 2000, 3000, 4000, 5000) mcg /mL.

The results showed that the ethanol extract of South African leaves (Vernonia amygdalina Del) had a very weak antioxidant activity with IC50 3489.1759 mcg/mL against DPPH free radicals. [22] Optimization of the ethanol extract gel of African leaves as an antibacterial for Pseudomonas aeuroginosa was carried out in a study using a factorial design method. Dried African leaves were macerated with 96% ethanol because it can dissolve the flavonoids from African leaves. African leaf ethanol extract is formulated in a gel dosage form, the base gel used is a mixture of propylene glycol and carbopol 940. This study aimed to determine the optimum amount of the combination of propylene glycol and carbopol 940 in the ethanol extract gel of African leaves as an antibacterial against Pseudomonas aeruginosa and Staphylococcus epididymis. Several amounts of carbopol are used in the range of 0.5-2% and 5-15% propylene glycol. The physical test of the gel included organoleptic observations and measurement of viscosity, adhesion, spreadability, and pH, while the antibacterial test used the well-diffusion method. Preliminary antibacterial test results for the ethanol extract of African leaves using four series of extract concentrations of 5%, 10%, 20%, and 40%, 20% concentration was chosen with an inhibition zone of 13 mm against Staphylococcus epidermidis and 12 mm against Pseudomonas aeruginosa as the active substance in the gel formula. The gel was optimized using the factorial design method with design expert trial software version 11, and the optimum formula value was obtained with the amount of carbopol 0.94 gram and propylene glycol 8.17 gram [23].

A study was also carried out on African leaves as an antibacterial against *Poryphomonas gingivalis*, repeating four times. From the research results, African leaf extract can inhibit *Poryphomonas gingivalis* bacteria in the absence of turbidity in all groups of experimental materials, so it is not representative in measuring MIC values. Determination of the minimum kill level (MBC) obtained that the experimental material can kill 99.9% of bacteria. This study showed that African leaf extract had antibacterial activity against Fusobacterium nucletatum with an MBC value of 12.5%. This study aims to compare the therapeutic and ethanol effects of African leaves in inhibiting electrolyte secretion in the intestines of mice. The mice used had a body weight of 150g – 200g and were given food for 18 hours, then divided into four groups and given castrol oil orally and left for a few hours. After that, they were given African leaf extract.

In vivo experiments showed a change in defecation in denser mice feces because anti-diarrheal drugs given at a dose of 40 mg/kg could not inhibit the frequency of defecation by anti-diarrheal drugs. It is presumably because the concentration of the viscous extract affects the physiology of the test animals, which results in the effect of the drug being less visible. Research conducted in Malaysia reported that African leaves were eaten as raw vegetables to prevent diarrhea. After further research, the results of phytochemical compounds in the form of phbatanins were inhibitors to reduce intestinal peristalsis so that intestinal contractions were reduced. Vernonia amygladina (African leaf) is traditionally used as an alternative medicine to treat pain, especially toothache. This study aimed to demonstrate the analgesic activity of the n-hexane extract of African leaves using two methods of determining the assay and its potent dose. The tail flick test method is used as a central analgetic activity tester, while the writhing test can test peripheral analgesic activity. The result of both methods is that the n-hexane extract of African leaves contains essential oils, and it is suspected that essential oils produce analgesics, namely steroids. Steroid compounds have an analgesic effect, although the process is not clear. It may be related to their activity as an anti-inflammatory, namely slowing the production of various inflammatory factors. The presence of anti-inflammatory activity reduces the productivity of inflammatory mediators as well as strengthens and maintains the perception of pain [24].

Research on the utilization of African leaf water extract gel as a medicine for second-degree burns in guinea pigs was carried out by Debi Meilani and Melati Yulia. This study aims to formulate African leaf water as a gel and obtain the optimum dose as a medicine for second-degree burns. The experimental animal used was the male guinea pig (Cavia cobaya). The stages of the research included characterization of African leaf simplicia, phytochemical screening, preparation of African leaf aqueous extract, formulating it in a gel dosage form, and obtaining the optimum dose of the gel as a medicine for second-degree burns.

The results of African leaf simplicia showed good simplicia quality and met the requirements with a water content of 7.99%, an extract content in water of 23.04%, an extract content in ethanol of 16.14%, an ash content of 10.90%, an ash content of insoluble in acid as much as 1.31%. The results of the phytochemical screening showed that African leaf simplicia showed the presence of flavonoids, saponins, and tannins. African leaf gel preparations (EADA) doses of 1%, 3%, and 5% are stable for up to 21 days. A gel (EADA) dose of 5% showed the results of measuring burns from 2cm to 0cm within 14 days and approached the results of bioplencenton as a positive control comparison [25]. In this study, fresh African leaves were taken in the Jati area, Bogor Regency. Fresh leaf samples were washed with clean water, dried, and then macerated with 96% ethanol using animals, namely 25 white male mice aged approximately 2.5 months and weighing 20-30 grams. Mice were acclimatized first and then given standard food for one week. Mice that are sick during acclimatization will not be used in the study. On the 8th day, healthy mice fasted for 3-4 hours, and their fasting blood sugar levels were measured. After that, the mice were hyperglycemia by injecting glucose. The 15th minute after the injection of glucose, the blood glucose level of the mice was measured again. The results of the above study showed that ethanol extract could reduce blood glucose levels in mice because, in the experiment given ethanol extract of African leaves orally, it was found that there was a decrease in glucose in the 2nd group of African leaf extract with a concentration of 20% w/v given metoformin.

Research on African leaf extract was also carried out based on the problem of the increasing prevalence of diabetes mellitus in the community, while synthetic drugs are increasingly expensive. One type of herbal medicine that has been traditionally used to treat this disease is African leaf (Vernonia amygdalina Del). Diabetes mellitus is characterized by hyperglycemia and progressive degenerative changes in the structure of pancreatic Langerhans β cells. This study aimed to determine the effective dose of boiled African leaf juice for reducing blood glucose levels. The results showed that the most effective African leaf decoction in reducing blood glucose levels in mice was boiling five pieces of African leaves until the boiled water turned green with a little added water at the beginning of boiling. For further research, it is necessary to replicate measurements of blood glucose levels at time intervals, toxicity tests, and further histological examination of the structure of pancreatic β cells [26]. *Salmonella typhi* is a group of gram-negative bacteria; this bacterium is rod-shaped, does not have spores, moves with a flagellum and has a capsule, and has a size of 2-4 micrometers. This germ has an out-layer component composed of lipopolysaccharide, which functions as endotoxin and does not have spores. Enterobacteria bacteria are resistant to sodium deoxycholate [27].

Gram-negative bacteria have components to help their survival of these bacteria. These components are the cell nucleus, membrane, cytoplasm, ribosomes, plasmids, peptidoglycan, lipoprotein, periplasmic cavity, and lipopolysaccharide [28]. These bacteria have a cell nucleus, also known as a nucleoid or a nuclear body, where the core contains genetic material and a small amount of RNA and protein. The nucleus of this bacteria is not surrounded by a nuclear membrane and does not contain mitotic tools. The bacterial nucleus comprises polyamine and magnesium ions bound to DNA and double-linked, negatively charged, coiled, double-stranded bits of RNA, RNA polymerase, and other proteins. The core part of bacteria that plays an important role is related to proteins in regulating, replication, functioning, and segregation of bacterial chromosomes [29]. The cytoplasm in bacteria does not contain organelles. The cytoplasm consists of cytosol (liquid cytoplasm) and organelles. The hydrophobic nature of the cytoplasm has a protective role, preventing cytoplasmic leakage and protecting cells from damage. The bacterial cytoplasmic membrane consists of a 2-layer (bilayer) of phospholipids and proteins that coat the contents of the bacteria. The cytoplasmic membrane functions include osmosis and passive diffusion, transmembrane and transporters for metabolites that must leave the cell, phagocytosis, and release of substances [30].

Bacterial ribosomes are cytoplasmic nucleoprotein particles whose main function is mRNA translation and protein synthesis. In protein synthesis according to m – RNA instructions, starting when the m – RNA binds to the 30S ribosome subunit known as Shine and Dalgrano (SD), the main sequence corresponds to the anti-SD sequence of 16S r – RNA. Plasmids are formed from circular double chains of DNA molecules which are replicated independently in the process of replication in host cells. Plasmid replication can be divided into three stages: initiation, elongation, and termination. The genes contained in the plasmid function as controllers of plasmid stability [31]. The structure of the cell wall is formed from several layers, namely murein, lipoprotein, phospholipids, proteins, and lipopolysaccharide. The peptidoglycan layer (murein) makes up 20% of the entire cell wall and is responsible for cellular rigidity, and this structure is similar to a net, composed of chains of N-acetyl glucosamine linked covalently with N-acetyl muramic acid

through bonds B 1-4 glycosides. The specific layers of phospholipids, proteins, and lipopolysaccharides, composed of specific polysaccharides, ensure antigenic properties and endotoxin activity [32].

Antibacterial is a drug used to eradicate microbes, especially types of microbes that are harmful to humans (pathogens). In this case, what bacteria means is limited to micro-organisms that do not belong to the group of parasites, viruses, or fungi. Substances that function as antibacterials can be derived from natural compounds, synthetic or semi-synthetic [33]. Antibacterials must have the highest possible selective toxicity, meaning that the drug must be highly toxic to bacteria but relatively non-toxic to the host. Based on the selective toxicity, antibacterials are divided into two antibacterials that inhibit growth (bacteriostatic) and antibacterials that kill bacteria (bactericidal). One of the antibacterials is antibiotics. Antibiotics are substances produced by microbes (especially fungi) that can inhibit the growth or eradicate other microbes, such as bacteria. Based on their mechanism of action, antibiotics are divided into three groups, namely: a) Antibiotics that affect cell walls, b) Antibiotics that disrupt and damage cell membranes, and c) Antibiotics that interfere with DNA function [34].

3. Research Method

This research is an experimental study with laboratory tests to test the antibacterial effectiveness of African leaf extract against *Salmonella typhi* bacteria. This research was conducted at the Microbiology Laboratory, Faculty of Medicine, Christian University of Indonesia. The process of extracting African leaves is carried out at the Bogor Spice and Medicinal Plants Research Institute (Balitro) and will begin as soon as this proposal is approved. The time needed to conduct this research is from September 2018 to December 2018. The samples from this study were *Salmonella typhi* bacteria obtained from the University of Indonesia. The samples used in the study were African leaf extract in various concentrations of 10%, 20%, 40%, 60%, 80%, and 100%, the antibiotic ceftazidime as a positive control, and sterile distilled water as a negative control. The formula used to determine the sample size (repeat) is the Feeder formula:

$$(n-1)\ (k-1) \geq 15$$

$$(n-1)\ (7-1) \geq 15$$

$$(n-1)\ 6 \geq 15$$

$$6n - 6 \geq 15$$

$$6n \geq 21$$

$$n \geq 3{,}5$$

So that the number of repetitions that can be done in each treatment group is four times as large, this sample size is used as a reference for duplication in research. The instruments used in this study were glassware, an alcohol meter, a rotatory evaporator, evaporating cup, an ose mushroom, an extractor, a cotton swab, a micropipette, bunsen, tweezers, an analytical balance, an oven, an autoclave, an incubator, and filter paper after planting the bacteria on Mueller Hilton Agar media and incubating for 24 hours. The bacteria were taken using a loop once, and the *Salmonella typhi* bacteria culture was wiped with sterile cotton on the media. Place filter paper soaked for 15 minutes with African leaf extract with concentrations of 10%, 20%, 40%, 60%, 80%, and 100% in petri dish media. As a positive control, a ceftazidime antibiotic disc was used, which was placed in the center of the media. The procedure was repeated four times in a petri dish. The media was incubated at 37°C for 24 hours, after which the inhibition zone formed on the filter paper was measured using a vernier caliper. Data analysis used to determine the antibacterial activity of African leaf extract on the growth of *Salmonella typhi* bacteria was a one-way ANOVA statistical test using the SPSS version 21 application.

4. Results and discussion

The results of the antibacterial activity test of African leaf extract against *Salmonella typhi* after 24 hours of incubation are presented in Figure 1 below.

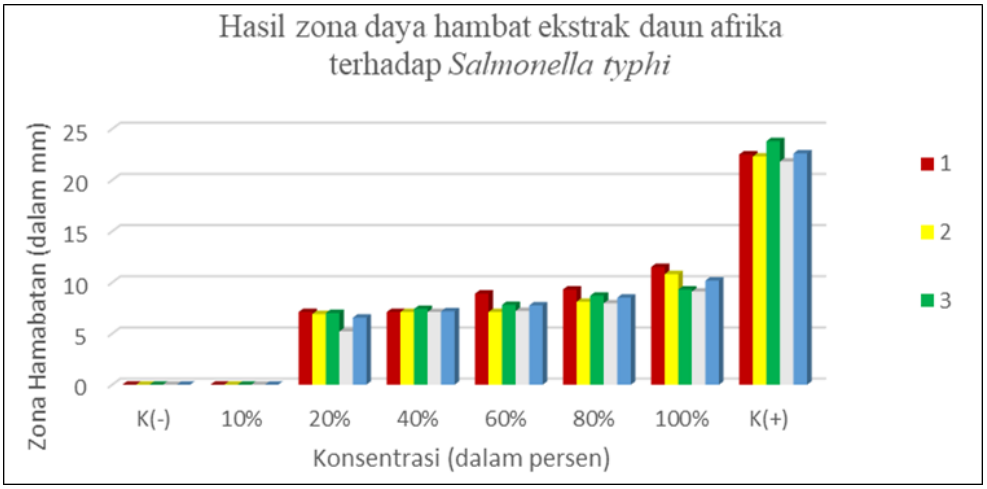
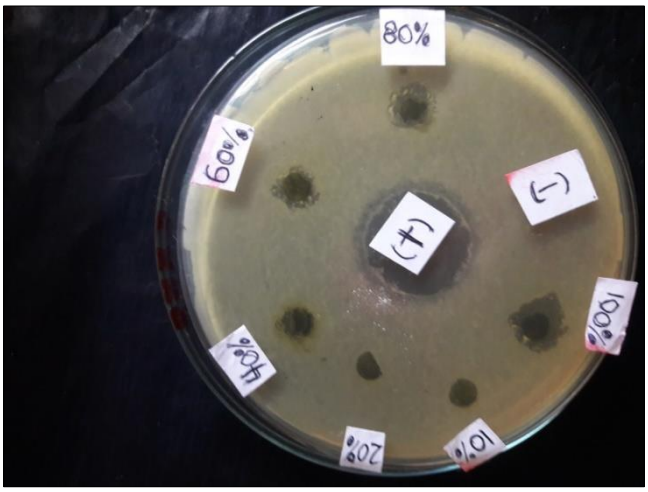


Figure 1 Diagram of the average results of growth inhibition zone measurements

Based on Figure 1, it can be seen that the greater the concentration of African leaf extract, the more it inhibits the growth of *Salmonella typhi* bacteria. In the negative control, an inhibition zone of 0 mm was formed, a concentration of 10% did not form an inhibition zone, a concentration of 20% formed an inhibition zone of 22.3 mm, a concentration of 40% formed an inhibition zone of 23.3 mm, a concentration of 60% formed an inhibition zone of 31.0 mm, 80% concentration formed a 28.0mm inhibition zone, 100% concentration formed a 33.8mm inhibition zone. In the positive control, an inhibition zone of 73.9mm was formed. The results of measuring the diameter of the inhibition zone of African leaf extract can be seen in Figure 2 below.



(Source: personal data)

Figure 2 Inhibition zone measurement results

Table 1 The results of the zone of inhibition of African leaf extract against *Salmonella typhi*

No	The average diameter of the African leaf inhibition zone (mm)							
	K(-)	10%	20%	40%	60%	80%	100%	K(+)
1	0	0	7.1	7.1	8.9	9.3	11.5	23.8
2	0	0	6.9	7.1	7.1	8.1	10.8	22.5
3	0	0	7.0	7.4	7.8	8.7	9.3	22.3
4	0	0	5.2	7.1	7.2	7.95	9.1	21.8
Average	0	0	22.3	23.3	25.6	28.0	33.8	73.9

Based on the category of inhibition zone according to Davis and Stout (2007), it is known that African leaf extract at concentrations of 10%, 20%, 40%, 60%, and 80% has weak inhibition, while a concentration of 100% has moderate inhibition of the test bacteria.

The inhibition test results of the African leaf extract for each concentration of African leaf extract had different diameters of inhibition zones, as shown in Table 1. African leaf extract concentration of 100% formed an average inhibition zone of 33.8 mm. It shows that the greater the concentration of African leaf extract, the greater the content of bioactive compounds with antimicrobial activity. Formation of the inhibition zone at the lowest concentration of African leaf extract (20%) formed an average inhibition zone of 22.3 mm. The inhibition of African leaf extract with a concentration of 40% was greater due to the treatment of the dilution process with sterile distilled water. The lower the concentration of African leaf extract, the smaller the diameter of the inhibition zone. At the same time, the antibiotics used as positive controls had greater inhibition than African leaf extracts. So antibiotics are better at inhibiting the growth of *Salmonella typhi*.

It can be seen in the following table what is the normality value of an inhibition zone. At a concentration of 100%, the inhibition zone of 33.8mm can be said to have a very strong inhibition against *Salmonella typhi* bacteria.

Table 2 Criteria for Activity of the Inhibitory Zone according to Greenwood

Light Zone Diameter	Growth Restraint Response
≥ 20 mm	Very Strong
16-20 mm	Strong
5-10 mm	Medium
≤ 5 mm	Nothing

In another study, an antibacterial test was carried out using soma leaves against *Salmonella typhi* bacteria using the Kirby-Bauaer Disc Diffusion method at a concentration of 0.125%; 0.25%; 0.5%; 1%; 2%; 4%; 8%; and 16%. The positive control was ciprofloxacin five µg/disc, and the positive control was 10% DMSO. Results Soma leaf methanol extract contains flavonoids, tannins, steroids, and phenols. Soma leaf methanol extract has antibacterial activity against *Salmonella typhi*. The concentration of 16% showed the greatest antibacterial activity, but ciprofloxacin five µg had better antibacterial activity.

According to research conducted by Jon Farizal in 2018, the inhibition test of garlic (Allium Sativum) against *Salmonella typhi* bacteria used a descriptive survey design method to determine the inhibitory power of garlic extract (Allium Sativum). Allicin is the main bioactive sulfur contained in garlic. This bioactive content will appear when the onion is cut and crushed. This study used concentrations of 100%, 75%, 50%, and 25%. The results of this study reported the concentrations that formed the growth inhibition zones of *Salmonella typhi* bacteria, namely concentrations of 100% and 75% - an average of 9.7 mm and 8.7 mm, while at concentrations of 50% and 25%, no inhibition zones were formed. From the results obtained, it was concluded that garlic (Allium Sativum) could be used as an alternative ingredient against *Salmonella typhi* bacterial infection.

Screening for antibacterial activity against *Salmonella typhi* used the disk diffusion method with a concentration of plant leaf extract of 10%. This study aimed to determine the antibacterial power of the ethanol extract of ten plant leaves against *Salmonella typhi* resistant to chloramphenicol and determine which compound group contained in the plant leaf extract had the best antibacterial activity. Extraction was carried out with 96% ethanol by maceration method.

Plant leaf extracts with the best antibacterial activity were determined using the liquid dilution method for minimal killing. The identification test for the compounds contained was analyzed by thin-layer chromatography (TLC), and bioautographic tests were carried out. The results showed that six extracts had antibacterial activity against chloramphenicol-resistant *Salmonella typhi*, namely Mahkota Dewa leaves, tea leaves, cherry leaves, clove leaves, tea leaves, and bay leaves. The minimum killing rate of clove leaf extract is 2.5%. The class of compounds contained in clove leaves are alkaloids, phenolics, flavonoids, terpenoids, triterpenoids, and saponins. Based on the results of bioautographic tests, phenolic compounds are estimated to inhibit the growth of *Salmonella typhi* bacteria resistant to chloramphenicol.

5. Conclusion

Based on the research results on the effectiveness of African leaves against *Salmonella typhi* bacteria, the largest inhibition zone diameter was 33.8 mm at a 100% African leaf extract concentration, and the smallest inhibition zone diameter was 22.3 mm at a concentration of 20% African leaf extract. Thus, it is necessary to carry out further research in clinical trials to obtain more significant results. In addition, it is necessary to research African leaves with other bacteria and test African leaf extracts using different types of solvents.

Compliance with ethical standards

Disclosure of conflict of interest

The authors (Hertina Silaban, Esther Elisabeth Bakujai and Dame Joyce Pohan) declare no conflict of interest.

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