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Literature Review on the Antibacterial Potential of African Leaf Extract (Vernonia Amygdalina Del) Against Gram-Positive and Gram-Negative Bacteria

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Abstract
Infections are typically caused by pathogenic microorganisms such as viruses, bacteria, and fungi. An antibacterial agent is a substance that inhibits disease-causing bacteria. Antibacterials are usually present in an organism as secondary metabolites. The Asteraceae family contains numerous genera and species that are scattered globally. This plant family has served a variety of functions, including as an antibacterial. African leaves (Vernonia amygdalina Del) contain many secondary metabolites that are useful as antibacterials, flavonoids, alkaloids, saponins, anthraquinones, and phenols are some examples. This literature search aims to collect and analyze research data regarding the antibacterial potency and active compound content of ethanol extracts of African leaves. African leaf extract was tested against both gram-positive and gram-negative bacteria, including Staphylococcus aureus, Streptococcus mutans, and Staphylococcus epidermidis. Inhibition of Gram-negative bacteria, especially Pseudomonas aeruginosa, was found to be more prevalent in the ethanol extract of African leaves, according to the research.

Keywords: Vernonia amygdalina, African Leaf, Gram-positive, Gram-negative, Antibacterial

Introduction

Infectious diseases are a significant public health concern in both advanced and developing nations. According to the World Health Organization (WHO), infection is one of the leading causes of death among children [1]. According to 2012 WHO data, infectious diseases were responsible for 20-30% of under-five child mortality in Indonesia. Infections are typically caused by pathogenic microorganisms and result in tissue damage and clinical symptoms in the patient [2]. Pathogenic microorganisms are parasitic microorganisms that can cause disease. There are 3 types of dangerous pathogens: viruses, bacteria, and fungi. Bacteria are prokaryotic organisms, which generally do not have chlorophyll and whose asexual production occurs through cell division. They have DNA, but bacterial DNA is not in the nucleus, which also does not have a cell membrane[2]. Propionibacterium, Lactobacillus, Peptostreptococcus, and Streptococcus are gram-positive bacteria. Gram-negative bacteria include species like Veillonella, E. coli, and Pseudomonas aeruginosa [3].

Antibacterial is a compound used to inhibit bacteria. Antibacterials are usually present in an organism as secondary metabolites. In general, antibacterial agents function by destroying cell walls, altering membrane permeability, interfering with protein synthesis, and inhibiting enzyme action [4]. Indonesia is a country with fertile land and abundant natural resources. There are numerous plant species that can be used as herbal medicine in Indonesia, such as the type of rhizome, stems, leaves, or other types [5]. An African leaf is one of the plants that can be used as herbal medicine (Vernonia amygdalina Delile). African leaf plants contain flavonoids, tannins, saponins, and terpenoids that can kill the parasites that cause schistosomiasis, leishmaniasis, and malaria, as well as antitumor and antimicrobial properties. Furthermore, African leaves have diabetes, malaria, blood pressure stabilization, and help cure insomnia and prevent disease stroke, cancer, and heart disease [6].

On the basis of the information provided, it is possible to formulate a problem statement, namely, what is the antibacterial potential of African leaf extract against Gram-negative bacteria? Positive or negative, based on the generated inhibition zone, as well as the active compounds that contribute to antibacterial activity. This study aims to determine the antibacterial potential of African leaf extract against Gram-positive and Gram-negative bacteria, as measured by the inhibition zone and active compounds. The benefit of this research is to provide information to the public regarding the potential of African leaves as an antibacterial. Furthermore, it is hoped that African leaf extract will be developed into alternative antibacterials derived from natural ingredients in the form of plants, which will increase the utilization power of surrounding plants.

Material and methods

This research was conducted through a literature study. Data searches were carried out online through official journal provider sites such as Google Scholar, Pubmed, Science Direct, and searches on various books. A search through the Internet was carried out using the keywords "Vernonia Amygdalina", "Vernonia Amygdalina + antibacterial", and "Extract Vernonia Amygdalina". The inclusion and exclusion criteria were determined first in the article selection process, and then nine articles matching the research topic were obtained. The potential of African leaf extract against Gram-positive and Gram-negative bacteria was determined based on a review and analysis of the obtained research journals.

Result and discussion

This research involved a literature review on the potential antibacterial activity of African leaves. African leaves have antibacterial, antifungal, antidiabetic, antiplasmodial, and anticarcinogenic properties, among others. The components in African leaves that are believed to be responsible for their antibacterial potential are flavonoids, tannins, saponins, and alkaloids [7].

Secondary Metabolite Content of African Leaves

Table 1: Secondary metabolite content in African leaves was detected from various journal sources

Compond	The presence of it in African leaves	Reference
Alkaloids	++++++	8, 9, 10, 11, 12, 13
Anthraquinone	++++	8, 10, 11, 12
Phenol	+	11
Flavonoids	++++++	10, 11, 12, 13, 14, 15
Cardiac Glycosides	+++	9, 12, 16
Polyphenolic	+	13
Tannins	++++++	16, 9, 11, 12, 13, 15
Terpenoids	++	10, 13
Saponins	+++++++	8, 9, 10, 11, 12, 13, 16
Steroids	+++++++	8, 10, 11, 12, 13, 15, 16

Based on table 1, the search found that African leaves have a lot of secondary metabolites that can be found. These include alkaloids, anthraquinones, phenols, flavonoids, cardiac glycosides, polyphenolics, tannins, terpenoids, saponins, and steroids.

Activity of African Leaf Extract Against Gram-Positive Bacteria

The gram-positive bacteria studied in this study were Staphylococcus aureus, Streptococcus mutans, and Staphylococcus epidermidis. Antibacterial activity is seen from the diameter of the inhibition zone formed. The potential antibacterial activity of African leaf extract can be seen in table 2.

In the search, it was found that two extraction methods were used, namely maseration and soxhlet. The extraction method has the principle "like dissolves like," which means a compound will be attracted or extracted if it has the same polarity as the solvent. Based on the results of a literature search, the extraction method that is commonly used is maceration. According to Harmita [17], maceration is a simple method that can be done by immersing simplicia powder in a solvent. The solvent will penetrate the cell wall and enter the cell cavity containing the active substances, so that the active substance will dissolve. Maceration was carried out for 24 hours, and then the filtrate was concentrated using a rotary vacuum evaporator to obtain a thick extract. This evaporation process is carried out to remove the solvent.

The soxhlet extraction method is based on the idea that repeated extraction is the best way to get perfect results. Organic solvents can repeatedly attract organic compounds in natural materials. According to Kadji *et al*. [18], the Soxhlet method of extraction produces a greater yield when compared to maceration. This is due to the existence of heat treatment, which can increase the ability of the solvent to extract compounds that are insoluble at room temperature conditions as well as the maximum withdrawal of compounds by the solvent, which is always circulating in the process of contact with simplicia so as to provide an increase in yield. Soxhlet extraction has several drawbacks; the extraction process can lasts for a long time, up to hours or even days. Then the thermolabile compounds can be degraded because the extract obtained is constantly at the boiling point [19].

Table 2: Activity of African Leaf Extract Against Gram-Positive Bacteria

Extraction	Bacteria	Test Method	Concentration (%)	Inhibition Zone (mm)	Reference
Maceration	Staphylococcus aureus	The well method	5	1	16
Maceration		The well method	10	2	16
Maceration		The well method	20	4.5	16
Maceration		The well method	1.25	12	8
Maceration		The well method	2.5	20	8
Soxhlet		The disc method	0.005	3	9
Soxhlet		The disc method	0.01	6	9
Soxhlet		The disc method	0.02	10.5	9
Maceration		The well method	0.01	6.5	10
Maceration		The well method	0.02	9	10
Maceration		The well method	50	6.67	11
Maceration		The well method	0.01	6.69	15
Maceration		The well method	0.025	7.04	15
Maceration		The well method	0.05	7.12	15
Maceration		The well method	0.075	7.31	15
Maceration		The well method	0.1	7.56	15
Maceration		The well method	1.25	11	8
Maceration	Staphylococcus epidermidis	The well method	2.50	15	8
Maceration	Streptococcus mutans	The well method	1.25	6	8

Maceration		The well method	2.50	11	8
Soxhlet		The disc method	0.005	1	9
Soxhlet		The disc method	0.01	2	9
Soxhlet		The disc method	0.02	4.5	9

In the search, it was found that there were 2 agar diffusion antibacterial test methods, namely wells and discs, but the method that was widely used in this study was the wells method. The working principle of the diffusion method is the diffusion of the antibacterial compound into the solid medium where the test microbe has been inoculated. Observations obtained were the presence or absence of clear areas formed around the wells, which indicated an inhibition zone for bacterial growth [20].

The greater the concentration used, the larger the inhibition zone that is generated, as shown by the antibacterial test results in table 2 compiled from all relevant literature. The study by Pratiwi and Gunawan [15] on the antibacterial effects of an ethanol extract of African leaves against Staphylococcus aureus at a concentration of 0.01% produced the largest inhibition zone, measuring 6.69 mm. The method used by Pratiwi and Gunawan [15] uses an extraction method using maceration and the diffusion antibacterial test method to use wells. But at a concentration of 0.02% in Anibijuwon [9], the resulting inhibition zone is 10.5 mm. Anibijuwon journal, *et al.* [9] employ the soxhlet extraction method as well as the agar diffusion antibacterial test method on paper discs.

Then in Akinyele's study, *et al.* [8], they tested Staphylococcus aureus, Streptococcus mutans, and Staphylococcus epidermidis at the same concentration of 1.25%, using a zone of inhibition of 12 mm, 6 mm, and 11 mm, respectively. The conclusion that can be drawn from these results is that the African leaf extract used in this study was more effective against Staphylococcus aureus.

Activity of African Leaf Extract Against Gram Negative Bacteria

The gram-negative bacteria studied in this study were E. coli and Pseudomonas aeruginosa. Antibacterial activity can be seen from the diameter of the inhibition zone formed. The potential antibacterial activity of African leaf extract against Gram-negative bacteria can be seen in table 3.

Table 3: Activity of African Leaf Extract Against Gram Negative Bacteria

Extraction	Bacteria	Test Method	Concentration (%)	Inhibition Zone (mm)	Reference
Maceration with ethanol 96%	Escherchia Coli	The well method	0.0025	7	10
			0.005	11	10
			0.01	13.5	10
			0.01	6.52	15
			0.02	14.5	10
			0.025	6.61	15
			0.05	6.63	15
			0.075	6.64	15
			0.1	6.74	15
			5	1	16
			10	6	21
			10	3.5	16
			20	8	16
			50	5.0	11
Maceration with ethanol 96%	Pseudomonas aeruginasa	The well method	0.005	11	10
			0.01	11.5	10
			0.02	16	10
			2.5	2	16
			5	3	16
			10	6	21
			10	6	16
			20	10.5	16

The solvent used is 96% ethanol. Differences in solvents and extraction methods used during research will affect the antibacterial activity of the extracts and optimize the withdrawal of compounds from extracts. Ethanol is used as a solvent because it is polar, universal, and easily available. Ethanol is a universal solvent capable of extracting flavonoids, saponins, tannins, anthraquinones, terpenoids, and alkaloids [22]. According to Septyaningsih [23], secondary metabolites that can dissolve in non-polar solvents are steroids and terpenoids. Compounds that are semi-polar are phenolic compounds, including flavonoids, while polar compounds are alkaloids, saponins, and tannins.

According to Evbouman *et al.* [10] and Pratiwi and Gunawan [15], the antibacterial activity test of African leaf extract on Escherichia coli bacteria at a concentration of 0.01% yielded successive results of 13.5 mm and 6.52 mm in table 3. At 0.02% concentration, the resulting inhibition zones measured 14.5 mm and 6.61 mm, respectively. So, it can be concluded that Evbouman's research, *et al.* [10], found an extract that was more effective in antibacterial testing. In the study of the bacterium Pseudomonas aeruginosa at a concentration of 10% conducted by Bukar *et al.* [21] and Adetunji [16], it produced the same inhibition zone of 6 mm.

When compared between studies conducted on Escherichia coli and Pseudomonas aeruginosa with a concentration of 0.01% in research according to Pratiwi and Gunawan [15] and Evbouman *et al.* [10], they produced inhibition zones of 6.52 mm and 11.5 mm, respectively. So, it can be concluded that this African leaf extract is more effective against Pseudomonas aeruginosa bacteria than Escherichia coli. The focus of the investigation is on determining whether or not an extract from an African leaf can kill gram-negative

bacteria; The range of inhibition increases with increasing concentration.

Antibacterial against Gram positive and Gram-negative bacteria

Based on table 4, if we compare the research between Pratiwi and Gunawan [15] and Evbouman *et al.* [10] on Staphylococcus aureus bacteria, E. coli, and Pseudomonas aeruginosa with the same concentration of 0.01%, the results are respectively 6.69 mm, 13.5 mm, and 11.5 mm. So, it can be concluded that African leaf extract with an extractant of 96% is more effective on Gram-negative bacteria, namely Escherichia coli. However, at a concentration of 0.02% Evbouman *et al.* [10], in a study of the bacteria Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa, had successive results of 9 mm, 14.5 mm, and 16 mm. So, both are more effective against Gram-negative bacteria, namely Pseudomonas aeruginosa.

Table 4: The activity of African leaf extract against Gram positive and negative bacteria according to concentration

Extraction	Bacteria	Concentration (%)	Inhibition Zone (mm)	Reference
Maceration with ethanol 96%	Pseudomonas aeruginosa	0.005	11	10
	Escherchia Coli	0.005	11	10
	Escherchia Coli	0.01	6.52	15
	Staphylococcus aureus	0.01	6.69	15
	Pseudomonas aeruginosa	0.01	11.5	10
	Escherchia Coli	0.01	13.5	10
	Staphylococcus aureus	0.01	6.5	10
	Pseudomonas aeruginosa	0.02	16	10
	Escherchia Coli	0.02	14.5	10
	Staphylococcus aureus	0.02	9	10
	Escherchia Coli	0.025	6.61	15
	Staphylococcus aureus	0.025	7.04	15
	Escherchia Coli	0.05	6.63	15
	Staphylococcus aureus	0.05	7.12	15
	Escherchia Coli	0.075	6.64	15
	Staphylococcus aureus	0.075	7.31	15
	Escherchia Coli	5	1	16
	Staphylococcus aureus	5	1	16
	Pseudomonas aeruginosa	5	3	16
	Escherchia Coli	10	3.5	16
	Staphylococcus aureus	10	2	16
	Pseudomonas aeruginosa	10	6	16
	Escherchia Coli	20	8	16
	Staphylococcus aureus	20	4.5	16
	Pseudomonas aeruginosa	20	10.5	16
	Escherchia Coli	50	5.0	11
	Staphylococcus aureus	50	6.67	11

Then in Adetunji's study [16], which compared Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa with a concentration of 5%, successive results were 1 mm, 1 mm, and 3 mm. Concentrate on 10% and 20% also showed that the largest inhibition zone was in Gram-negative bacteria, namely Pseudomonas aeruginosa. So, it can be concluded that this African leaf extract is more effective on Gram-negative bacteria, namely Pseudomonas aeruginosa. This can happen because the cell walls of the two types of bacteria have differences. Gram-positive bacteria have a peptidoglycan layer that makes up the cell wall, consisting of a thick and rigid structure that contains a cell wall substance called teichoic acid. Meanwhile, Gram-negative bacteria have a peptidoglycan layer that contributes to a thinner cell wall that is easily damaged. Peptidoglycan is a component used to maintain cell integrity, making Gram-negative bacteria's cell walls more vulnerable to damage when exposed to an antibacterial [24].

The results of the comparison of the effectiveness of African leaf extracts are still diverse. Many factors influence the results of the antibacterial activity test, such as the extraction method, the presence of other contaminants, the turbidity level of the tested bacterial suspension, the concentration of the tested extract, the incubation temperature, the thickness of the media, the length of the incubation time, the composition of the media, and the impregnation time of the tested bacterial suspension into the media [25].

The mechanism of action of flavonoids as antimicrobials can be divided into three categories: inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism [26]. The mechanism of action of flavonoids in inhibiting the function of cell membranes is by forming complex compounds with extracellular and dissolved proteins so that they can damage the bacterial cell membrane and be followed by the release of intracellular compounds [27]. Flavonoids can inhibit energy metabolism by inhibiting the use of oxygen by bacteria. Flavonoids inhibit cytochrome C reductase, so the formation of metabolism is inhibited. Energy is needed by bacteria for macromolecular biosynthesis [28].

Saponins act as antibacterials by altering the cellular membrane's permeability and leading to cell death[29]. Saponins' antibacterial mechanism of action involves the release of intracellular proteins and enzymes. Saponins are antibacterial because their surface-active substances are similar to detergents; consequently, saponins reduce the surface tension of the bacterial cell wall and damage the membrane's permeability. Cell membrane damage is extremely detrimental to bacterial survival [30]. Saponins diffuse through the outer membrane and the weakened cell wall before attaching to the cytoplasmic membrane, thereby

disrupting and decreasing the membrane's stability. This substance causes the cell's cytoplasm to leak out, resulting in cell death. Bactericidal are antimicrobial agents that disrupt the cytoplasmic membrane [31].

Alkaloids act as antibacterials by interfering with the constituent components of peptidoglycan in bacterial cells, preventing the formation of the cell wall layer and causing cell death. Alkaloid antibacterials are also effective because some of their constituents, known as DNA interchelators, inhibit the activity of bacterial cell topoisomerase enzymes [32]. The antibacterial mechanism of steroids involves lipid membranes and sensitivity to steroid components that induce liposome leakage. Steroids can interact with cell membrane phospholipids, which are permeable to lipophilic substances. Consequently, decreased membrane integrity and altered cell membrane morphology result in brittle cells and cell death.

As an antibacterial, anthraquinone inhibits the synthesis of Escherichia coli bacterial cells. Anthraquinone is a phenolic compound that, like the phenol group, inhibits bacteria by denaturing their proteins [33]. Terpenoid compounds have been hypothesized to have an antibacterial mechanism of action based on the premise that they cause membrane damage via the addition of lipophilic compounds. Terpenoids can react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall to form strong polymeric bonds and damage the porins, reducing the permeability of the bacterial cell wall and starving the bacteria of nutrients, halting their growth, or killing them [33].

Conclusion

According to the study's findings, African leaf extract is more effective against Gram-negative bacteria, specifically Pseudomonas aeruginosa. The potential for antibacterial activity is suspected because African leaf extract contains chemical compounds consisting of flavonoids, tannins, saponins, steroids, anthraquinones, and terpenoids.

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