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Antibacterial Activity of Moringa Plants (*Moringa oleifera* Lam.) to Overcome Antibiotic Resistance: A Systematic Review

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ABSTRACT

Antibiotic resistance This causes the performance of synthetic antibiotics to decrease, so it is necessary to search for natural antibiotics. Moringa plant (*Moringa oleifera* Lam.) has antibacterial activity proven to inhibit and kill pathogenic bacteria and Multi-Drug Resistance (MDR) article review aims to collect data related to testing the antibacterial activity of Moringa plants as an effort to overcome antibiotic resistance. The method follows the PRISMA-2020 protocol by searching data on PubMed and ScienceDirect media using the keywords: "Antibacterial activity" and "*Moringa oleifera*". The data selection uses the Rayyan application by considering the inclusion and exclusion criteria that have been set. The final results obtained are 95 data which are experimental journals of the Randomized Control Trial (RCT) type published in 1981-2021. Moringa plants began to be investigated for their activity against MDR bacteria in 2012 and became a sign that research was already in the direction of finding solutions. Antibacterial testing was carried out in vitro (10 studies) and in vivo (1 study). The leaves have been thoroughly tested for their activity against *Methicillin-Resistant Staphylococcus aureus* (MRSA). The results of in vitro study obtained the range of zone of inhibition, minimum inhibitory concentration, and minimum kill concentration, respectively: 8.3-12 mm; 0.25-20 mg/ml; 0.50-20 mg/ml study in vivo showed that there was the healing of MRSA infection wounds in test animals.

1. Introduction

Antibiotic resistance is the pinnacle of the bacterial revolution due to the irrational use of drugs. The world health organization provides a strict watch list for bacteria that are resistant to more than 3 kinds of antibiotics, known as Multi-Drug Resistant (MDR).¹ *Staphylococcus aureus* B3 was shown to be resistant to 10 of the 12 antibiotics tested.² The data confirms that there is a scarcity of synthetic antibiotics that are

effective against pathogenic and MDR bacterial infections, so it is necessary to search for new active substances.

Natural ingredients that can be used as a source of natural antibiotics are Moringa plants (*Moringa oleifera* Lam.). The antibacterial activity of this plant comes from gallic acid, isothiocyanate, niazimicin, tannins, saponins, alkaloids, and flavonoids.^{4,5} Found

compound 4(α -L-rhamnopyranosiloxy-isothiocyanate), which can inhibit bacterial growth by interfering with essential enzymes.⁵ Based on content analysis, many researchers have continued to test the antibacterial properties of Moringa plants in vitro and in vivo. Generally, the test uses one part of the plant, and it is rarely found that examines the whole part. It is difficult to find literature that systematically discusses the antibacterial activity of Moringa plants. From these problems, it is necessary to conduct research using a Systematic Literature Review (SLR) approach as the main method.

SLR is a method for identifying, assessing, and interpreting related topics and answering predetermined research questions.⁶ This method is said to be systematic because it is regulated by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).⁷ Based on the description above, this article aims to examine the antibacterial activity of Moringa plants using the SLR method. This article is expected to provide an overview of the antibacterial development of Moringa plants to overcome antibiotic resistance.

2. Methods

Data search

Data search used 2 electronic databases, including PubMed and ScienceDirect. To increase the sensitivity in the search, a combination of the terms Free Word and Medical Subject Heading (MESH) was used. Combinations of terms are combined with Boolean Operators (OR; AND; NOT). The keywords in the search were: "*Moringa oleifera*" and "Antibacterial activity." The data search was carried out in October

2021 so that the journals published afterward were not included in the category.

Data selection

The accepted data inclusion criteria are i) RCT type research journal in English; ii) Complete writing format (introduction, method, results, and discussion); iii) Antibacterial testing of the Moringa plant should be carried out either in vitro or in vivo. The selection in this article follows the PRISMA protocol and uses the help of the Rayyan application.

Data extraction

The main information extracted in the form of antibacterial activity from Moringa plants can be in the form of the zone of inhibition, Minimum Inhibitory Concentration (MIC), and Minimum Kill Concentration (KBM). In addition, information related to the types of bacteria evaluated, active substances, antibacterial mechanisms, test methods, solvents, and types of extraction were also provided. Additional information in the form of the name of the author of the article, the year of publication, and the country of origin of the research.

Data synthesis

Data synthesis was carried out qualitatively (meta-synthesis) with reference to antibacterial activity, active substance, and the antibacterial mechanism of the Moringa plant. This article features Milestone to describe the development of antibacterial studies. Synthesis related to the active substance as well as the antibacterial mechanism is presented in the form of a flow chart.

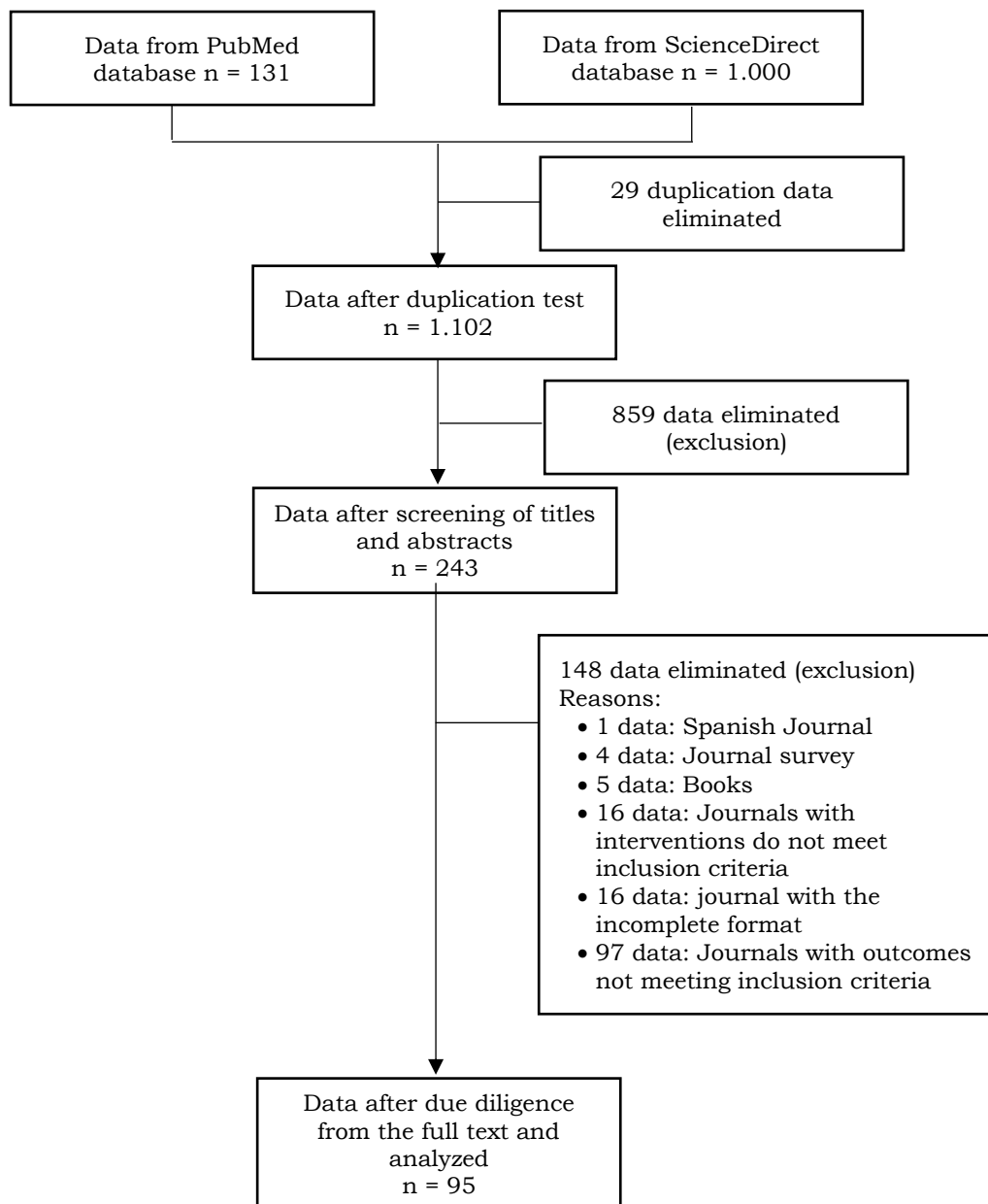


Figure 1. PRISMA flow diagram of systematic review

3. Results

Search data in this article using 2 electronic databases, PubMed and Science Direct. combination of electronic databases Proven has more optimal performance than single use.⁸ The data search results from this article are presented in Table 1. The total data obtained were 1,131 data searched on 10/10/21.

The data selection in this article uses the help of an online application called Rayyan. The results of the data selection are summarized in the prism diagram of

the study (Figure 1). The data went through 3 stages, namely: duplication test, selection of titles and abstracts, and selection of full text.⁶ At the end of the selection, 95 data were obtained according to the inclusion criteria and entered the next stage.

Data extraction shows that antibacterial testing of *Moringa* plants has been carried out from 1981–2021. This indicates that *Moringa* has great potential as an antibacterial, so the plant has been tested for its ability. The data obtained were 95 grouped by year of

publication and presented in Table 2. A summary of data extraction by the method is presented in Table 3. From the data extraction, it can be seen that antibacterial research carried out at least 3 basic methods, namely: sample extraction, content analysis, and antibacterial test.

The data synthesis in this article is divided into several parts, namely: the overall development of antibacterial research (Figure 2); antibacterial studies with MDR bacteria (Table 4); plant parts tested (Table 5); antibacterial testing in vitro (Table 6); in vivo antibacterial testing (Table 7); antibacterial content of the Moringa plant (Table 8).

Table 1. Data search results

Databases	Search term	Total
PubMed	("Moringa"[Mesh] OR "Moringa oleifera"[Mesh] OR "moringa oleifera Lam."[tw] OR "moringa oleifera"[tw] OR moringa[tw] OR "Drumstick tree"[tw]) AND ("Anti-Bacterial Agents"[Mesh]) OR "Microbial Sensitivity Tests"[Mesh]) OR "Drug Resistance, Multiple, Bacterial"[Mesh] OR "antibacterial agent"[tw] OR "antibacterial agents"[tw] OR "antibacterial compound"[tw] OR "antibacterial compounds"[tw] OR "antibacterial activity"[tw] OR "antibacterial activities"[tw] OR "antibacterial activity tests"[tw] OR "antibacterial effects"[tw] OR antibacterial[tw])	131
ScienceDirect	("moringa oleifera Lam." OR "moringa oleifera" OR moringa OR "Drumstick tree") AND ("antibacterial agent" OR "antibacterial activity" OR "antibacterial activities" OR "antibacterial activity tests" OR antibacterial)	1.000
Total		1.131

Table 2. Data extraction results

Year of publication	Number of publications	References
1981	1	18
1991	1	19
1997	1	20
2001	1	21
2005	1	22
2007	1	23
2010	2	24, 25
2011	2	26, 27
2012	6	28, 29, 3, 30, 31, 32
2013	3	33, 34, 35
2014	3	36, 37, 38
2015	6	10, 39, 40, 16, 41, 42
2016	7	43, 44, 45, 46, 47, 48, 49
2017	3	50, 51, 52
2018	7	53, 54, 55, 56, 57, 58, 59
2019	12	60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71
2020	23	2, 14, 72, 73, 55, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91
2021	15	92, 93, 95, 96, 97, 98, 99, 100, 101, [102, 103, 104, 99, 105

Table 3. Summary of data extraction

Outcome	Method
Sample extract	Cold way
	Hot way
Antibacterial content	Analysis with Spectrophotometry
	Analysis with Chromatography
Antibacterial activity	Antibacterial test in vitro
	Antibacterial test in vivo

Table 4. Antibacterial testing of Moringa plants against MDR bacteria

No.	Plant parts	MDR bacteria	Testing antibacterial		References
			in vitro	in vivo	
1.	Leaf	<i>Staphylococcus aureus</i>	✓	-	2
2.	Leaf	<i>Staphylococcus aureus</i>	✓	-	97
3.	Leaf	<i>Staphylococcus aureus</i>	✓	-	70
4.	Leaf	<i>Staphylococcus aureus</i>	✓	-	84
5.	Leaf	<i>Staphylococcus aureus</i> <i>Enterococcus faecalis</i>	✓	-	31
6.	Leaf	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i>	✓	-	86
7.	Leaf	<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Enterobacter aerogenosa</i>	✓	-	43
8.	Leaf	<i>Escherichia coli</i> <i>Enterobacter aerogenosa</i> <i>Klebsiella pneumoniae</i> <i>Providencia stuartii</i>	✓	-	47
9.	Leaf	<i>Staphylococcus aureus</i>	-	✓	10
10.	Seed	<i>Staphylococcus aureus</i>	✓	-	68
11.	Sap	<i>Staphylococcus aureus</i>	✓	-	92

Table 5. In vitro and in vivo testing of MDR bacteria

In vitro antibacterial activity testing				
Plant parts	MDR bacteria	Test results		
		ZH (mm)	KHM (mg/ml)	KBM (mg/ml)
Leaf	<i>Staphylococcus aureus</i>	8.3-12	0.25-10	0.50-20
	<i>Eschericia coli</i>	8-9	0.12-8.19	-
	<i>Klebsiella pneumoniae</i>	12	0.25	-
	<i>Enterococcus faecalis</i>	15	-	-
	<i>Enterobacter aerogenes</i>	-	8.19	-
	<i>Providencia stuartii</i>	-	1.02	-
Seed	<i>Staphylococcus aureus</i>	26.75	-	-
Sap	<i>Staphylococcus aureus</i>	6	-	-
In vivo antibacterial activity testing				
Plant parts	Animal modeling test	Test results		
Leaf	Cut wounds on rats that have been inoculated by MDR bacteria (<i>Staphylococcus aureus</i>)	Oral administration of Moringa leaf solution can heal infectious wounds		
References				
31: 10; 43: 47; 70: 68; 2: 84; 86; 92				

Table 6. In vitro antibacterial testing of pathogenic bacteria and MDR

Plant parts	Bacterial test	Test results		
		ZH (mm)	KHM (mg/ml)	KBM (mg/ml)
Leaf	<i>Staphylococcus aureus</i>	3.53-32	0.012-12.5	0.6-12.5
	<i>Staphylococcus aureus</i> (MDR)	8.3-12	0.25-10	0.50-20
References				
76; 71; 49; 31; 2; 70; 84				

Table 7. Summary of in vivo antibacterial testing

Animal model test	Sample	Treatment	Research results
Cut wounds on rats those have inoculated MRSA	Moringa leaf extract	Oral administration of a dose of 150 mg, 2 times a day for 8 days	<ul style="list-style-type: none"> • Observation of wounds: the 11th day of dry wounds. • Histopathology: tissue regeneration, fibroblast proliferation.
Cut wounds on rats	Moringa seed fraction ointment	Topical administration of fraction ointment on the wound, once a day for 21 days	<ul style="list-style-type: none"> • Observation of wounds: the 14th day of the wound is dry, on the 21st day the scar is greatly faded. • Histopathology: the presence of tissue regeneration, fibroblast proliferation, tissue granulation.
Cut wounds on rats	Moringa seed extract gel	Topical administration of the gel to the wound, once a day for 14 days	<ul style="list-style-type: none"> • Wound observation: the 4th day the wound is dry, the 12th day the scar fades, the 14th day the scar disappears completely • Histopathology: tissue regeneration, proliferation of fibroblasts, tissue granulation, increased collagen.
References			
10; 49; 11			

Table 8. Antibacterial content of the Moringa plant

Antibacterial content	References
Saponins; Glycosides; Tannins; Alkaloids; Terpenoids; polyphenolics; Pterygospermin; Quercetin; Kaempferol; Benzyl sotiosinate; Benzyl glucosinolate; Gallic acid; and lectins	104 93 14 64 13

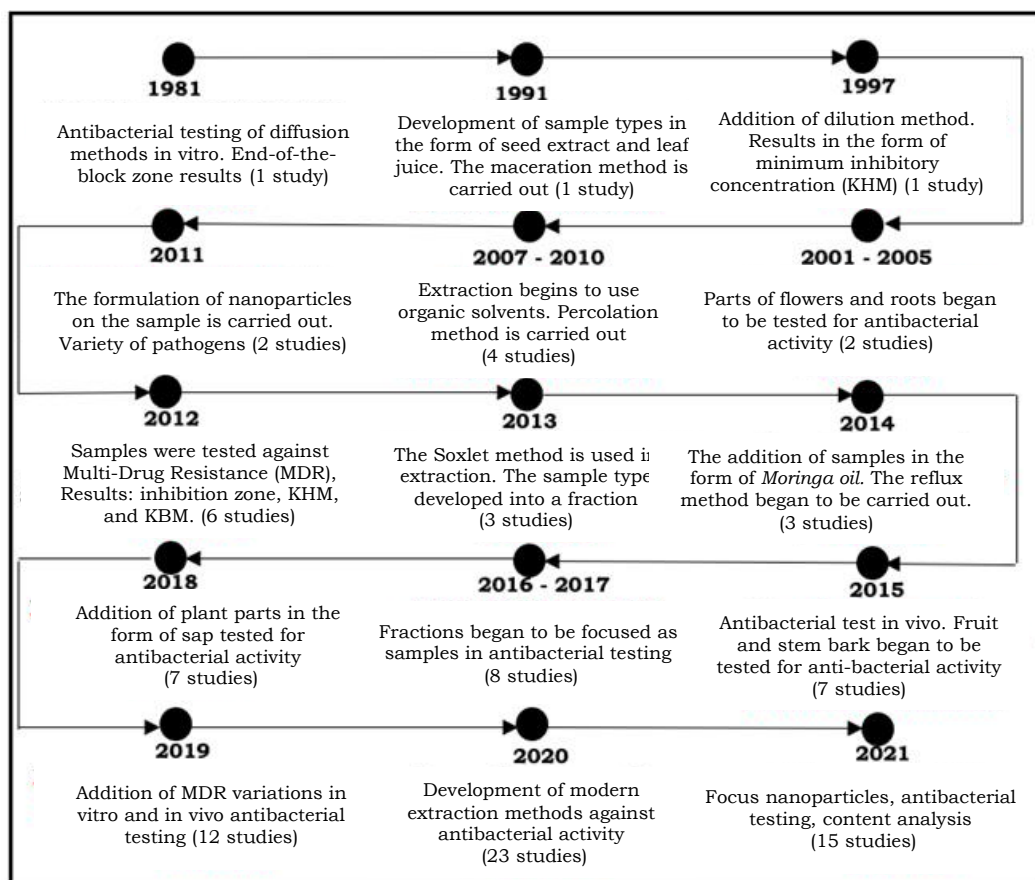


Figure 2. Antibacterial research milestones of the Moringa plant

4. Discussion

Antibacterial research from Moringa plants has been carried out for 40 years (1981-2021). To see the development of the research is designed in the Milestone (Figure 2). The diagram shows the exploration of the antibacterial activity of the Moringa plant so that all parts of the plant have been investigated. In general, compounds and secondary metabolites are evenly distributed but sometimes distributed in certain parts. The diagram also shows the development of research from Moringa plants towards finding solutions to overcome antibiotic resistance.

Research to overcome antibiotic resistance from Moringa plants has been carried out since 2012. This is evidenced by the use of Multi-Drug Resistance (MDR) bacteria. Details of antibacterial testing of

Moringa plants against MDR bacteria are presented in Table 4. Tests have been carried out for 9 years from 2012-2021, in vitro (10 studies) and in vivo (1 study). The duration of testing for MDR bacteria is indeed shorter than for pathogenic bacteria. This causes the use of parts of the Moringa plant to be incomplete but has shown the development of research towards finding solutions to cases of antibiotic resistance.

The parts of the Moringa plant that were tested for antibacterial activity against Multi-Drug Resistance (MDR) bacteria were: leaves, seeds, and sap. The test results of the three parts of the Moringa plant against MDR bacteria are presented in Table 5. Details of the results indicate that the leaf part has been studied for its activity against *Staphylococcus aureus* (MDR) completely in vitro and in vivo. Antibacterial testing parameters In vitro were Inhibited Zone, MIC, and

KBM, while in vivo were wound healing due to MDR bacterial infection.

Antibacterial testing in vitro not only tested the activity of Moringa plants against pathogens but on MDR bacteria as well. The authors compared the results of testing the antibacterial activity of Moringa plants against pathogenic bacteria and MDR, which are presented in Table 6. This comparison was compiled based on all the results of antibacterial testing from the leaves taken from the lowest value and the highest value. Based on the data, the leaf is the part that has been tested for its activity in vitro against pathogenic bacteria and MDR. Moringa plant, as an antibacterial, has a strong category against *Staphylococcus aureus* and moderate against *Staphylococcus aureus* (MDR). The selection of this category based on the inhibition zone was said to be very strong (≥ 20 mm) and moderate (5-10 mm).⁹

Antibacterial testing In vivo uses test animals that have been inoculated with bacteria. Antibacterial testing is *in vivo*, presented in Table 7. The initial procedure injures the skin of the previously anesthetized mice. The wound is then inoculated with the bacteria to be tested, such as Methicillin Resistant *Staphylococcus aureus* (MRSA).¹⁰ MRSA is a group of *Staphylococcus aureus* that has been resistant to *Methicillin*. Treatment will start when the wound in the mouse has pus which indicates an infection. During treatment, wound observations were made, and the results showed that the mice's wounds healed in less than 21 days. The results of antibacterial studies from Moringa plants also show that there is an increase in testing not only limited to in vitro but has begun to switch to in vivo and will continue to increase.^{3,12}

Antibacterial content is the most important factor because the ability to inhibit or kill comes from these ingredients. Moringa content that affects its antibacterial activity is presented in Table 8. Surendra's research carried out the separation of phenolic compounds from Moringa plants and analysis of the content.^{13,14} This compound is able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. Coriolano's research carried out the

separation of lectin compounds from Moringa seeds and antibacterial tests.¹⁴ Lectins are bacteriostatic against *Micrococcus luteus*, which means that these compounds are able to kill about 99.9% of bacteria.

Lectins are compounds that work by attaching to glycans (components of bacterial cell membranes). The attachment will form pores so that ions, nucleic acids, proteins, and other cell organelles come out.¹⁵ This mechanism was confirmed by Moura's research, proving that lectins destroy the cell wall integrity of *Serratia marcescens*, causing protein leakage.¹⁶ Lectins have also been shown to inhibit the growth of bacterial biofilms, so that cell viability decreases.¹⁷ Decreased viability will cause bacteria to be more susceptible to antimicrobial agents because the protective layer is lost.

Decision-making of a plant said to be able to overcome antibiotic resistance must go through complete testing. Moringa plant testing until 2021 is still limited to in vitro and in vivo based on data obtained from 2 databases. The more databases that can be accessed is hoped the results of further SLR research will increase.

5. Conclusion

Antibacterial studies from Moringa plants have been going on for 40 years, but the focus on overcoming antibiotic resistance has only been running for 9 years since 2012. The use of Multi-Drug Resistance (MDR) as test bacteria is strong evidence. Antibacterial testing of Moringa plants against MDR bacteria has been carried out in vitro and in vivo. Article review proves that Moringa plants can overcome pathogenic bacterial infections and MDR based on the Randomized Controlled Trial (RCT) study obtained by the authors.

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