

ROSELLE PLANT ANTIBACTERIAL ACTIVITY TEST (*Hibiscus sabdariffa* L.): (A REVIEW ARTICLE)

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ABSTRACT:

Background: Infections are caused by bacteria, viruses, and fungi. It is still the leading cause of death in the world. Infectious diseases can be overcome by using antibiotic therapy, but inappropriate use of antibiotics can cause resistance. Roselle plant (*Hibiscus sabdariffa* L.) contains secondary metabolites including organic acids, polysaccharides, flavonoids, saponins, tannins, and alkaloids which have medicinal properties where these compounds are thought to have antibacterial effects.

Objective: Assessing the content of roselle plant compounds that show antibacterial activity

Materials and methods: In preparing this review article, the technique used is to use literature study by searching for sources or literature in the form of primary data or in the form of official books and international journals in the last 10 years. Find the main references used in this review article through trusted websites such as ScienceDirect, PubMed, NCBI, ResearchGate, and Google Scholar which focus on antibacterial activity in rosella (*Hibiscus sabdariffa* L.)

Result: Studies published from 2010-2020 were selected. Roselle (*Hibiscus sabdariffa* L.) is a plant that has antibacterial properties. The content of antibacterial compounds in roselle such as alkaloids, flavonoids, saponins, and tannins. These compounds are found in the roots, leaves, and petals. Rosella flowers have been shown to inhibit bacterial growth. High the concentration of roselle flower, leaf, and root extracts, the greater the inhibition zone formed so that the inhibition of bacterial growth will be stronger.

Conclusion: Roselle plant (*Hibiscus sabdariffa* L.) is a medicinal plant that has antibacterial properties by using various concentrations and having different inhibition diameters.

Keywords: Roselle (*Hibiscus sabdariffa* L.), Antibacterial Activity, Zone of Inhibition

INTRODUCTION:

The use of herbal plants tends to increase from year to year.[1]. The use of herbs in the world as much as \pm 80%, especially in developing countries for health maintenance efforts and help in treating various diseases.[2]. One of them is an infectious disease which is the biggest health problem in the world. According to WHO 2015 based on YLL (Years Of Life Lost) data in developing countries, infectious diseases are still the main cause of death.[3]. Infections are caused by bacteria, viruses, fungi, and parasites.[4].

Infection occurs due to the entry of microorganisms into body tissues which then multiply. To overcome infectious diseases, therapy can be done using various antibiotics.[5] Many types of antibiotic classifications, germ sensitivity patterns, and the discovery of new antibiotics often make it difficult to determine the right antibiotic choice when dealing with a case of the disease. This is one of the factors triggering resistance, but the main cause of antibiotic resistance is its widespread and irrational use.[6]

One of the natural ingredients that can be used for traditional medicine is the roselle plant (*Hibiscus sabdariffa* L.).[7]. Rosella flowers contain secondary metabolites that are thought to have antibacterial effects. Roselle plants contain organic acids, polysaccharides, flavonoids, saponins, tannins, and alkaloids which have medicinal properties.[8]. Roselle leaves contain flavonoids, saponins, phenolics, tannins, steroids, and glycosides. Flavonoids, saponins, phenolics, tannins, steroids, glycosides, and alkaloids are also present in the stems and roots of the roselle plant. Tartaric acid and saponins are also present in the roots. This can be a reference that the compound content of the flowers, leaves, and roots of roselle can be used as antibacterial. [9] Antibacterials are

substances that can interfere with growth or even kill bacteria by interfering with the metabolism of harmful microbes.[10]. Roselle (*Hibiscus sabdariffa* L.) is a plant of the Malvaceae family, including a plant that is relatively easy to grow and is used as a source of food and fiber which is widely used as alternative medicine.[11]. Rosella plants are erect, dense, can grow up to 2.4 m, with smooth or almost smooth stems, cylindrical, and usually red. Roselle flowers are bright. The petals of roselle flowers are dark red. The part of rosella flowers that can be processed into food is the petals which have a very sour taste, can also be processed into drinks, jelly, powder (tea), or roselle sweets.[12].



Figure 1. Roselle plant(*Hibiscus sabdariffa* L.) [13].

Based on the many benefits contained in the rosella plant (*Hibiscus sabdariffa* L.) so that a review is needed aims to provide information regarding The antibacterial activity of roselle extract using the disc diffusion method against several types of bacteria is expected to be used as an alternative treatment to improve the health and quality of life of the community, especially in overcoming infectious diseases to minimize the use of antibiotics.

MATERIALS AND METHODS:

Reporting: All complete studies carried out on antibacterial activity are available for free on the internet.

Inclusion and Exclusion Criteria: All full original articles are included in this review. The study period 2010-2019 is selected. Studies that report antibacterial activity are included. Studies that have irrelevant titles, objectives, methods, or statistical tests are excluded. All studies that had incomplete or confusing content, methods, references, and author information are also excluded. To ensure the completeness of the desired attributes, a checklist has been created containing the parts of the plant, the parts tested, the type of bacteria, the concentration and diameter of resistance in the water, and ethanol solvents used.

Search Strategies and Information Sources: In preparing this review article, the technique used is to use literature study by searching for sources or literature in the form of primary data or in the form of official books and international journals in the last 10 years. Find the main references used in this review article through trusted websites such as

ScienceDirect, PubMed, NCBI, ResearchGate, and Google Scholar which focus on antibacterial activity roselle (*Hibiscus sabdariffa* L.)

Study Selection: In the first step, the study was carried out in a reference management software called MENDELEY for storage and duplication avoidance. The studies taken are then assessed via the checklist mentioned above. Irrelevant or ambiguous studies were excluded. In the second step, the author critically analyzes the article content. Articles that do not match the title have irrelevant variables, or analyzes that are incorrectly excluded will be excluded.

Data Extraction: A structured data extraction form was created to extract information from selected studies. Plant part, part tested, type of bacteria, concentration, the diameter of resistance in water and ethanol solvents used, and reference. The two reviewers independently extracted data from the articles. Any discrepancies in the reported data were reviewed, and corrected by a third reviewer.

RESULTS:

Table 1 Diameter of plant antibacterial inhibition rosella(*Hibiscus sabdariffa* L.)

No.	Part	Part tested	Bacteria	Concentration	Resistance Diameter (mm)			Ref		
					Water	Ethanol 70%	Ethanol 96%			
1	Root	Extract thick	<i>Staphylococcus aureus</i>	10% 20% 30%	-	10.48 13.2 13.73	-	[14]		
	Leaf	Extract Thick	<i>Staphylococcus aureus</i>	10% 20% 30%	-	12.4 16.73 21.87	-			
	Flower	Extract Thick	<i>Staphylococcus aureus</i>	10% 20% 30%	-	17.43 21.6 24.23	-			
2	Flower petals	Extract thick	<i>Streptococcus mutans</i>	5%	1	-	-	[15]		
				10%	2					
				15%	5					
				20%	7					
				25%	8					
				30%	9					
				35%	11					
				40%	13					
45%	15									
50%	18									
3	Flower petals	Thick extract	<i>Streptococcus mutans</i>	5%	1.47	-	-	[16]		
				10%	1.66					
				20%	2.20					
				40%	2.28					
				80%	3.50					
4	Flower petals	Extract water	<i>Streptococcus pneumoniae</i>	10%	17	-	-	[17]		
				30%	21					
				50%	22					
				70%	24.3					
		Extract thick	<i>Streptococcus pneumoniae</i>	10%	-				14	-
				30%	-				20	-
				50%	-				22.6	-
70%	-	25.6	-							
5	Flower	Extract Water	<i>Staphylococcus aureus</i>	0.80%	10.98	-	-	[18]		
				1.43%	11.11					
				1.63%	11.59					

				0.84%	11.00			
				1.38%	11.08			
				1.63%	11.60			
				1.41%	11.47			
6	Flower	Extract thick	<i>Staphylococcus aureus</i>	6.25%	-	10.4	-	[19]
				12.5%		14.2		
				25%		20.2		
				50%		28		
			<i>Staphylococcus epidermidis</i>	6.25%	-	12.4	-	
				12.5%		17.4		
				25%		21.4		
				50%		26.6		
			<i>Salmonella thypi</i>	6.25%	-	7.5	-	
				12.5%		11		
				25%		12		
				50%		18		
7	Flower petals	Extract	<i>Staphylococcus aureus</i>	70%	-	14.4	-	[20]
				75%		15.7		
				80%		16.4		
				85%		19.6		
8	Flower petals	Extract Thick	<i>Staphylococcus aureus</i>	20%	-	2.5	2.5	[21]
				40%		2.8	3.1	
				60%		3.1	4.5	
				80%		4.5	5	
9	Flower	Extract	<i>Escherichia coli</i>	1: 1	3.02	3.82	5.43	[22]
			<i>Salmonella typhimurium</i>		2.12	2.42	2.83	
			<i>Enterobacter clocae</i>		3.12	3.22	3.43	
			<i>Klepsiella pneumonia</i>		1.12	1.32	1.83	
			<i>Staphylococcus aureus</i>		3.12	3.42	4.83	
			<i>Yersinia enterocolitica</i>		-	-	1.47	

**DISCUSSION:
ANTIBACTERIAL ACTIVITIES OF ROSELLA
ROOTS AND LEAVES (*Hibiscus sabdariffa* L.)**

For the antibacterial activity of the ethanol extract of roots, leaves, and flowers of the roselle plant (*Hibiscus sabdariffa* L.) against bacteria *Staphylococcus aureus* using the disc diffusion method. According to Greenwood, the classification of the response to bacterial growth inhibition is as follows: inhibition zone diameter <10 mm is categorized as no barrier, inhibition zone 10-15 mm is categorized as weak, inhibition zone 16-19 mm is categorized as moderate and inhibition zone > 20 mm is categorized as strong. The results of the antibacterial inhibition of the ethanol extract of roselle root in the three concentration variations showed that there was a weak antibacterial activity against the growth of *Staphylococcus aureus* bacteria by inhibition of 10.48 mm, 13.2 mm and 13.73 mm. The results of the antibacterial inhibition of the ethanol extract of roselle leaves were moderate with inhibition of 12.4 mm, 16.73 mm, and 21.87

mm and the results of the antibacterial inhibition of ethanol extract of roselle flowers on *Staphylococcus aureus* bacteria were strong with 17.43 mm, 21.6 mm and 24,23 mm.[14]. The inhibition zone is influenced by the content of the active compounds contained in the ethanol extract of the flower, leaf, and root of roselle (*Hibiscus sabdariffa* L.), which among them act as antibacterial agents, namely alkaloids, flavonoids, saponins, and tannins. Where these compounds can inhibit bacterial activity. Alkaloid compounds can interfere with the peptidoglycan components of bacterial cells so that the cell wall layer is not formed intact and causes cell death. Flavonoid compounds are able to inhibit nucleic acid synthesis, disrupt the function of the cytoplasmic membrane, and metabolize bacterial energy. Saponins have hydrophilic and lipophilic molecules so they can reduce the surface tension of cells which can lead to the destruction of bacteria. Tannins can attack cell wall polypeptides, causing cell damage [7]. The higher the extract concentration, the greater the inhibition against bacterial growth. This is indicated by

the larger diameter of the formed resistance zone, which indicates that the activity of the test material against microbes is getting better. [14].

ANTIBACTERIAL ACTIVITIES OF THE ROSELLA FLOWER (*Hibiscus sabdariffa* L.)

Streptococcus mutans is one of the gram-positive bacterial pathogens that causes corrosion of tooth enamel. It can be seen that this extract has a Minimum Inhibitory Concentration (MIC) of 5% and the effective concentration is at a water extract concentration of 45% and 50% roselle. This shows that the water extract of roselle petals can inhibit the growth of *Streptococcus mutans* in vitro. The active substances contained in roselle flower water extract at various concentrations can produce antibacterial effects. This is because the water solvent in this study functions to dissolve the active substances in roselle flower petals in the form of flavonoids and anthocyanins. Anthocyanins can inhibit glucose oxidation and bind to iron, which is needed by bacteria, thus inhibiting bacterial metabolism. The flavonoids in the roselle plant have hydroxyl groups that can cause changes in organic components and nutrient transport, which will result in toxic effects on bacteria. [15].

Roselle flower petal extract is often tested against *Streptococcus mutans* bacteria because this bacteria causes dental caries, which is an infectious disease that damages the tooth structure and causes cavities. Tests were carried out at a concentration of 5%, 10%, 20%, 40%, and 80% of the roselle petal extract with positive and negative controls, namely 0.12% chlorhexidine and aqua dest. At a concentration of 40%, it shows an inhibitory concentration that is close to positive control in inhibiting *Streptococcus mutans* bacteria, so that this roselle petal extract can replace the antibacterial content (chlorhexidine 0.12%) present in some mouthwash products. The formation of an inhibitory zone in the roselle petal extract has something to do with the content of its chemical compounds or secondary metabolites which have an antibacterial effect.[16].

The ethanol extract and water extract of rosella flower petals were tested against *Streptococcus pneumoniae* bacteria, which is one of the bacteria that cause disease in the respiratory tract. The upper respiratory tract is the main gate, through which bacteria enter the body. Common respiratory diseases are influenza, pneumonia, tuberculosis, and chickenpox. Ethanol extract and roselle water extract have the same effectiveness in inhibiting the growth of *Streptococcus pneumoniae* bacteria. At a concentration of 70%, the ethanol extract and roselle aqueous extract had the widest inhibition zone compared to the other extract concentrations. Meanwhile, the concentration of ethanol extract and roselle water had different effects in inhibiting the growth of *Streptococcus pneumoniae* bacteria. Phytochemical test results of ethanol extract and water extract of rosella flower petals contained tannins, saponins, and flavonoids. Tannins and saponins found in plants can act as antibacterial. Flavonoids which are lipophilic in nature may also damage mucous membranes, phenols are very easily absorbed through the tissue. Systemically, phenol stimulates the central nervous system and causes paralysis due to muscle spasms [17].

The potential as a traditional medicine for rosella plants is caused by the active ingredients. Rosella plants contain organic acids, polysaccharides, cardiac glycosides, flavonoids, saponins, tannins, and alkaloids that have medicinal properties. The material used was dried roselle flower with 3 brands (A, B, and C) in circulation. Each brand was tested with different concentrations, brewed using hot water as much as 180 ml or the same as one glass, and let stand for 20 minutes. Testing of roselle infusion water (*Hibiscus sabdariffa* L.) against *Staphylococcus aureus* bacteria showed a zone of inhibition (clear area), which means that the infused water from rosella flowers can inhibit *Staphylococcus aureus* bacteria. According to Elgayar, Plant extracts can be grouped based on the resulting inhibition diameter into three categories, namely high (> 11 mm), medium (> 6 - <11 mm), low (<6 mm). From the results obtained, the rosella flower infusion water can be classified into materials that have a moderate inhibiting ability and can be used as first aid or prevention of diarrhea. [18].

Diseases that are commonly suffered by people are typhus and infections caused by *Salmonella thypi* and skin diseases such as ulcers, acne, and arthritis caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*. These bacteria naturally live on human skin and mucous membranes. The chemical content of the powder and extract of roselle flowers is known to contain flavonoids, saponins, tannins, phenolics, triterpenoids, and glycosides, among which these compounds have antibacterial activity. The extract concentrations used in determining the inhibition zone were 50%, 25%, 12.5% , and 6.25%. This is intended to determine which concentration can provide an inhibition zone for the three bacteria. It was shown that the ethanol extract of roselle flowers had stronger antibacterial power against *Staphylococcus aureus* and *Staphylococcus epidermidis* (gram-positive) than *Salmonella thypi* (gram-negative). At a concentration of 50%, roselle extract can inhibit the growth of the three bacteria compared to other concentrations. In determining the Minimum Inhibitory Concentration (KHM) starting from a concentration of 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, and 3% are intended to know the lowest concentration of the solution that can inhibit bacteria where at that value there is no more bacterial growth. Of the three bacteria, *Staphylococcus aureus* gave the highest inhibition zone diameter (28 mm) with a MIC value of 5% to inhibit bacterial growth compared to *Staphylococcus epidermidis* (26, 6 mm) with a MIC value of 4% and *Salmonella thypi* (18 mm) with a MIC value of 7%. [19] *Staphylococcus aureus* is the main cause of festering infections in humans that are found in the nasal cavity and skin of most of the human population. It can be seen that the ethanol extract of rosella flower petals (*Hibiscus Sabdariffa* L.) can inhibit the growth of *Staphylococcus aureus* bacteria and the inhibition zone results are obtained with a strong category for a concentration of 70%, strong for a concentration of 75%, strong for a concentration of 80% and strong for a concentration. 85%. The greater the concentration of roselle flower petal extract, the greater the concentration of the inhibition zone formed in the growth of *Staphylococcus aureus* bacteria. The formation of a clear area

or what is called the inhibition zone around the disc paper indicates the inhibition of bacterial colony growth due to the influence of compounds contained in the ethanol extract of roselle flower petals (*Hibiscus Sabdariffa* L.). Roselle flower petals are known to have antibacterial properties. The content contained in roselle flower petals is phenol or polyphenol compounds, saponins, and tannins, which are included in the flavonoid class. [20]

Roselle ethanol extract against *Staphylococcus aureus* bacteria using 30% ethanol solvent and 96% ethanol solvent to compare the antibacterial activity. *Staphylococcus aureus* bacteria are the main pathogens for humans, are gram-positive bacteria, which are found on the skin, nose, mouth, mucous membranes, ulcers, and wounds. The treatments used were ethanol extract concentrations of 30% and 96% of roselle petals with concentrations of 20%, 40%, 60%, and 80%, respectively. Antibacterial activity of 30% ethanol extract and 96% of roselle petals (*Hibiscus sabdariffa* L) against *Staphylococcus aureus* bacteria showed growth-stopping activity. This can be seen from the results of measuring the width of the inhibition area that is formed in the form of a clear area around the disc paper containing ethanol extract of roselle flower petals. The highest concentration was in the ethanol extract of 96% roselle petals (*Hibiscus sabdariffa* L) with an average width of the inhibition area of 5 mm while in the ethanol extract of 30% the average width of the inhibition area was 4.5 mm. The ratio of the width of the inhibition area produced by the ethanol extract of 96% roselle flower petals was directly proportional to the addition of the concentration and resulted in a larger inhibition area width than the 30% ethanol extract. This shows that the ethanol extract of 96% roselle petals is more active in killing *Staphylococcus aureus* bacteria colonies compared to the ethanol extract concentration of 30%. The difference in the killing power of the ethanol extract of 30% and 96% of roselle flower petals was probably due to the difference in the active components absorbed in the extraction process. The ethanol extract of 30% and 96% of rosella flower petals can kill bacteria due to the presence of chemical compounds contained in the ethanol extract of roselle flower petals such as anthocyanin compounds which are flavonoids compounds. [21]

The antibacterial activity of roselle extract (*Hibiscus sabdariffa* L.) was carried out on several bacteria, namely *Escherichia coli*, *Salmonella typhimurium*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Yersinia enterocolitica*. Using solvents, namely water, and 96% ethanol. The test results showed that the increase in inhibitory value occurred when using 96% ethanol in *Escherichia coli* with an average value of 5.43 mm followed by *Staphylococcus aureus* with a value of 4.83 mm. However, *Yersinia enterocolitica* has the smallest inhibitory value, which is only 1.47 mm. In the use of water solvents, the inhibition of bacteria is low. The difference in inhibition value could be caused by the concentration of the extract, the solvent used and the resistance of the bioactive compounds of each bacterium studied. [22]

CONCLUSION:

The Roselle plant (*Hibiscus sabdariffa* L.) is a medicinal plant that has antibacterial properties by using various concentrations and having different inhibition diameters.

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