

## A Review: Antibacterial Activity of Roselle (*Hibiscus sabdariffa*) Calyx Extract against Pathogenic Bacteria

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### **Abstract:**

**Background:** Roselle (*Hibiscus sabdariffa*) contains secondary metabolites that have an antibacterial activity such as tannins, alkaloids, and flavonoids. Generally, the compounds found in Roselle that exhibit antibacterial activity are polyphenolic compounds, some of which have also been shown to exhibit antioxidant activity. These compounds are delphinidin-3-sambubiside and cyanidin-3-sambubioside. The purpose of this review article is to determine the potential of Roselle calyx extract as an antibacterial.

**Methods:** In this article review uses literature studies from various international journal sources in the last 10 years (2010-2020). The search keywords "Hibiscus sabdariffa" and "Antibacterial Activity of Hibiscus Sabdariffa Extract".

**Results:** Roselle (*Hibiscus sabdariffa*) calyx extract has antibacterial activity against Gram-positive and Gram-negative bacteria. The antibacterial activity of Roselle extract can be seen from the diameter of the inhibition formed.

**Conclusion:** Antibacterial activity of Roselle calyx extract was influenced by the solvent and the concentration used. More polar solvents have better antibacterial activity than semi-polar and non-polar solvents.

**Key Word:** Roselle, extract, antibacterial, bacteria

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### I. Introduction

Herbalism (herbal medicine) is the use of plants for medical purposes and botanical studies. Plants have been the basis of medical medicine based largely on human experience and their use is still widely practiced until now (1). One example is the use of herbal products as antibacterial agents which are the best alternative in the unwise use of antibiotics. Therapy using herbal products is increasing in both developing and developed countries. This increase occurred due to the recognition that herbal products are natural products, non-narcotics, and easily biodegradable, produce minimum environmental hazards, have no adverse side effects, and are easily available at affordable prices (2).

Roselle (*Hibiscus sabdariffa* L.) is a plant that belongs to the kingdom Plantae, division Magnoliophyta, class Magnoliopsida, order Malvaceae, family Malvaceae, genus Hibiscus. (3). Roselle is a plant that has long been used as herbal medicine and cultivated throughout the world, especially in tropical and subtropical areas, Africa and Asia. Roselle is a wood-based perennial or shrub with serrated leaves and red calyx (4). This plant can be found in almost all tropical countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt, and Mexico (5). This plant is known as *Roselle* (UK), *Loisoi* (France), *Karkade* (Arabic), and *Bissap* (Wolof). In some countries, Roselle is used for decorative purposes, the seeds and calyx are used for human consumption. Roselle is consumed in the form of tea, sauce, and jam or syrup (6).

Roselle is a woody perennial or sub-shrub, growing to 2-2.5 m tall. The leaves are very wide 3-5 lobes and 8-15 cm long, arranged alternately on smooth red cylindrical stems. The flowers are 8-10 cm in diameter and have fat fleshy calyxes at the base, 1–2 cm wide, enlarge to 3–3.5 cm, fleshy and bright red when the fruit is ripe. Roselle fruit takes about six months to ripen. Roselle is cultivated at the beginning of the rainy season, during mid-April and the calyxes are harvested from the fruit, after 3 weeks from the start of flowering. (7).

Rosella plant is rich in various secondary metabolites such as tannins, alkaloids, and flavonoids, which have been proven in vitro to have antibacterial properties. Generally, the compounds found in roselle calyx that exhibit antimicrobial activity are polyphenol compounds, some of which have also been shown to exhibit antioxidant activity. One of the polyphenol compounds present in Roselle calyxes extract are flavonoids, which include plant pigments called anthocyanins. The two anthocyanins that have the highest amounts have been identified in Roselle as Delphinidin-3-sambubiside and Cyanidin-3-sambubioside, which are responsible for the

dark red pigment of the calyx and are also found to be major contributors to antioxidant activity. Other compounds found in Roselle calyx are phenolic acids such as gallic and protocatechuic acids (8).

The dried flowers of the Roselle plant contain Gossipetine and Hibiscin (anthocyanins); the calyx produce glucoside hibiscritin (flavanol); and rich in riboflavin, ascorbic acid, niacin, carotene, calcium and iron. Roselle is an important source of vitamins, minerals and bioactive compounds, such as organic acids, phytosterols, and polyphenols, some of which have antioxidant effects. The phenolic content in this plant consists of anthocyanins such as Delphinidin-3-glucoside, Sambubioside, and Cyanidin-3-sambubioside; other flavonoids such as Gossypetin, Hibiscetin, and glycosides; Protocatechuic acid, eugenol, and sterols such as  $\beta$ -cytoesterol and ergosterol (9).

There have been several reports of the antibacterial activity of different herbal extracts, the herbal product Roselle which is commonly used to make cold or hot drinks, is also used in traditional medicine for hypertension, liver disease, fever (10), diuretics, laxatives, and medications for heart and nerve disease and cancer (11). Hibiscus anthocyanin is a natural phenolic pigment found in dried *H. sabdariffa* flowers, which has cardioprotective, hypocholesterolemic, antioxidant, and hepatoprotective effects. (12). *H. sabdariffa* has been reported to be antiseptic, astringent, diuretic, emollient, laxative, sedative, stomachic, and tonic. Roselle is also a traditional medicine for abscesses, bilious conditions, cancer, coughs, and scurvy as well as anti-cancer properties (13).

*H. sabdariffa* extract has been shown to have antibacterial activity against many strains of bacteria. Methanol extract from Rosella petals has been shown to have antimicrobial activity against *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia marcescens*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, dan *Pseudomonas* sp. (14) at a concentration of  $0.30 \pm 0.2$  to  $1.30 \pm 0.2$  mg / mL. The MICs for the aqueous calyx extract of *H. sabdariffa* were 0.5 and 1.0 mg / mL for *S. aureus* ATCC 6358 and *E. coli* ATCC 8937 (8).

## II. Method

The preparation of this review article is based on literature studies by searching for sources or literature in the form of primary data in the form of official books and international journals for the last 10 years (2010-2020). The search for data in this review article uses online media through trusted sites such as Google Scholar, Scencedirect, and NCBI with the search keywords "*Hibiscus sabdariffa*" and "Antibacterial Activity of *Hibiscus Sabdariffa* Extract".

## III. Result

The data obtained from the literature show that Roselle extract has antibacterial effects against several bacteria, both Gram-positive and Gram-negative bacteria in various solvents and concentrations. The antibacterial effect of Roselle extract can be seen from the diameter of the inhibition formed. The following are the search results for a literature study on the antibacterial effects of Roselle extract :

**Table 1.** Inhibition diameter of Rosella calyx extract

No	Bacteria	Concentrations	Extract	Inhibition Diameter (mm)	Reference
1.	<i>Pseudomonas aeruginosa</i> <i>Klebsiella pneumoniae</i> <i>Staphylococcus aureus</i> <i>E.coli</i>	250 mg/ml	Ethanol	29 mm 33 mm 22 mm 23 mm	(10)
2.	<i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	250 mg/ml	Water	13.55 mm 11.61 mm 8.45 mm	(14)
		500 mg/ml	Water	17.47 mm 15.34 mm 9.52 mm	
3.	<i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Streptococcus mutans</i> <i>Pseudomonas aeruginosa</i>	10 mg/mL	Ethanol	46 mm 20 mm 30 mm 17 mm	(9)
			Water	40 mm 40 mm 28 mm 27 mm	
4.	<i>Corynebacterium diphtheria</i> <i>Proteus mirabilis</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Serratia marcescens</i> <i>Enterococcus faecalis</i>	100 mg/ml	Methanol	26 mm 14 mm 23 mm 24 mm 17 mm 33 mm	(11)

	<i>Listeria monocytogenes</i> <i>Escherichia coli</i> <i>Escherichia coli</i> (ATCC 25922) <i>Klebsiella pneumoniae</i> (ATCC 70063) <i>Klebsiella pneumoniae</i> <i>Pseudomonas aeruginosa</i> (ATCC 27853) <i>Proteus vulgaris</i> <i>Bacillus cereus</i>			36 mm 20 mm 19 mm 25 mm 18 mm 28 mm 19 mm 28 mm	
5.	<i>Corynebacterium diphtheria</i>	800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup> 100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>	Ethanol	17 mm 12 mm 12 mm 9 mm 8 mm - -	(15)
800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup> 100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>		Water	15 mm 16 mm 16 mm - - - -		
<i>Staphylococcus aureus</i>	800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup> 100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>	Ethanol	21 mm 18 mm 16 mm 9 mm 8 mm 7 mm 7 mm		
	800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup> 100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>	Water	17 mm 12 mm 12 mm 8 mm 8 mm - -		
<i>Staphylococcus capitis</i>	800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup> 100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>	Ethanol	19 mm 15 mm 14 mm 9 mm 8 mm 7 mm -		
	800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup> 100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>	Water	16 mm 15 mm 10 mm 9 mm 7 mm 7 mm 7 mm		
<i>Pseudomonas aurogenosa</i>	800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup> 100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>	Ethanol	17 mm 10 mm 10 mm 9 mm 7 mm 7 mm -		
	800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup> 100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>	Water	16 mm 10 mm 7 mm 8 mm 7 mm - -		
<i>Protus merabeles</i>	800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup> 100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>	Ethanol	15 mm 16 mm 14 mm 9 mm - - -		
	800 mg/c <sup>3</sup> 400 mg/c <sup>3</sup> 200 mg/c <sup>3</sup>	Water	13 mm 13 mm 12 mm		

		100 mg/c <sup>3</sup> 50 mg/c <sup>3</sup> 25 mg/c <sup>3</sup> 12.5 mg/c <sup>3</sup>		9 mm 8 mm 8 mm -	
6.	<i>S. Typhimurium</i> Z1 <i>S. Typhimurium</i> Z2 <i>S. Typhimurium</i> Z3 <i>S. Typhimurium</i> Z4 <i>S. Typhimurium</i> Z5 <i>S. Montevideo</i> Z6 <i>S. Gaminara</i> Z7 <i>S. Typhi</i> Z8 <i>S. Typhimurium</i> Z9 <i>S. Typhimurium</i> Z10 <i>S. Typhimurium</i> Z11 <i>S. Typhimurium</i> Z12 <i>S. Typhimurium</i> Z13	50 mg/mL	Methanol	12±0.2 mm 12±0.4 mm 12±0.2 mm 12±0.2 mm 12±0.3 mm 13±0.2 mm 12±0.2 mm 13±0.2 mm 11±0.2 mm 10±0.2 mm 12±0.2 mm 12±0.3 mm 11±0.4 mm	(16)
			Water	10±0.4 mm 10±0.6 mm 10±0.6 mm 10±0.6 mm 10±0.8 mm 10±0.5 mm 10±1.0 mm 10±0.4 mm 10±0.8 mm 10±0.4 mm 10±0.6 mm 10±0.8 mm 10±0.8 mm	
7	<i>Streptococcus mutans</i> <i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i>	1000 µg/mL 500 µg/mL 250 µg/mL	Methanol	- 9 mm 9 mm 9 mm - - 6 mm 7 mm - - 6 mm -	(17)
8	<i>A. baumannii</i> Ab1 Ab2 Ab3 Ab4 Ab5	10 mg/disc	Methanol	11.3±0.3 mm 13.6±0.3 mm 11.6±0.3 mm 13.3±0.3 mm 13.3± 0.3 mm	(18)
9	<i>Bacillus subtilis</i> (ATCC 6633) <i>Staphylococcus aureus</i> (ATCC 6538) <i>Escherichia coli</i> (ATCC 8739)	25 mg/mL 50 mg/mL 100 mg/mL 200 mg/mL	Ethanol Water Ethanol Water Ethanol Water Ethanol Water	10.70 ± 0.87 mm 18.37 ± 0.60 mm 11.73 ± 0.61 mm 10.40 ± 0.00 mm 16.90 ± 0.20 mm 13.63 ± 1.00 mm 12.37 ± 0.51 mm 24.70 ± 3.60 mm 13.63 ± 0.74 mm 11.97 ± 1.67 mm 22.53 ± 1.18 mm 13.93 ± 0.60 mm 15.17 ± 1.04 mm 30.23 ± 1.01 mm 17.90 ± 0.92 mm 13.27 ± 0.83 mm 27.63 ± 1.42 mm 15.37 ± 1.23 mm 18.67 ± 1.10 mm 34.17 ± 1.82 mm 23.93 ± 0.47 mm 18.17 ± 0.74 mm 32.20 ± 0.26 mm 19.97 ± 1.31 mm	(19)
10	<i>Staphylococcus aureus</i>	5 mg/ml	Methanol	16±0.5 mm	(20)

	<i>Bacillus subtilis</i> <i>Escherichia coli</i> <i>Klebsiella pneumonia</i>		18±1.0 mm 13±0.5 mm 11±0.6 mm
		Water	16±0.6 mm 18±0.5 mm 13±0.3 mm 11±0.3 mm

#### IV. Discussion

In Table 1. it can be seen that from the references obtained, almost all Roselle extracts used have antibacterial activity. In general, the antibacterial activity of Roselle extract was highest in ethanol extract. The highest inhibition of Roselle extract was in the *Escherichia coli* bacteria with an inhibitory diameter (46 cm) and a concentration of 10 mg / mL.

The antibacterial activity of *Hibiscus sabdariffa* extract can be related to the chemical compounds it contains, especially those that are phenolic such as gallic acid, quercetin, routine, luteolin, and analogs detected in it (10). Roselle calyx contains many compounds that have antimicrobial activity, such as organic acids, phenolic acids, alkaloids, and anthocyanins. Gossypetin and protocatechuic acid are two compounds found in *H. sabdariffa* that have been shown to have antimicrobial activity (21). The antimicrobial activity of Roselle extract is related to flavonoid compounds because it can form complexes with bacterial cell walls and the permeability of the bacterial cell surface to the extract. The mechanisms of action that occur include inhibition of translocation of electron transport proteins, phosphorylation, and other enzyme reactions followed by increased plasma membrane permeability, which results in ion leakage from bacterial cells. (7).

Solvents and extraction procedures are important things to consider in evaluating the antibacterial effect of a plant compound. Types of solvents for example, methanol, ethanol, hexane, acetone, water, chloroform, and others are known to affect the antimicrobial effect of plant extracts due to differences in polarity between solvents extracts obtained with polar solvents (methanol and water) showed relatively better antibacterial activity than semi-polar (ethyl acetate) and non-polar (petroleum ether) extracts (22). In addition, the antibacterial test method affects. Where the open well diffusion method is more sensitive which can be seen from the wider zone of inhibition for most of the test organisms (23).

Different extraction methods can be applied to obtain Roselle extract. Variations and extraction conditions used (the type of solvent, concentration, time, and temperature) have the potential to affect the polyphenolic compounds from the extract. (24). The difference observed for antimicrobial activity using the hydroethanol extract and the infusion could be attributed to the different concentrations of phenolic compounds found in the two extracts, which can be ascribed to the different polarity of the solvents used to obtain the extracts. Besides, in general the antibacterial and antifungal properties show the same concentration range of inhibition (25). Antibacterial test results showed that alcohol is a better solvent than water for the extraction of phenolic compounds from the calyx of *H. sabdariffa* L. The solubility of phenolic compounds is regulated by the chemical properties of the plant, as well as the polarity of the solvent used. Ethanol is a good solvent for polyphenol extraction and is safe for human consumption (9).

The results of the current study clearly indicate that the aqueous and alcoholic extracts of *H. sabdariffa* L. inhibited the growth of the test microorganisms, however, the effect varied depending on the test microorganisms. The results of the study are following Olaleye (2007) that methanol aqueous extract from *H. sabdariffa* calyx has been found to show antibacterial activity against *S. aureus*, *B. stearothemophilus*, *Micrococcus luteus*, *Serratia mascentes*, *Clostridium sporogenes*, *E. coli*, *Klebsiella pneumoniae*, *B. cereus dan P. fluorescence* (9). Similar reports of the antimicrobial activity of *H. sabdariffa* demonstrated various inhibitory effects against Gram-positive and negative bacteria. Gram-positive bacteria are less sensitive to extracts than Gram-negative bacteria. This is presumably due to differences in the composition of the bacterial cell wall (26).

The results of the *H. sabdariffa* calyx extract antibacterial test provide information regarding the potential of this plant as a safe and edible antibacterial agent candidate. Current research strongly supports the use of *H. sabdariffa* calyx in the treatment of coughs, abscesses, and bilious conditions especially those caused by microbial infections. However, further research is needed to ascertain the in vivo antibacterial effect of Roselle extract (10). The use of Roselle calyx is generally considered safe and has even been approved as a coloring agent by the U.S. Food and Drug Administration. (21 CFR 172,510). In addition, Roselle extract also has a very low level of toxicity and the lethal dose of 50% Roselle calyx extract in mice is above 5,000 mg/kg. (27).

#### V. Conclusion

Roselle contains many secondary metabolite compounds that have antibacterial activity. Roselle calyx extract has excellent antibacterial potential against Gram-positive and Gram-negative bacteria, so it can be developed as an alternative to herbal products in overcoming the problem of antibiotic resistance that occurs.

The antibacterial activity of Roselle calyx extract was influenced by the solvent and the concentration used. More polar solvents have better antibacterial activity than semi-polar and non-polar solvents.

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