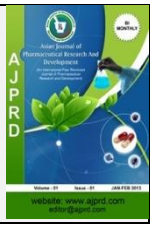
iAvailable online on 15.08.2021 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research



Open Access

Review Article

Potential of Piperaceae Plants as Antibacterial against *Staphylococcus aureus* and *Escherichia coli*: A Review

Hottria Magdalena, Anzharni Fajrina*, Aried Eriadi, Ridho Asra

Sekolah Tinggi Ilmu Farmasi (STIFARM) Padang, Indonesia 25147

A B S T R A C T

Background: Infectious disease was one of the most common health problems in developing countries which are caused by microorganism, such as bacteria. Piper (Piperaceae) was a medicinal plant that has potential as an antibacterial. Some plants of the piperaceae family have antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, such as *Piper cubeba*, *Piper nigrum*, *Piper betle*, *Piper crocatum*, *Piperomia pellucida*, *Piper longum*, *Piper sarmentosum*, and *Piper aduncum*.
Purpose: This article described plant of the Piperaceae family that has antibacterial potential. This article reviewed the study of the antibacterial potential of the Piperaceae plant from various articles.
Data source: The database sources used include Google Scholar, Science Direct, and Media Published from 2010 to 2021.
Conclusion: It carried out by reviewing several libraries such as indexed national journals, international journals, and scientific articles regarding the potential of the Piperaceae plants as antibacterial against *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity of the Piperaceae plant originated from secondary metabolites of these plants including alkaloids, tannins, saponins, phenolic compounds, and flavonoids.

Keywords: Piperaceae, Antibacteria, *Staphylococcus aureus*, *Escherichia coli*

A R T I C L E I N F O: Received 23 March 2021; Review Complete; 15 July 2021 Accepted; 03 August 2021 Available online 15 August 2021

Cite this article as:

Magdalena H, Fajrina A*, Eriadi A, Asra R, Potential of Piperaceae Plants as Antibacterial against *Staphylococcus aureus* and *Escherichia coli*: A Review, Asian Journal of Pharmaceutical Research and Development. 2021; 9(4):83-100.
DOI: http://dx.doi.org/10.22270/ajprd.v9i4992

***Address for Correspondence:**

Anzharni Fajrina, Sekolah Tinggi Ilmu Farmasi (STIFARM) Padang, Indonesia.

INTRODUCTION

Infectious disease is one of the most common health problems in developing countries [1]. The way to deal with bacterial infection is with antibiotic. Rational application of antibiotic is expected to reduce morbidity, mortality, economic losses, and reduce bacterial against antibiotic. The irrational of using antibiotic in various fields of medical science is one of the causes of acquired Resistance[2].Some microbiologists found alternatives to new antimicrobial medicine that can cure infectious diseases. New alternative antimicrobial medicine can be sourced from nature, which is by utilizing secondary metabolite compounds derived from plant [3]. Secondary metabolite compounds derived from plant has antibacterial effects including phenol, quinone, flavonoid, tannin, terpenoid, alkaloid, and other compounds such as stilbenoid, lectin and polypeptide [4]. These compounds have the potential as natural antibacterial against pathogenic bacteria, for example against *Staphylococcus aureus* and *Escherichia coli* [5]. Example of gram-positive bacteria is *Staphylococcus aureus*, these bacteria is one of the bacteria that causes the most common infections in the world. The severity of infection also varies, ranging from minor skin infection (furunculosis and impetigo), urinary tract infection, respiratory tract infection, eye infection and the Central Nervous System (CNS) [6]. *Escherichia coli* is a Gram-negative bacterium, rod-shaped (2.0–6.0 mm length and 1.1–1.5 mm wide) with rounded tips. Some *Escherichia*

coli serogroup are known to be mostly non-pathogenic, however, some groups can cause severe, sometimes fatal diarrheal disease [7]. Some of the clinical syndromes due to *Escherichia coli* infection are urinary tract infection, meningitis, septicemia and diarrheal diseases [8].

The Piperaceae family is reported to have antimicrobial effects. Family Piperaceae is a plant that is often used in traditional medicine by people such as thrush medication, cough, and traditional antiseptics. These tribes contain alkaloid/amides, lignans, neolignans, and terpenoids, some alkaloids derived compounds that have the potential as an antimicrobial [3]. Family Piperaceae or pepper contain about 3600 species in 13 general, mainly divided into two main types, namely Piper (2000 species) and *Peperomia* (1600 species) [9].

Traditionally, the Piperaceae plant has been used to treat various diseases such as respiratory problem, seizure, pain and rheumatism, diabetes, infection, malaria, gynecological disorder and inflammation [9]. Some plant parts used as an antibacterial Piperaceae include *Piper cubeba* [10], *Piper nigrum* [11], *Piper betle* [12], *Piper crocatum* [13], *Piperomia pellucid* [14]. Some plants with the Piperaceae family that have been studied and used as antibacterial include water overlap (*Piperomia pellucida*), red betel (*Piper crocatum*), betel (*Piper betle*), cubeb (*Piper cubeba*), Javanese chili (*Piper longum*), pepper (*Piper nigrum*), forest betel (*Piper aduncum*) and karuk leaf (*Piper sarmentosum*).

The cubebin plant (*Piper cubeba*) contains the main compound in the form of cubebin. Cubebin compounds from the Piper cubeba plant have anti-inflammatory, antiparasitic, antibacterial, anti-tumor, and erectile dysfunction effects [15]. In India and China, the *Piper cubeba* plant used to treat various bacterial infections such as dysentery, diarrhea, gonorrhea and syphilis. In addition to treating infectious diseases in bacteria, cubeb also has antiviral activity, where this plant can inhibit the activity of the hepatitis C virus [16].Based on the benefits of plants with the Piperaceae family against various types of bacteria, especially *Staphylococcus aureus* and *Escherichia coli*, a study is needed which aimed to provide information about the antibacterial activity of plants with the Piperaceae family against *Staphylococcus aureus* and *Escherichia coli* bacteria and it is hoped will be used as an alternative treatment material for improve the health and quality of life, especially in the prevention of infectious disease by minimizing the use of antibiotics. Thus far, no comprehensive literature was available on its use as an antibacterial. Therefore, it is important to preserve valuable herbal knowledge that could be of use for future medicine discovery efforts. The purpose of this article was to gather evidence of the extensive literature studies reported antibacterial activity of the Piperaceae plant.

METHOD

This article reviewed the study of the antibacterial effect of plant with the Piperaceae family from various articles. The database sources used include Google Scholar, Science Direct, and Media Published from 2010 to 2021. In this article conducted by reviewing several literatures such as indexed national journals, international journals, and scientific articles on the potential of the Piperaceae plants as antibacterial against *Staphylococcus aureus* and *Escherichia coli*. There were no restrictions according to the language used or the type of publication. This article reviewed the antibacterial effect of plant with the Piperaceae family, so the data taken included the method used, the zone of inhibition, the minimum inhibitory concentration and the minimum bactericidal concentration.

The Piper genus in the taxonomy of plants was classified as follows[9] :

Kingdom : Plantae
Subkingdom : Tracheobionta
Superdivision : Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Magnoliidae
Order : Piperales
Family : Piperaceae
Genus : Piper

Piperaceae Morphology

Piper (Piperaceae) is an aromatic plant which is usually used as a medicinal plant and ornamental plant [17]. Piperaceae consist of plant, shrub, vine, or tree. The leaves are spiral-shaped, simple, firm (speckled against the petiole) or exstipulate. The inflorescence is nail or hilt. The flower is very small, bisexual or unisexual, actinomorphic, bracteate with peltate bract and hypogynous. The gynoecium consists of a single pistil with a superior ovary, having 1 or 3–4 carpels and 1 locule. The seed is starchy perisperm (slightly endosperm). This plant has round, aromatic (smooth) oil cells in the parenchyma and blood vessel similar to atactostel (but with an outer cambium) [18]. The morphology of the piperaceae family is a group of dicot plant. In general, the piperaceae family is characterized by a tap root system, characterized by a twisted stem (vobubilis) accompanied by a seemingly knotty (nodes) on the stem accompanied by tendril, but there are also herbaceous stem that grow upright with monopodial branching. The characteristics of the Piperaceae plant was single leaf with alternating leaves that grow on each leaf. Part of curved leaves with edges leaf is usually wavy and flat. Another feature if flowering is in the Plagiotrophic (horizontal) branches arranged in spikes (spica) or strands (amentum) [19]. The Piperaceae or Pepper contains about 3600 species in 13 general, mainly divided into two main types namely Piper (2000 species) and *Peperomia* (1600 species) [9]. Some of the morphology of the Piperaceae family:

The cubeb plant (*Piper cubeba*) has a rounded character segmented rod, 4-12 m length. The root of this plant is in the form of sticky roots and makes cubeb to grow sticky or vine. The leaves on the cubeb plant are single-stemmed leaves with tapered leaf tips. The leaf has 8-15 cm length

and 3-5 cm width. The cubical plant has compound interest. The stalk fruit has 2-3 cm length with light green round fruit shape [20].

Peperomia pellucida is characterized by a smooth leaves, heart-shaped fleshy, juicy stem, shallow root and small flower, which eventually grew into a small seed spikes stuck to it like a cable [21].

Piper crocatum (red betel) is a vine or creeper, can reach about 5-10 m length, round stems, red-purple green, segmented with internodes 3-8 cm, each book grows one leaf. Single leaf, stiff, sitting alternating leaves, leaf shape hanging - rounded eggs - oval, flat upper leaf blade surface - slightly convex, shiny, curved lower leaf surface with prominent leaf repetition, 6.1–14,6 cm length leaf, 4–9.4 cm width leaf, base color of leaf is green on both surfaces, green top with reddish pink stripes, underside is green dark red-purple. The stalk is green red-purple, 2.1–6.2 cm length, the base of the stalk on the leaf blade slightly to the middle of about 0.7–1 cm from the bottom edge of the leaf [22].

Piper betle (betel green) is a plant that can grow spread on another tree using root sticking on stem segment. Green betel stem has a greenish brown color with a round shape, edged, and at each segment to grow one leaf. The length of the trunk can reach 6-15 m. Green betel leaf in the form of single leaves are heart-shaped, shiny surface, the tip of a pointed leaf, sits leaves alternate between segments of the stem, can reach 6-12 cm in length and 5-9 cm in width. Betel flower is a compound flower with a small round shape and a bow. There is an elliptical protective leaf that serves to protect the flower. The fruit is hidden, small round shape, fleshy, and a yellowish green color [20].

Piper longum (Chili Jawa) has round stems and can reach 5-10 m in length. The color of the Javanese chili is red when ripe or green to yellowish when young. The fruit has 3.5 - 4 cm length, with a long round shape. The leaves are dark green, 12-14 cm length, 4-5 cm width. Javanese chili leaves are round and elongated, tapered leaf tips, rounded leaf base, flat leaf edge and slightly shiny leaf surface [20].

Piper nigrum (Pepper or pepper) has a slightly flattened and segmented stem with a length about 4-7 cm, and the stem can reach 6-14 m length. On the stem segment, the roots are attached to object and there is a leaf of each book. Pepper leaves are single, 10-14 cm length, 5-9 cm width and 3-5 cm stalk. Pepper leaves are oval, tapered leaf tips, curved, and the leaves are alternately located. Flowers on pepper plants are compound flowers and usually grow in the armpit of the leaves. The seed is brownish with a diameter of 3-5 mm and are inside the pulp [20].

Piper sarmentosum is a dioecious plant, usually growing as a small shrub sometimes climbing to as high as 30 cm. Leaf stalk 1-2.5 cm in length and the shape and size of the leaves varies; thin to thick leaf, light green to dark, ovate wide to elliptical with a length of 7.5-95 cm and a width of 4.5-6 cm, present with an aromatic smell and pungent taste. Plant ferns grow with male and female flowers perpendicular to cylindrical with a length 1-1.5 cm, diameter 0.3-0.5 cm. These fruit has 1-2 cm length and 0.5-1 cm in diameter [23].

Piper aduncum has the shape of a woody stem, ovate, pointed tip, rounded base, flat edge of each book, fuzzy stalk, 5-10 mm cylindrical, leaf has 10-14 cm length, 5-6 cm width, finger repetition, and light green. Compound leaves, grain shape, one or two sexually, protective leaves with ½ - 1 ¼ mm stem, curved, short stamens, small anthers, seated ovaries, two to three pistils, short white, yellowish white. Buni flower, short-stemmed, 12-14 cm length, greenish yellow after dark green. Fruit is boxy, round, flat, ± 2 mm in diameter, purplish green. Small seed is brown. Brownish white taproot [24]. Some of the piperaceae plant species can be seen in Figure.

(a) (b)

Figure 1: Species of Piperaceae a. *Piper aduncum*, b. *Piper sarmentosum*[17]

Chemical Content of Piperaceae species

Piperomia pellucida contains active compounds such as secondary metabolites: alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides, reducing sugars, anthraquinones, carbohydrates, resins and cardiac glycosides [14]. *Piper crocatum* is an herbal medicinal plant with a large number of benefits [25]. *Piper crocatum* contains various secondary metabolites such as alkaloids, phenolic compounds, monoterpenes, sesquiterpenes, monolignans, and flavonoid C-glycosides [26]. Flavonoid compound has antimicrobial activity [27]. The secondary metabolites content of the *Piper cubeba* plant include alkaloids, glycosides, tannins, and flavonoids contained in the crude ethanol extract of *Piper cubeba* [28].

The secondary metabolites content of the *Piper longum* plant are alkaloids, steroids, flavonoids, anthraquinones, glycosides, cardiac gycosides, and essential oils [29]. The secondary metabolites found in *Piper nigrum* include tannins, alkaloids, cardiac glycosides, and flavonoids [11]. *Piper nigrum* is a plant in the piperaceae family with the main ingredient being piperine, cubebine, eugenol, and piperacide. Cubebein compound has an antibacterial effect. In addition, the *Piper nigrum* plant also has other compounds contained in *Piper nigrum* extract, namely alkaloids, flavonoids, anthraquinones, reducing sugars, tannins, saponins, and terpenoids, where these compounds has antibacterial activity against gram-positive and gram-negative bacteria, especially against *Saphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa* [16]. Another study also reported that the *Piper nigrum* plant is rich in biologically active compounds such as monoterpenes, sesquiterpenes, and other volatile compounds [30].

The content of *Piper betle* includes tannins, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, proteins and saponins [31]. *Piper sarmentosum* is stated to contain glycosides, flavonoids, terpenoids, alkaloids, and phenolics [32]. *Piper aduncum* also contains essential oils, phenolic compounds, flavonoids, and sesquiterpenes[33].

Testing Antibacterial ActivityMethod

One of the methods of testing the antibacterial activity of the articles reviewed was the diffusion method. The diffusion method was the most widely used method, this was possible because the diffusion method was easy to do because it does not have special tools and includes greater flexibility in choosing the medicine to be examined [34], another advantage was that the resulting diameter of the inhibition was clearer and the test compound direct contact with the media so it diffused quickly into the agar medium, while the disadvantage was the concentration of the test compound required was large and cannot determine the minimum kill concentration [35]. This diffusion method was used to determine the sensitivity of the test microbes to antimicrobial agents [34].

Table: 1 Inhibition zone diameter criteria

No	Criteria	Inhibition zone diameter
1	Weak	<5 mm
2	Mediate	6-10 mm
3	Strong	11-20 mm
4	Very strong	>20 mm

Source: [36]

Potential Antibacterial Activity of Piperaceae Plants against *Staphylococcus aureus* and *Escherichia coli*

Plants with the piperaceae family are widely used as antibacterial. Several studies have shown that Piperaceae plants have antibacterial effects, both gram-positive bacteria such as *Staphylococcus aureus* and gram-negative ones such as *Escherichia coli*. The results of testing the antibacterial activity of the Piperaceae plants as antibacterial against *Staphylococcus aureus* and *Escherichia coli*, which are shown in the table below as follows:

Table 2: Results of Testing the Antibacterial Activity of Several Plants of the Piperaceae against *Staphylococcus aureus* and *Escherichia coli*

Types of plants	Part of plants used	Method	Solvent	Concentration	Inhibition zone diameter	Bacteria	Respond of inhibition zone diameter	Reference
Piperomia pellucida	Leaves	Agar Diffusion	Crude Methanol fractions	200 mg/ml	10 mm	*Staphylococcus aureus*	Mediate	[14]
					10 mm	*Escherichia coli*	Mediate	
				100 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				50 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				25 mg/ml	-	*Staphylococcus aureus*	Null	

					-	*Escherichia coli*	Null	
				12,5 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				6,25 mg/ml	-		Null	
					-		Null	
			n-hexane fractions	200 mg/ml	16 mm	*Staphylococcus aureus*	Strong	
					16 mm	*Escherichia coli*	Strong	
				100 mg/ml	14 mm	*Staphylococcus aureus*	Strong	
					14 mm	*Escherichia coli*	Strong	
				50 mg/ml	12 mm	*Staphylococcus aureus*	Strong	
					12 mm	*Escherichia coli*	Strong	
				25 mg/ml	10 mm	*Staphylococcus aureus*	Mediate	
					10 mm	*Escherichia coli*	Mediate	
				12,5 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				6,25 mg/ml	-		Null	
					-		Null	
			Ethyl acetate fractions	200 mg/ml	14 mm	*Staphylococcus aureus*	Strong	
					10 mm	*Escherichia coli*	Mediate	
				100 mg/ml	12 mm	*Staphylococcus aureus*	Strong	
					-	*Escherichia coli*	Null	
				50 mg/ml	10 mm	*Staphylococcus aureus*	Mediate	
					-	*Escherichia coli*	Null	
				25 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				12,5 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				6,25 mg/ml	-		Null	
					-		Null	
			Butanol extract	200 mg/ml	22 mm	*Staphylococcus aureus*	Very strong	
					12 mm	*Escherichia coli*	Strong	
				100 mg/ml	18 mm	*Staphylococcus aureus*	Strong	
					10 mm	*Escherichia coli*	Mediate	
				50 mg/ml	16 mm	*Staphylococcus aureus*	Strong	
					-	*Escherichia coli*	Null	
				25 mg/ml	14 mm	*Staphylococcus aureus*	Strong	
					-	*Escherichia coli*	Null	
				12,5 mg/ml	10 mm	*Staphylococcus aureus*	Mediate	
					-	*Escherichia coli*	Null	

				6,25 mg/ml	-		Null	
					-		Null	
			Aqueous fractions	200 mg/ml	14 mm	*Staphylococcus aureus*	Strong	
					12 mm	*Escherichia coli*	Strong	
				100 mg/ml	12 mm	*Staphylococcus aureus*	Strong	
					10 mm	*Escherichia coli*	Mediate	
				50 mg/ml	10 mm	*Staphylococcus aureus*	Mediate	
					-	*Escherichia coli*	Null	
				25 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				12,5 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				6,25 mg/ml	-		Null	
					-		Null	
	Leaves	Disc diffusion	Ethyl acetate	1 mg/ml	8,4 mm	*Staphylococcus aureus*	Mediate	[37]
					8,2 mm	*Eschericia coli*	Mediate	
				2 mg/ml	12,2 mm	*Staphylococcus aureus*	Strong	
					10,2 mm	*Eschericia coli*	Strong	
				5 mg/ml	13,5 mm	*Staphylococcus aureus*	Strong	
					12,0 mm	*Eschericia coli*	Strong	
				10 mg/ml	14,5 mm		Strong	
					16,2 mm		Strong	
			Chloroform	1 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Eschericia coli*	Null	
				2 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Eschericia coli*	Null	
				5 mg/ml	-	*Staphylococcus aureus*	Null	
					9,3 mm	*Eschericia coli*	Weak	
				10 mg/ml	-		Null	
					12,3 mm		Strong	
			Ethanol	1 mg/ml	10,5 mm	*Staphylococcus aureus*	Mediate	
					7,3 mm	*Eschericia coli*	Mediate	
				2 mg/ml	11,7 mm	*Staphylococcus aureus*	Strong	
					9,4 mm	*Eschericia coli*	Mediate	
				5 mg/ml	13,8 mm	*Staphylococcus aureus*	Strong	
					12,3 mm	*Eschericia coli*	Strong	
				10 mg/ml	16,3 mm		Strong	

					14,0 mm		Strong	
			Aqueous	1 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				2 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				5 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				10 mg/ml	-		Null	
					-		Null	
			n-hexane	1 mg/ml	-	*Staphylococcus aureus*	Null	
					3 mm	*Escherichia coli*	Weak	
				2 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				5 mg/ml	8,8 mm	*Staphylococcus aureus*	Weak	
					6 mm	*Escherichia coli*	Weak	
				10 mg/ml	9,7 mm		Weak	
					12,3 mm		Strong	
	The Whole Plant	Agar well diffusion	N-hexane extract	25 mg/ml	10 mm	*Staphylococcus aureus*	Mediate	[38]
					10 mm	*Escherichia coli*	Mediate	
				50 mg/ml	12 mm	*Staphylococcus aureus*	Strong	
					12 mm	*Escherichia coli*	Strong	
				100 mg/ml	14 mm	*Staphylococcus aureus*	Strong	
					14 mm	*Escherichia coli*	Strong	
				200 mg/ml	16 mm		Strong	
					16 mm		Strong	
			Crude methanol extract	25 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				50 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				100 mg/ml	-	*Staphylococcus aureus*	Null	
					-	*Escherichia coli*	Null	
				200 mg/ml	10 mm		Mediate	
					10 mm		Mediate	

			Crude ethyl acetate	25 mg/ml 50 mg/ml 100 mg/ml 20 mg/ml	- - 10 mm - 12 mm - 14 mm 14 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Null Null Mediate Null Strong Null Strong Strong	
Piper betle	Leaves	Agar cup diffusion	Chloroform	0,208 mg/ml 0,199 mg/ml	18,5 mm 22,75 mm	*Staphylococcus aureus* *Escherichia coli*	Strong Very strong	[31]
			Ethyl acetate	0,234 mg/ml 0,242 mg/ml	20,25 mm 20,5 mm	*Staphylococcus aureus* *Escherichia coli*	Strong Strong	
			Methanol	0,312 mg/ml 0,232 mg/ml	25,25 mm 20,25 mm	*Staphylococcus aureus* *Escherichia coli*	Very strong Strong	
		Disc diffusion	Chloroform	0,208 mg/ml 0,199 mg/ml	14,25 mm 18,25 mm	*Staphylococcus aureus* *Escherichia coli*	Strong Strong	
			Ethyl acetate	0,234 mg/ml 0,242 mg/ml	17,5 mm 18,75 mm	*Staphylococcus aureus* *Escherichia coli*	Strong Strong	
			Methanol	0,312 mg/ml 0,232 mg/ml	24,75 mm 23,75 mm	*Staphylococcus aureus* *Escherichia coli*	Very strong Very strong	
	Leaves	Agar Diffusion	Ethanol	50 µl/ml 100 µl/ml	9,7 mm 8,9 mm 18,0 mm 11,0 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Mediate Mediate Strong Strong	[39]
			Water	50 µl/ml 100 µl/ml	5,4 mm - 4,9 mm 8,5 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Weak Null Weak Mediate	
			Chloramphenicol	50 µl/ml 100 µl/ml	15,6 mm 18 mm 17,4 mm 15,4 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Strong Strong Strong Strong	
Piper crocatum	Leaves	Diffusion	Ethanol extract	25 mg/ml 50 mg/ml 75 mg/ml	- - - - - -	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus*	Null Null Null Null Null Null	[25]

				100 mg/ml	- - -	*Escherichia coli*	Null Null Null	
	Leaves	Paper disc diffusion	Water extract	0% 40% 60% 80%	0,15 mm 0,45 mm 0,54 mm 0,7 mm	*Staphylococcus aureus* *Staphylococcus aureus* *Staphylococcus aureus* *Staphylococcus aureus*	Weak Weak Weak Weak	[40]
	Leaves	Well diffusion	Ethanol extract	2,5% 5% 10% 20% 40% 80% 100%	6 mm 6 mm 6 mm 6 mm 6,3 mm 6 mm 10,66 mm 6 mm 12,6 mm 6,3 mmm 16,6 mm 7,6 mm 17,6 mm 7,6 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Strong Mediate Mediate Mediate Mediate Mediate Mediate Mediate Strong Mediate Strong Mediate Strong Mediate	[41]
	Leaves	Kirby-bauer Diffusion	Ethanol extract	3,125% 6,25% 12,5% 25% 50% 100 %	9 mm 9,4 mm 9,6 mm 12,0 mm 15,1 mm 16,3 mm	*Staphylococcus aureus* *Staphylococcus aureus* *Staphylococcus aureus* *Staphylococcus aureus* *Staphylococcus aureus*	Strong Mediate Mediate Strong Strong Strong	[2]
Piper nigrum	Leaves	Agar well diffusion	Ethanol extract	1 mg/ml 2 mg/ml 3 mg/ml 4 mg/ml	6 mm 11 mm 8 mm 13 mm 9 mm 20 mm 11 mm 22 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Weak Strong Mediate Strong Mediate Very strong Strong Very	[11]

							Strong	
			chloroform extract	1 mg/ml 2 mg/ml 3 mg/ml 4 mg/ml	11 mm 15 mm 13 mm 16 mm 15 mm 17 mm 16 mm 18 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Strong Strong Strong Strong Strong Strong Strong	
	Leaves	Disc diffusion	PNET (Piper nigrum ethanol extract)	100 mg/ml	12,33 mm 14,67 mm	*Staphylococcus aureus* *Escherichia coli*	Strong Strong	[42]
			PNMT (Piper nigrum methanol extrat)	100 mg/ml	16,33 mm 20,00 mm	*Staphylococcus aureus* *Escherichia coli*	Strong Strong	
			PNPE (Piper nigrum petroleum ether extract)	100 mg/ml	10,67 mm 11,33 mm	*Staphylococcus aureus* *Escherichia coli*	Mediate Strong	
			PNAQ (Piper nigrum aqueous extract)	100 mg/ml	8,00 mm 9,33 mm	*Staphylococcus aureus* *Escherichia coli*	Mediate Mediate	
	Leaves	Paper disc method	Cold water leave	1 %	12,52 mm 12,61 mm	*Staphylococcus aureus* *Escherichia coli*	Strong Strong	[43]
			Hot water leave		13,16 mm 13,57 mm	*Staphylococcus aureus* *Escherichia coli*	Strong Strong	
			Pepper soup extract leave		13,46 mm 13.60 mm	*Staphylococcus aureus* *Escherichia coli*	Strong Strong	
Piper sarmentosum	Leaves	Disc diffusion	Ethanol extract	1 mg 10 mg 100 mg	- - - - 11 mm 8 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Null Null Null Null Strong Mediate	[44]
	Leaves	Disc diffusion	Methanol extract	25 mg/ml 50 mg/ml	- - 7 mm 6,5 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Null Null Weak Weak	[32]
	Leaves	Disc diffusion	Methanolic extract	1,0 mg/ml 1,5 mg/ml	11,1±0,7 mm 6,3±0,3 mm	*Staphylococcus aureus* *Staphylococcus aureus*	Strong Weak	[45]
Piper longum	Fruit	Disc diffusion	Hexane fraction	14 $\mu g/ml^{-1}$ 152 $\mu g/ml^{-1}$	6,33±1,52 mm 10,66±1,52 mm	*Staphylococcus aureus* *Escherichia coli*	Mediate Strong	[46]
			Chloroform fraction	14 $\mu g/ml^{-1}$	11,33±1,15 mm 20,33±2,08 mm	*Staphylococcus aureus* *Escherichia coli*	Strong	

				152 µg/ml^{-1}			Very Strong	
			Ethyl acetate fraction	14 µg/ml^{-1} 152 µg/ml^{-1}	14,33±0,57 mm 24,33±1,52 mm	*Staphylococcus aureus* *Escherichia coli*	Very strong Strong	
			Methanol fraction	14 µg/ml^{-1} 152 µg/ml^{-1}	9,33±1,15 mm 16,66±1,52 mm	*Staphylococcus aureus* *Escherichia coli*	Mediate Strong	
	Piper longum extract catkin	Disc diffusion	Ag NPs synthesis using *piper longum* extract	10 µg/ml 20 µg/ml 30 µg/ml 40 µg/ml	6,23 mm 8,16 mm 10,23 mm 12,45 mm	*Staphylococcus aureus* *Staphylococcus aureus* *Staphylococcus aureus* *Staphylococcus aureus*	Weak Weak Mediate Strong	[47]
	Fruit	Agar well diffusion	Ethyl acetate extract	0,5 mg 1,0 mg	20 mm 22 mm	*Staphylococcus aureus* *Staphylococcus aureus*	Strong Very strong	[48]
			Methanol extract	0,5 mg 1,0 mg	25 mm 27 mm	*Staphylococcus aureus* *Staphylococcus aureus*	Very strong Very strong	
			Water extract	0,5 mg 1,0 mg	25 mm 27 mm	*Staphylococcus aureus* *Staphylococcus aureus*	Very strong Very strong	
	Fruit	Disc diffusion	Chloroform extract	16 mg/ml 64 µg/ml	22 mm 20 mm 8 mm 6 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Very strong Very strong Weak Weak	[49]
			Acetone extract	16 mg/ml 64 µg/ml	21 mm 19 mm 5 mm 4 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Very strong Strong Weak Weak	
			Ethanol extract	16 mg/ml 64 µg/ml	20 mm 22 mm 5 mm 4 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Strong Very strong Weak Weak	
			Aqueous extract	16 mg/ml 64 µg/ml	24 mm 17 mm 3 mm 4 mm	*Staphylococcus aureus* *Escherichia coli* *Staphylococcus aureus* *Escherichia coli*	Very strong Strong Weak Weak	
Piper aduncum	Leaves	Disc diffusion	Ethanol extract	1 mg/ml	10 mm 8 mm 7 mm 8 mm 7 mm	*Staphylococcus aureus* ATCC 25923 *Escherichia coli* ATCC 25922 *Escherichia coli* J96 (ATCC 700336) *Escherichia coli* 185^{0} *Escherichia coli*MNF^{d}	Mediate Mediate Mediate Mediate Mediate	[50]
	Leaves	Disc diffusion	Aduncum piper extract	10 %	11,3 mm	*Staphylococcus aureus* *Escherichia coli*	Strong	[51]

			macerated using Alcohol 70%		12 mm	*Staphylococcus aureus*	Strong	
				30%	12 mm	*Escherichia coli*	Strong	
					13 mm	*Staphylococcus aureus*	Strong	
				60%	14 mm	*Escherichia coli*	Strong	
					14 mm		Strong	

Antibacterial Activity

Piperomia pellucida

The crude methanol fraction of *Piperomia pellucida* leaves has antibacterial activity using the agar diffusion method at a concentration of 200 mg/ml with an inhibition zone diameter of 10 mm for *Staphylococcus aureus* and 10 mm for *Escherichia coli*. Whereas at concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml, there was no inhibition zone diameter obtained. At this concentration, it did not show any antibacterial activity against the tested bacteria. The n-hexane fraction of *Piperomia pellucida* leaves has antibacterial activity using the agar diffusion method showed that the diameter of the inhibition zone with a concentration of 200 mg/ml was 16 mm *Staphylococcus aureus* and 16 mm *Escherichia coli*, concentration of 100 mg/ml was 14 mm for *Staphylococcus aureus* and 14 mm for *Escherichia coli*, concentration of 50 mg/ml was 12 mm for *Staphylococcus aureus* and 12 mm for *Escherichia coli*, concentration of 25 mg/ml was 10 mm in for *Staphylococcus aureus* and 10 mm for *Escherichia coli* and at a concentration of 12.5 mg/ml and 6.25 mg/ml, there was no inhibition zone diameter obtained. The ethyl acetate fraction of *Piperomia pellucida* has antibacterial activity using the agar diffusion method showed the diameter of the inhibition zone with a concentration of 200 mg/ml was 14 mm in *Staphylococcus aureus* and 12 mm in *Escherichia coli*, concentration of 100 mg/ml was 12 mm in *Staphylococcus aureus* and there was no inhibition zone in *Escherichia coli*, concentration of 50 mg/ml was 10 mm in *Staphylococcus aureus* and no inhibition zone in *Escherichia coli*, concentration of 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml, there was no inhibition zone diameter obtained. The butanol fraction of *Piperomia pellucida* has antibacterial activity using the agar diffusion method showed that the diameter of the inhibition zone with a concentration of 200 mg/ml was 22 mm in *Staphylococcus aureus* and 12 mm in *Escherichia coli*, concentration of 100 mg/ml was 18 mm in *Staphylococcus aureus* and 10 mm *in Escherichia coli*, a concentration of 50 mg/ml was 16 mm in *Staphylococcus aureus*and no inhibition zone diameter in *Escherichia coli*, a concentration of 25 mg/mL was 14 mm in *Staphylococcus aureus* and no inhibition zone diameter in *Escherichia coli*, concentration 12,5 mg/mL was 10 mm in *Staphylococcus aureus* and there was no inhibition zone diameter obtained in *Escherichia coli* , a concentration of 6.25 mg/ml and there was no inhibition zone diameter obtained in testing for *Staphylococcus aureus* and *Escherichia coli*. The water fraction of *Piperomia pellucida* leaves has antibacterial activity using the agar diffusion method showed that the diameter of the inhibition zone with a concentration of 200 mg/ml was 14 mm in *Staphylococcus aureus* and 12 mm in *Escherichia coli*, 100 mg/mL concentration was 12 mm in *Staphylococcus aureus* and 10 mm in *Escherichia coli*, a concentration of 50 mg/ml was 10 mm in *Staphylococcus aureus* and there was no inhibition zone diameter obtained in *Escherichia coli*, and at a concentration of 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, there were no inhibition zone diameter obtained in testing for *Staphylococcus aureus* and *Escherichia coli*. Different fractions were examined to inhibit the micro-organisms in *Escherichia coli* and *Staphylococcus aereus* generally the activity observed at higher concentrations of 25-200 mg/mL. The metabolite compounds that found in *Piperomia pellucida* are alkaloids, tannins, resins, steroids, phenols and carbohydrates [52].

Testing the ethyl acetate activity of *Piperomia pellucida* leaves with disc diffusion method with concentration of 1 mg/mL against *Staphylococcus aureus* was 8.4 mm and 8.2 mm *in Escherichia coli*, a concentration of 2 mg/mL against *Staphylococcus aureus* was 12,2 mm and 10.2 mm in *Escherichia coli*, the concentration of 5 mg/mL against *Staphylococcus aureus* was 13.5 mm and 12.0 mm in *Escherichia coli*, at a concentration of 10 mg/mL against the *Staphylococcus aureus* was 14, 5 mm and 16.2 mm in *Escherichia coli*.Testing the chloroform antibacterial activity of *Piperomia pellucida* leaves against *Staphylococcus aureus* and *Escherichia coli* with the disc diffusion method at the concentration of 1 mg/mL and 2 mg/mL showed that there was no inhibition zone diameter obtained, while the concentration of 5 mg/ml, there was no inhibition zone diameter obtained at *Staphylococcus aureus* and in *Escherichia coli* the inhibition zone diameter obtained was 9.3 mm, and a concentration of 10 mg/mL in *Staphylococcus aureus* showed there was no inhibition zone diameter obtained and *Escherichia coli* obtained inhibition zone diameter was 12.3 mm. Testing the antibacterial activity of *Piperomia pellucida* leaves ethanol using the disc diffusion method with a concentration of 1 mg/mL against *Staphylococcus aureus* was 10.5 mm and 7.3 mm in *Escherichia coli*, a concentration of 2 mg/ml against *Staphylococcus aureus* was 11.7 mm and 9.4 mm in *Escherichia coli*, the concentration of 5 mg/mL against *Staphylococcus aureus* was 13.8 mm and 12.3 mm in *Escherichia coli*, and at a concentration of 10 mg/ml against *Staphylococcus aureus*was 16,3 mm and 14.0 mm in *Escherichia coli*. Testing antibacterial activity of *Piperomia pellucida* of leaves water against *Staphylococcus aureus* and *Escherichia coli* with the disc diffusion method at all concentrations did not show any inhibition zone diameter. Then, in testing the antibacterial activity of n-hexane leaves of *Piperomia pellucida* against *Staphylococcus aureus* at a concentration of 1 mg/mL, there was no inhibition zone diameter and in *Escherichia coli* was 3 mm, the concentration of 2 mg/mL did not show

any inhibition zone diameter, a concentration of 5 mg/mL against *Staphylococcus aureus* was 8.8 mm and for *Escherichia coli* was 6 mm, a concentration of 10 mg/mL against *Staphylococcus aureus* was 9.7 mm and on *Escherichia coli* was 12.3 mm. The result of phytochemical testing of *Piperomia pellucida* extract showed the presence of phytochemicals such as carbohydrates, steroids, alkaloids, tannins and flavonoids [37].

N-hexane extract of all *Piperomia pellucida* plants with the agar well diffusion method has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, the inhibition zone diameter obtained at a concentration of 25 mg/ml was 10 mm in *Staphylococcus aureus* and 10 mm in *Escherichia coli*, the concentration of 50 mg/ml was 12 mm for *Staphylococcus aureus* and 12 mm in *Escherichia coli*, concentration of 100 mg/ml was 14 mm in *Staphylococcus aureus* and 14 mm in *Escherichia coli* , and concentration of 200 mg/ml was 16 mm in *Staphylococcus aureus* and 16 mm in *Escherichia coli*. Crude methanol extract of all *Piperomia pellucida* plants using the agar well diffusion method has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at a concentration of 25 mg/ml, 50 mg/ml, 100 mg/ml did not show any inhibition zone diameter and a concentration of 200 mg/ml showed that the diameter of the inhibition zone obtained in *Staphylococcus aureus* was 10 mm and 10 mm in the *Escherichia coli*.Then, the ethyl acetate extract of all *Piperomia pellucida* plants with the agar well diffusion method has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at a concentration of 25 mg/ml did not show any inhibition zone diameter, a concentration of 50 mg/ml of *Staphylococcus aureus* was 10 mm and there is no inhibition zone diameter in *Escherichia* coli, the concentration of 100 mg/ml *Staphylococcus aureus* was 12 mm and there was no inhibition zone diameter in *Escherichia coli*, and the concentration of 200 mg/ml has inhibition zone diameter in *Staphylococcus aureus* was 14 mm and 14 mm in *Escherichia coli*.The antimicrobial activity of plant extracts was tested in vitro against five types of organisms *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhi* using the agar diffusion method showed that antibacterial activity with methanol extract showing the least potential while N-hexane extract showed the strongest potential. The N-hexane and ethyl acetate extracts had an inhibition zone indicating the susceptibility of organisms between 10 and 12 mm at a concentration of 25 µg/ml when compared. The methanol extract showed antibacterial activity with an inhibition zone of 10 mm at 200 µg/ml. The minimum inhibitory concentration (MIC) was shown where it was clearly observed that at higher concentrations there was a stronger activity against microorganisms. The minimum inhibitory concentrations of plant extracts were evaluated in the range 25-200 mg/ml. This article revealed that the metabolites contained from *Piperomia pellucida* such as tannins, saponins, cardenolides, flavonoids, essential oils and carotols [38].

Piper betle

Antibacterial activity of chloroform leaves of *Piper betle* with agar cup diffusion method against *Staphylococcus aureus* with a concentration of 0.208 mg/ml, the diameter of the inhibition zone obtained was 18.5 mm. In *Escherichia coli* with a concentration of 0.199 mg/ml, the diameter of the inhibition zone obtained was 22.75 mm. Antibacterial activity of *Piper betle* leaves ethyl acetate with agar cup diffusion method against *Staphylococcus aureus* with a concentration of 0.234 mg/ml, the diameter of the inhibition zone obtained was 20.25 mm. In *Escherichia coli* with the concentration was 0.242 mg/ml, the diameter of the inhibition zone was 20.5 mm. Then, in the antibacterial activity methanol leaves of *Piper betle* with the agar cup diffusion method against *Staphylococcus aureus*, a concentration of 0.312 mg/ml, the diameter of the inhibition zone was 25.25 mm, and in the *Escherichia coli* a concentration of 0.232 mg/ml, the diameter of the inhibition zone was 20.25 mm. While the antibacterial activity chloroform leaves of *Piper betle* with a different method, namely the disc diffusion method against *Staphylococcus aureus* with a concentration of 0.208 mg/ml, the diameter of the inhibition zone obtained was 14.25 mm, in *Escherichia coli* with the concentration was 0.199 mg/ml was 18.25 mm. In the ethyl acetate of *Piper betle* leaves with the disc diffusion method against *Staphylococcus aureus* with a concentration of 0.234 mg/ml, the diameter of the inhibition zone obtained was 17.5 mm, in *Escherichia coli* with the concentration was 0.242 mg/ml, the diameter of the inhibition zone obtained was 18.75 mm. Then, in the methanol leaves of *Piper betle* with the disc diffusion method against *Staphylococcus aureus* with a concentration of 0.312 mg/ml, the diameter of the inhibition zone obtained was 24.75 mm, and in the *Escherichia coli* with a concentration of 0.232 mg/ml, the diameter of the inhibition zone obtained was 23.75 mm. Phytochemical analysis showed that there was phenols, tannins and steroids in the plant extracts. The literature showed that *Piper betle* extract contains secondary metabolites such as phenols (chavicol, hydroxychavicol), essential oils (saforle, eugenol, isoeugenol, methyl ester), fatty acids (steraic and palmatic) [31].Testing for the antibacterial activity of ethanol leaves of *Piper betle* with the agar diffusion method against *Staphylococcus aureus*, concentration of 50 µl/ml was 9.7 mm and for *Escherichia coli* was 8.9 mm, concentration of 100 µl/ml against *Staphylococcus aureus* was 18.0 mm and the *Escherichia coli* was 11.0 mm. In testing the antibacterial activity of water leaves of *Piper betle* with the agar diffusion method with the concentration of 50 µl/ml, inhibition zone diameter was 5.4 mm against *Staphylococcus aureus* and the *Escherichia coli* showed there was no inhibition zone diameter, concentration of 100 µl/mL against *Staphylococcus aureus* was 4.9 mm and *Escherichia coli* was 8.5 mm. Then, in testing the antibacterial activity of chlorampenicol of leaves of *Piper betle* with the agar diffusion method with concentration of 50 µl/mL against *Staphylococcus aureus* was 15.6 mm and for *Escherichia coli* was 18 mm, concentration of 100 µl/mL against *Staphylococcus aureus* was 17.4 mm and in*Escherichia coli* was 15.4 mm. Secondary metabolite compounds contained in *Piper betle* leaf extract such as alkaloids, tannins, phenolic substances and glycosides [39].

Piper crocatum

The ethanol extract of *Piper crocatum* leaves against *Staphylococcus aureus* and *Escherichia coli* with diffusion

method at a concentration of 25 mg/mL, 50 mg/mL, 75 mg/mL, 100 mg/mL was not found in any inhibition zone diameter. Red betel leaf extract (*Piper crocatum*) contained alkaloids, steroids, and tannins which were shown positive results in phytochemical tests and show negative results on flavonoids, saponins, and triterpenoids [25]. The water leaves extract of *Piper crocatum* has antibacterial activity against *Staphylococcus aureus* using paper disc diffusion method with the inhibition zone diameter obtained at a concentration of 0% was 0.15 mm, concentration of 40% was 0.45 mm in *Staphylococcus aureus*, concentration of 60% was 0.54 mm in *Staphylococcus aureus*, and concentration of 80% was 0.7 mm in *Staphylococcus aureus*. The result showed that the concentration of red betel leaf extract was the highest in inhibiting the growth of *Staphylococcus aureus* at a concentration of 80% with an average inhibition zone yield (0.70 ± 0.15).The result of four types of treatment of red betel leaf extract (*Piper crocatum* Ruiz & Pav), namely 0%, 40%, 60%, and 80% can affect the growth of the inhibition zone of *Staphylococcus aureus*. It showed that the red betel leaf extract was effective as an antibacterial agent for *Staphylococcus aureus*. Substances that affect the large growth of the inhibition zone in red betel leaf extract include saponins, flavonoids, polyphenols, tannins, and triterpenes. Meanwhile, the chemical compounds contained in red betel leaf extract include tannins, flavonoids, polyphenols, saponins, alkaloids, and essential oils [40].

Antibacterial activity of ethanol extract of *Piper crocatum* leaves with the well diffusion method obtained the inhibition zone diameter at a concentration of 2.5% against *Staphylococcus aureus* ATCC 6538 was 6 mm and *Escherichia coli* ATCC 11229 was 6 mm, concentration of 5% against *Staphylococcus aureus* ATCC 6538 was 6 mm and *Escherichia coli* ATCC 11229 was 6 mm, the concentration of 10% against *Staphylococcus aureus* ATCC 6538 was 6.3 mm and *Escherichia coli* ATCC 11229 was 6 mm, concentration of 20% against *Staphylococcus aureus* ATCC 6538 was 10.66 mm and *Escherichia coli* ATCC 11229 was 6 mm, a concentration of 40% against *Staphylococcus aureus* ATCC 6538 was 12.6 mm and *Escherichia coli* ATCC 11229 was 6.3 mm, a concentration of 80% against *Staphylococcus aureus* ATCC 6538 was 16.6 mm and *Escherichia coli* ATCC 11229 was 7.6 mm, the concentration of 100% against *Staphylococcus aureus* ATCC 6538 was 17.6 mm and *Escherichia coli* ATCC 11229 was 7.6 mm. The ethanol extract of red betel leaf (*Piper crocatum*) has an inhibitory power against the growth of *Staphylococcus aureus* ATCC 6538 at concentrations of 10%, 20%, 40%, 80% and 100%, while against the growth of *Eschericia coli* ATCC 11229 ethanol extract of red betel leaf (*Piper crocatum*) has inhibitory power at concentrations of 40%, 80%, and 100% although it was not statistically significant. The difference inhibition zone in the resulting between *Staphylococcus aureus* and *Eschericia coli* caused by the diameter of the inhibition zone formed was strongly influenced by several factors, including the toxicity of the test material, the diffusion ability of the test material on the media, the interaction between the components of the medium and in vitro micro environmental conditions. The difference inhibition zone was also due to the difference in cell wall structure between the two bacteria which affects the work of the ethanol extract of red betel leaf as an antibacterial compound. The leaves of red betel contained chemical compounds such as alkaloids, polyphenolic compounds, flavonoids, tannins, saponins, and essential oils [41].

Testing of the ethanol extract of *Piper crocatum* leaves against *Staphylococcus aureus* with the kirby-bauer diffusion method, the diameter of the inhibition zone was obtained at a concentration of 3.125% was 9 mm in *Staphylococcus aureus*, concentration of 6.25% was 9.4 mm in *Staphylococcus aureus*, concentration 12,5% was 9.6 mm in *Staphylococcus aureus*, concentration of 25% was 12.0 mm in *Staphylococcus aureus*, concentration of 50% was 15.1 mm in *Staphylococcus aureus* and concentration of 100% was 16.3 mm in *Staphylococcus aureus*.In this article, it was shown that the antibacterial activity of red betel leaf extract against *Staphylococcus aureus*. Red betel leaf extract at all concentrations namely 3.125%, 6.25%, 12.5%, 25%, 50%, and 100% could inhibit the growth of gram-positive *Staphylococcus aureus* and gram-negative bacteria. Red betel leaf extract was stronger in inhibiting the growth of gram-positive bacteria than gram-negative bacteria. The properties of red betel were caused by the presence of a number of active compounds it contained, including flavonoids, alkaloids, tannins, and essential oils [2].

Piper nigrum

The ethanol extract of *Piper nigrum* leaves with the agar diffusion method at a concentration of 1 mg/ml, the inhibition zone diameter obtained in *Staphylococcus aureus* was 6 mm and *Escherichia coli* was 11 mm, concentration of 2 mg/ml the inhibition zone diameter was 8 mm against *Staphylococcus aureus* and 13 mm for *Escherichia coli*, concentration of 3 mg/ml the zone of inhibition was 9 mm in *Staphylococcus aureus* and 20 mm for *Escherichia coli*, and at a concentration of 4 mg/ml the diameter of the inhibition zone found against *Staphylococcus aureus* was 11 mm and *Escherichia coli* was 22 mm. Antibacterial activity of leaf chloroform extract of *Piper nigrum* with the agar diffusion method at a concentration of 1 mg/ml the inhibition zone diameter was 11 mm against *Staphylococcus aureus* and 15 mm against *Escherichia coli*, concentration of 2 mg/ml was 13 mm in the *Staphylococcus aureus* and 16 mm of *Escherichia coli*, concentration of 3 mg/ml inhibition zone diameter was 15 mm in *Staphylococcus aureus* and *Escherichia coli* was 17 mm, and concentration of 4 mg/ml of inhibition zone diameter was 16 mm in *Staphylococcus aureus* and 18 mm of *Escherichia coli*. In the *Piper nigrum* plant there were secondary metabolite compounds such as alkaloids, steroids, tannins, phenolic compounds, flavonoids, resins and fatty acids [11].

Antibacterial activity of the ethanol extract of *Piper nigrum* leaves using the disc diffusion method with a concentration of 100 mg/ml inhibition zone diameter was 12.33 mm against *Staphylococcus aureus* and 14.67 mm against *Escherichia coli*. In the methanol extract of *Piper nigrum* with a concentration of 100 mg/ml, the diameter of the inhibition zone obtained was 16.33 mm for *Staphylococcus aureus* and 20.00 mm for *Escherichia coli*. Then, in the

petroleum ether extract of *Piper nigrum* using the disc diffusion method with a concentration of 100 mg/ml the inhibition zone diameter obtained was 10.67 mm in *Staphylococcus aureus* and 11.33 mm in *Escherichia coli*. In aqua extract of *Piper nigrum* using the disc diffusion method with a concentration of 100 mg/ml the inhibition zone diameter was found 8.00 mm against *Staphylococcus aureus* and 9.33 mm. *Piper nigrum* extract contained reducers, anthraquinones, terpenoids, flavonoids, saponins, tannins and alkaloids [42].Testing antibacterial activity of cold water of *Piper nigrum* with paper disc method at a concentration of 1% of the inhibition zone diameter obtained against *Staphylococcus aureus* was 12.52 mm and on *Escherichia coli* was 12.61 mm. Testing on hot water of *Piper nigrum* the inhibition zone diameter obtained against the *Staphylococcus aureus* was 13.16 mm and the *Escherichia coli* bacteria was 13.57 mm, in testing the *Piper nigrum* extract the inhibition zone diameter obtained in the *Staphylococcus aureus* bacteria was 13.46 mm and in *Escherichia coli* was 12.60 mm. This article examined the phytochemical and an antimicrobial activity of *Piper nigrum* extract was different. The result showed that significant antimicrobial effect on selected microorganisms. Phytochemical screening revealed the presence of tannins, flavonoids, cardiac glycosides and alkaloids in *Piper nigrum* [43].

Piper sarmentosum

Antibacterial activity of methanol extract of *Piper sarmentosum* with the disc diffusion method against *Staphylococcus aureus* and *Escherichia coli* was not found in the inhibition zone diameter at a concentration of 25 mg/ml, while at a concentration of 50 mg/mL the inhibition zone diameter was found 7 mm in *Staphylococcus aureus* and 6, 5 mm in *Escherichia coli*. *Piper sarmentosum* extract contained glycosides, flavonoids, terpenoids, alkaloids, and phenolics [32].

Testing the antibacterial activity of methanol extract of *Piper sarmentosum* with the disc diffusion method against *Staphylococcus aureus* at a concentration of 1.0 mg/ml, the diameter of the inhibition zone was 11.1 ± 0.7 mm in *Staphylococcus aureus* and at a concentration of 1.5 mg/ml against *Escherichia coli* was 6.3 ± 0.3 mm. *Piper sarmentosum* contained phenylpropanoid, phenylpropanoyl amide, dihydroflavones and essential oils [45].

Piper longum

The hexane fraction of *Piper longum* leaves has antibacterial activity using the Kirby-Bauer disc diffusion method at a concentration of 14 µg/ml-1 against *Staphylococcus aureus*, the inhibition zone diameter was 6.33 ± 1.52 mm and at a concentration of 152 µg/ml-1 against *Escherichia coli* with an inhibition zone diameter was 10.66 ± 1.52 mm. The chloroform fraction of *Piper longum* has antibacterial activity using the disc diffusion method at concentration of 14 µg/ml-1 against *Staphylococcus aureus*, the diameter of the inhibition zone obtained was 11.33 ± 1.15 mm and concentration of 152 µg/ml-1 against *Escherichia coli* with the inhibition zone diameter was 20.33 ± 2.08 mm. The ethyl acetate fraction of *Piper longum* has antibacterial activity with the disc diffusion method at a concentration of 14 µg/ml-1 against *Staphylococcus aureus*, the inhibition zone diameter was 14.33 ± 0.57 mm and at a concentration of 152 µg/ml-1 against *Escherichia coli* with the inhibition zone diameter was 24.33 ± 1.52 mm. Then, in the methanol fraction of *Piper longum* also has antibacterial activity with the disc diffusion method at a concentration of 14 µg/ml-1 against *Staphylococcus aureus*, the inhibition zone diameter obtained was 9.33 ± 1.15 mm and at a concentration of 152 µg/ml-1 against *Escherichia coli* with an inhibition zone diameter was 16.66 ± 1.52 mm. Among all the organic extracts, ethyl acetate extract showed important activity against the test microbes. Disc diffusion test showed various growth inhibitions of different organic solvent extracts against the tested bacterial strains. The result showed that there was the antibacterial activity of different fractions. Petroleum ether fraction was found to be ineffective against bacterial strains, whereas hexane extract had the least inhibition (6.33 ± 1.52) among the extracts against all test strains. Several secondary metabolites derived from *Piper longum* such as alkaloids and flavonoids [46].

The antibacterial activity of synthesized Ag NPs using *Piper longum* extract with the disc diffusion method against *Staphylococcus aureus* at concentrations of 10 µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml resulted the inhibition zone diameter was 6.23 mm, 8.16 mm, 10.23 mm, and 12.45 mm. *Piper longum* extract was rich in tannins, dihydrostigmasterol, piperidine alkaloids, terpenins, sesamim, and isobutyl deca-trans-2-trans-4 dienamide and several other active phytoconstituents with medicinal properties [47].

Ethyl acetate extract of *Piper longum* fruit has antibacterial activity with the agar diffusion method with concentration of 0.5 mg against *Staphylococcus aureus*, the inhibition zone diameter obtained was 20 mm, and at a concentration of 1.0 mg against *Escherichia coli*, the inhibition zone diameter obtained was 22 mm. The methanol extract of *Piper longum* fruit has antibacterial activity with the agar diffusion method with concentration of 0.5 mg against *Staphylococcus aureus*, the inhibition zone diameter was 25 mm and concentration of 1.0 mg against *Escherichia coli*, the inhibition zone diameter was 27 mm. There were compounds in the *Piper longum* plant such as volatiles, tannins, phenols and alkaloids [48].

Testing for the antibacterial activity of chloroform extract of *Piper longum* fruit used the agar diffusion method against *Staphylococcus aureus* and *Escherichia coli* at a concentration of 16 mg/mL with the inhibition zone diameter was 22 mm in *Staphylococcus aureus* and 20 mm in *Escherichia coli*, at a concentration of 64 mg/mL with the inhibition zone diameter was 8 mm in *Staphylococcus aureus* and 6 mm in *Escherichia coli*. Testing acetone extract of *Piper longum* fruit using the agar diffusion method has antibacterial activity at a concentration of 16 mg/mL with inhibition zone diameter was 21 mm in *Staphylococcus aureus* and 19 mm in *Escherichia coli*, at a concentration of 64 mg/mL with inhibition zone diameter was 5 mm at *Staphylococcus aureus* and 4 mm in *Escherichia coli*. Testing the ethanol extract of *Piper longum* fruit using the agar diffusion method has antibacterial activity at a concentration of 16 mg/mL, the

diameter of the inhibition zone was 20 mm in *Staphylococcus aureus* and 22 mm in *Escherichia coli*, at a concentration of 64 mg/mL with the inhibition zone diameter was 5 mm at *Staphylococcus aureus* and 4 mm in *Escherichia coli*. Then, in testing the antibacterial activity of water extract of *Piper longum* fruit using the agar diffusion method at a concentration of 16 mg/mL with the inhibition zone diameter was 24 mm in *Staphylococcus aureus* and 17 mm in *Escherichia coli*. At a concentration of 64 mg/mL with the diameter of the inhibition zone obtained was 3 mm in *Staphylococcus aureus* and 4 mm in *Escherichia coli*. *Piper longum* extract was rich in flavonoid compounds [49].

Piper aduncum

Antibacterial activity of ethanol extract of *Piper aduncum* using the disc diffusion method found the inhibition zone diameter at a concentration of 1 mg/mL against *Staphylococcus aureus* ATCC 25923 was 10 mm, *Escherichia coli* ATCC 25922 was 8 mm, *Escherichia coli* J96 (ATCC 700336) was 7 mm, *Escherichia coli* 1850 was 8 mm, and *Escherichia coli*MNFd was 7 mm [50].

Piper aduncum extract macerated using alcohol with the disc diffusion method has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at concentration of 10% were 11.3 mm and 12 mm, concentration of 30% diameter inhibition zone were 12 mm and 13 mm, and at concentration of 60% with inhibition zone diameter were 14 mm and 14 mm. *Piper aduncum* extract contained several compounds, namely flavonoids, saponins, tannins and alkaloids [51].

Some plants from the piperaceae family have very strong activity, such as *Piper cubeba*, *Piper nigrum*, *Piper betle*, *Piper Crocatum*, *Piperomia pellucida*, *Piper longum*, *Piper sarmentosum*, and *Piper aduncum*. The antibacterial activity of this plant was due to the content of secondary metabolites such as alkaloids, flavonoids, saponins, phenols and tannins [4].

Alkaloid was plant secondary metabolites that have been shown to have strong pharmacological activity. These alkaloids could function as potential compounds that could act as lead compounds for the development of antibacterial and/or auxiliary plant-based compounds [53].

The work mechanism of flavonoids was they could inhibit the function of the cell membrane by forming complex compounds that that will be related with proteins causing damage to bacterial cell membranes [54]. Flavonoids worked as antibacterial with several mechanisms of action, including inhibiting nucleic acid synthesis, inhibiting cytoplasmic membrane function and inhibiting energy metabolism from bacteria [55]. Phenol or corbalic acid was a colorless crystalline substance to bright pink that has a pungent and distinctive smell. The largest group of phenolic compounds was flavonoids and tannins. Phenolic compounds has antibacterial activity which worked by interacting with bacterial cells through an absorption process involving hydrogen bonds [56]. The mechanism of action of tannins was by binding proteins and forming ion H+ that caused the pH to become acidic. So that protein denaturation occurred and activates enzymes in bacteria which cause damage in bacterial cells [57].The mechanism of antibacterial action of saponins was by increasing the permeability of the cell membrane so the membrane became unstable and caused cell hemolysis [58]. Saponins formed a complex compound with bacterial proteins that bonded to hydrogen so the permeability of bacterial cell membrane was disturbed [59].

CONCLUSION

Based on the result of this article, the Piperaceae family plants has an antibacterial effect, where plants that has antibacterial activity include *Piperomia pellucida*, *Piper crocatum*, *Piper cubeba*, *Piper longum*, P*iper nigrum*, *Piper betle*, *Piper sarmentosum* and *Piper aduncum*. Most of the plants in the piperaceae family has strong inhibition against *Staphylococcus aureus* and *Eschericia coli*. Based on the table of the antibacterial activity test of the eight piperaceae family plants showed that the diameters of inhibition zone depend on the solvent and the concentration used. The smaller the concentration used, the larger the diameter of the inhibition zone was obtained.

The antibacterial activity of plants in the piperaceae family came from secondary metabolites of these plants, including alkaloids, tannins, saponins, phenolic compounds, and flavonoids.

REFERENCE

1. Noor Mutsaqof AA, - W, Suryani E. Sistem Pakar Untuk Mendiagnosis Penyakit Infeksi Menggunakan Forward Chaining. J Teknol Inf ITSmart. 2016; 4(1):43.
2. Soleha TU, Carolina N, Kurniawan SW. The Inhibition Test of Red Betel Leaves (*Piper crocatum*) Towards *Staphylococcus aureus* and *Salmonella typhi*. Majority. 2015;4(5):117–22.
3. R DN, Sasongko H. Perbandingan Aktivitas Antimikroba Ekstrak Infusa dari Sembilan Jenis Suku Piperaceae terhadap *Staphylococcus aureus* dan *Cnadida albicans*. J Ilmu Alam dan Tek Terap. 2019;1:1–5.
4. Egbuna C, Kumar S, Ifemeje JC, Ezzat SM, Kaliyaperumal S. Phytochemicals as Lead Compounds for New Drug Discovery. London: Susan Dennis; 2020.
5. Septiani, Dewi EN, Wijayanti I. Aktivitas Antibakteri Ekstrak Lamun (*Cymodocea rotundata*) terhadap Bakteri *Staphylococcus aureus* dan *Escherichia coli* Antibacterial Activities of Seagrass Extracts (Cymodocea rotundata) Against *Staphylococcus aureus* and *Escherichia coli*. Indones J Fish Sci Technol. 2017;13(1858–4748):1–6.
6. Deleo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet [Internet]. 2010;375(9725):1557–68. Available from: http://dx.doi.org/10.1016/S0140-6736(09)61999-1
7. Percival SL, Williams DW. *Escherichia coli* [Internet]. Second Edi. Microbiology of Waterborne Diseases: Microbiological Aspects and Risks: Second Edition. Elsevier; 2013. 89–117 p. Available from: http://dx.doi.org/10.1016/B978-0-12-415846-7.00006-8
8. Desmarchelier P, Fegan N. Pathogens in Milk: *Escherichia coli* [Internet]. Reference Module in Food Science. Elsevier; 2016. 1–8 p. Available from: http://dx.doi.org/10.1016/B978-0-08-100596-5.00989-6
9. Kuete V. African medicinal spices of genus piper [Internet]. Medicinal Spices and Vegetables from Africa: Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases. Elsevier Inc.; 2017. 581–597 p. Available from: http://dx.doi.org/10.1016/B978-0-12-809286-6/00027-3
10. Al-tememy TMK. Extracts Against Selected Bacterial Pathogens in Basrah City. 2013;12(1):142–51.

11. Ganesh P, Suresh Kumar R, Saranraj P. Phytochemical analysis and antibacterial activity of Pepper (*Piper nigrum* L.) against some human pathogens. Sch Res Libr Cent Eur J Exp Biol [Internet]. 2014;3(2):36–41. Available from: http://scholarsresearchlibrary.com/archive.html

12. Surdojawdojo P, Saputra TF, Ridhowi A. Antimicrobial activity of *Piper betle* L. against some mastitis disease bacteria at different temperatures and extraction times. Drug Invent Today [Internet]. 2019;11(10):2620–4. Available from: http://www.biomedcentral.com/1472-6882/11/104

13. Hartini SY, Diaseptana SMY, Putri NR, Susanti EL. Antagonistic Antibacterial Effect of Betel and Red Betel Combination against Gram-positive and Gram-negative Bacteria. Int J Curr Microbiol Appl Sci. 2018;7(05):267–72.

14. Oloyede G, Onocha P. of *Peperomia pellucida* from Nigeria. Adv Environ Biol. 2011;5(12):3700–9.

15. Lima R godoy de, Barros MT, da Silva Laurentiz R. Medicinal Attributes of Lignans Extracted from *Piper Cubeba*: Current Developments. ChemistryOpen. 2018;7(2):180–91.

16. Hanif MA, Nawaz H, Khan MM, Byrne HJ. Medicinal Plants of South Asia. Amsterdam: Susan Dennis; 2020.

17. Munawaroh E, Yuzammi. The Diversity and Conservation of Piper (Piperaceae) in Bukit Barisan Selatan National Park, Lampung Province. Media Konserv. 2017;22(2):118–28.

18. Simpson MG. Plant Pystematics. Plant Syst. 2010;i–iii.

19. Sarjani TM, Mawardi M, Pandia ES, Wulandari D. Identifikasi Morfologi dan Anatomi Tipe Stomata Famili Piperaceae di Kota Langsa. J ipa pembeajaran ipa. 2017;1(2):182–91.

20. A'tourrohman M, Ulfah M. Etnobotany Study of The Utilization of Sirih (Famili: Piperaceae) In Kalijambe Village, Bener Sub-district, Purworejo District. 2020;14(3):268–78.

21. Ooi DJ, Iqbal S, Ismail M. Proximate composition, nutritional attributes and mineral composition of *Peperomia pellucida* L. (ketumpangan air) grown in Malaysia. Molecules. 2012;17(9):11139–45.

22. Astuti PI, Munawaroh E. Karakteristik morfologi sirih merah: 2011;83–5.

23. Nurul ', Sanusi A, Adawiyah Umar R, Zahary MN, Adzim M, Rohin K, et al. Chemical Compositions and Antimicrobial Properties of *Piper Sarmentosum*-A Review. IOSR J Dent Med Sci e-ISSN [Internet]. 2017;16(8):62–5. Available from: www.iosrjournals.org

24. Anonim. Inventaris Tanaman Obat Indonesia (1) Jilid 2. Departemen Kesehatan dan Kesejahteraan Sosial RI Badan Penelitian dan Pengembangan Kesehatan; 2001. 269–270 p.

25. Puspita PJ, Safithri M, Sugiharti NP. Antibacterial Activities of Sirih Merah (*Piper crocatum*) Leaf Extracts. Curr Biochem. 2019;5(3):1–10.

26. Li HX, Widowati W, Azis R, Yang SY, Kim YH, Li W. Chemical constituents of the *Piper crocatum* leaves and their chemotaxonomic significance. Biochem Syst Ecol [Internet]. 2019;86(March):103905. Available from: https://doi.org/10.1016/j.bse.2019.05.013

27. Adjatin A, Dansi A, Badoussi E, Loko YL, Dansi M, Gbaguidi F, et al. Phytochemical screening and toxicity studies of Crassocephalum rubens (Juss. ex Jacq.) S. Moore and Crassocephalum crepidioides (Benth.) S. Moore consumed as vegetable in Benin. J Chem Pharm Res. 2013;5(6):160–7.

28. Nahak G, Sahu RK. Phytochemical Evaluation and Antioxidant activity of *Piper cubeba* and *Piper nigrum*. 2011;01(08):153–7.

29. Saraf A, Saraf A. Phytochemical and Antimicrobial Studies of Medicinal Plant Piper. 2014;6(2).

30. Meghwal M, Goswami TK. *Piper nigrum* and piperine: An update. Phyther Res. 2013;27(8):1121–30.

31. Jayalakshmi B, Raveesha KA, Murali M, Amruthesh KN. Phytochemical, antibacterial and antioxidant studies on leaf extracts of Piper Betle L. Int J Pharm Pharm Sci. 2015;7(10):23–9.

32. Yusof H. Qualitative Phytochemical Analysis and Antimicrobial Activity of *Piper sarmentosum* Leaves Extract Against Selected Pathogens. sains Kesihat malaysia. 2019;17(1):67–72.

33. Pacheco FV, de Paula Avelar R, Alvarenga ICA, Bertolucci SKV, de Alvarenga AA, Pinto JEBP. Essential oil of monkey-pepper (*Piper aduncum* L.) cultivated under different light environments. Ind Crops Prod [Internet]. 2016;85:251–7. Available from: http://dx.doi.org/10.1016/j.indcrop.2016.03.016

34. Katrin D, Idiawati N, Sitorus B. Uji Aktivitas Antibakteri dari Ekstrak Malek (*Litsea graciae Vidal*) terhadap Bakteri *Stapylococcus aureus* dan *Escherichia coli*. Jkk. 2015;4(1):7–12.

35. Fatimah S, Nadifah F, Burhanudin I. Uji Daya Hambat Ekstrak Etanol Kubis (Brassica oleracea var. capitata f. alba) Terhadap Bakteri Staphylococcus aureus Secara In Vitro. Biog J Ilm Biol. 2016;4(1):102–6.

36. Maimunah S. Uji Aktivitas Antibakteri Ekstrak Sintrong (*Crassocephalum crepidiodies*) Terhadap Bakteri *Staphylococcus aureus*. 2020;6(1):103–11.

37. Zubair. In Vitro Investigation Of Antdiarrhoeal, Antimicrobial And Thrombolytic Activities Of Aerial Parts Of *Peperomia Pellucida*. 2015;3:5–13.

38. Idris OO, Olatunji BP, Madufor P. In vitro Antibacterial Activity of the Extracts of *Peperomia pellucida* (L). 2016;11(4):1–7.

39. Kaveti B, Tan L, Kuan TS, Baig M. Antibacterial Activity Of Piper Betel Leaves. 2011;2(3):129–32.

40. Affif FE. Efektifitas Ekstrak Mengkudu (*Morinda citrifolia* L.) dan Sirih Merah (*Piper crocatum* Ruiz & Pav) Terhadap Zona Hambat Pertumbuhan *Staphylococcus aureus*. jounal Sci. 2017;10 (April):12–6.

41. In S, Candrasari A, Romas MA, Hasbi M, Astuti OR. Uji Daya Antimikroba Ekstrak Etanol Sirih Merah (*Piper Crocatum* Ruiz & Pav.) Terhadap Pertumbuhan *Staphylococcus aureus* ATCC 6538 , *Eschericia coli* ATCC 11229 dan Candida. 2012;4:9–16.

42. Akhtar SM. Antimicrobial activity of *Piper nigrum* L . and C assia didymobotyra L . leaf extract on selected food borne pathogens. 2014;4 (Suppl 2).

43. Kigigha LT, Kalunta CG. Antimicrobial efficacy of leaf extracts of *Piper nigrum* against *Escherichia coli* , *Staphylococcus aureus* and *Candida albicans*. :32–6.

44. Saad R. Determination Of Minimum Inhibitory Concentration Utilizing Microtitreplate Bioassay For Three Malaysian Herbal Medicines. 2014;(March).

45. Ibrahim MA, Mohd Nazir SS. Antibacterial Activities Of *Piper sarmentosum* (Kaduk) Methanolic Extract. Acta Sci Malaysia. 2019;3(2):21–4.

46. Singh C, Singh SK, Nath G, Rai NP. Anti-mycobacterial activity of *Piper longum* L . fruit extracts against multi drug resistant Mycobacterium Spp . 2011;3:353–61.

47. Jayapriya M, Dhanasekaran D, Arulmozhi M, Nandhakumar E, Senthilkumar N, Sureshkumar K. longum catkin extract irradiated by sunlight : antibacterial and catalytic activity. Res Chem Intermed [Internet]. 2019;(0123456789). Available from: https://doi.org/10.1007/s11164-019-03812-5

48. Sawhney SS. Evaluation Of Bactericidal And Anticancer Properties Of Fruits Of *Piper Longum*. 2019;(August).

49. Ahmad T, Kamruzzaman M, Ahmed A, Paul DK. In Vitro Antimicrobial Activity of Different Extracts of Long pepper (*Piper longum*) and Water cress (*Enhydra fluctuans*) against different Pathogenic Bacterial Strains. Curr Res Microbiol Biotechnol. 2016;4(3):241–7.

50. Abreu OA, Sanchez I, Pino J, Barreto G. Antimicrobial Activity of *Piper aduncum* sub sp ossanum essensial oil. 2015;7:205–8.

51. Hallianah IP, Lambui O, Ramadanil. Uji Daya Hambat Ekstrak Sirih Hutan (*Piper aduncum* L.) Terhadap Pertumbuhan Bakteri *Saphylococcus aureus* dan *Escherichia coli*. J Chem Inf Model. 2019;53(9):1689–99.

52. Oloyede G, Onocha P. Phytochemical, toxicity, antimicrobial and antioxidant screening of leaf extracts of *Peperomia pellucida* from Nigeria. Adv Environ Biol. 2011;5 (12)(May):3700–9.

53. Mabhiza D, Chitemerere T, Mukanganyama S. Antibacterial Properties of Alkaloid Extracts from Callistemon citrinus and Vernonia adoensis against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Int J Med Chem. 2016;2016:1–7.

54. Lutpiatina L, Amaliah NR, Dwiyanti RD. Daya Hambat Ekstrak Kenikir (*Cosmos caudatus Kunth*) Terhadap *Staphylococcus aureus*. Meditory. 2017;5(2):83–91.

55. Manik DF, T H, H A. Dengan Aktivitas Antibakteri Ekstrak Etanol Dan Fraksi-Fraksi Kersen. 2014;4:1–11.

56. Putri DD, Nurmagustina DE, Chandra AA. Kandungan Total Fenol dan Aktivitas Antibakteri Kelopak Buah Rosela Merah dan Ungu Sebagai Kandidat Feed Additive Alami Pada Broiler Phenol Content and Antibacterial Activity of *Red Roselle Calyces* and *Purple Roselle Calyces* and To Determine The Type of R. J Penelit Pertan Terap. 2014;14(3):174–80.

57. Rosalina Yuliana Ayen RM. Aktivitas Antibakteri Ekstrak Metanol Sembung Rambat (*Mikania micrantha* H . B . K) Terhadap Pertumbuhan Bakteri Bacillus cereus IHB B 379 dan Shigella flexneri. Protobiont. 2017;6(3):123–9.

58. Sulistiyono FD, Sofihidayati T, Lohitasari B. Antibacterial and Phytochemical Activity Test of Onion Leather (*Allium cepa* L.) Extraction Result Method Microwave Assisted Extraction (MAE). Mandala Heal A Sci J. 2018;11(2):70–8.

59. Dwiyanti W, Ibrahim M, Trimulyono G. Pengaruh Ekstrak Kenikir (*Cosmos caudatus*) terhadap Pertumbuhan Bakteri Bacillus cereus secara In Vitro The Effect of Kenikir Leaves (*Cosmos caudatus*) Extract on In Vitro Growth of Bacillus cereu s. LenteraBio. 2012;3(1):1–5.