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Author for correspondence:
Robert N Weinreb
e-mail: robert_nw11@gmail.com

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Review of Antibacterial Activity of Green Betel Leaf Extract (*Piper Betle L.*) Methods of Diffusion and Microdilution

Robert N Weinreb

Shiley Eye Institute, La Jolla, United States

Green betle plant (*Piper betle L.*) is a type of plant that is widely used for medicine. Parts of the betel plant (*Piper betle L.*) such as roots, seeds, and leaves have potential for treatment, but the most frequently used are the leaves. Empirically betel leaves are used by the community as an antibacterial. There are many literature and research studies that report the antibacterial activity of green betel leaves. In this review, we will discuss green betel leaf extract using various solvents using two antibacterial methods, namely the diffusion and microdilution methods. Library searches were carried out using secondary data with databases in the form of Pubmed, Google Scholar, Elsevier, and Science Direct. The results of the literature review stated that in the diffusion method, the highest antibacterial activity of green betel leaf extract was shown in the use of ethanol solvent with which had the highest growth inhibition of 33 mm against *Staphylococcus aureus* bacteria. Whereas with the microdilution method, the antibacterial activity of green betel leaf extract using ethanol and metanol solvents showed MIC value at 312 µg/mL.

1. Introduction

Plants are an important component in human life, especially as a source of food and as medicine. Plants empirically have antimicrobial activity and have traditionally been widely used for medicine. Indonesia is one of the tropical countries that has a diversity of biological flora that can be used for traditional medicine (Su et al., 2018). Traditional medicine has been recognized for its existence since ancient times both in Indonesia and other countries and until now it is still used and even tends to increase.

Medicinal plants are used to improve health (promotive), restore health (rehabilitative), disease prevention (preventive) and wound healing (curative), even some natural materials have been fabricated on a large scale (Chairunnisa et al., 2015; Damayanti, 2008).

Infection is one of the causes of diseases that often occur in tropical climates such as Indonesia. Infection is defined as a process when organisms (eg: bacteria, viruses, fungi) capable of causing disease enter the body or tissue and cause trauma or damage (Menaldi, 2016). At first, some bacterial infections can still be treated with a variety of antibiotics. However, since the last fifty years treatment against this infection has begun to have difficulties, because bacteria have become resistant to antibiotics (Hostettmann, 2014).

The chemical content of the betel plant is saponins, flavonoids, polyphenols, and essential oils. Saponin compounds can work as antimicrobials. This compound will damage the cytoplasmic membrane and kill the cell. Flavonoid compounds are thought to have a mechanism of action to denature bacterial cell proteins and damage cell membranes irreparably. Of these various ingredients, in essential oils there are natural phenols that have antiseptic power 5 times stronger than ordinary phenols (bactericides and fungicides) but are not sporadic. Essential oil from betel leaf contains 30% phenol (Agustina et al., 2018).

The phenol compounds that make up betel leaf essential oil consist of two phenol components, namely phenol betel isomers from kavikol and eugenol with various phenol combinations such as allyl pyrocatekol, cavibetol, carvakrol, metal eugenol, sineol and estragol.

Chemical compounds other than phenol consist of cadinene, karyophyllene, terpene, terpinene, metal ether, menthon and sesquiterpenes (Bustanussalam et al., 2015).

Green betel plant (*Piper betle* L.) is one type of plant that is widely used for treatment. Parts of the betel plant (*Piper betle* L.) such as roots, seeds, and leaves have potential for treatment, but the most often used is the leaf, empirically betel leaf is used by the community as antibacterial (Surjawiria, 2005).

Green betel leaf plants have antibacterial properties that can be used as antiseptics and used as an alternative therapy for *Acne Vulgaris* / acne, even giving a decoction of betel leaves and turmeric can reduce pathological vaginal discharge in adolescents (Zahid and Ismi, 2015). Since 600 BC betel leaves have been known to contain antiseptic substances that can kill bacteria so it is widely used as antibacterial and antifungal.

Based on this, the author is interested in reviewing several literatures to determine the antibacterial potential of green betel leaf.

2. Research Method

The method used in the review process of this article is narrative review through literature review from national and international libraries. In the preparation of this review, the data used were secondary data with databases in the form of Pubmed, Google Scholar, Elsevier, and ScienceDirect regarding antibacterial activity in green betel leaf plants. Searching library sources is carried out using keywords or keywords, namely Antibacterial, *Piper betle* and Green Betel Leaf.

The journal chosen in this article is a journal that contains some information including pharmacognosy descriptions and classifications of Green Betel Leaf plants and journals that contain antibacterial activity data by diffusion and dilution methods or microdilution. In addition, the article used in compiling a narrative review of antibacterial activity is the original article. The selected literature is then evaluated and compiled in the form of a review of scientific articles.

3. Result and Discussion

Library Search Results

In the preparation of *this review*, 51 libraries were obtained. Articles obtained on google scholar number 38, *Elsevier* 5, *Pubmed* 3, *Science Direct* 4, *Google Books* 7. The number of articles used for pharmacognosy description and classification is 10 articles, antibacterial activity of diffusion methods is 23 articles and microdilution methods is 8 articles.

Green Betel Plant (*Piper betle* L.)

Betel plant is a plant that thrives throughout tropical Asia to East Africa, spreading almost throughout Indonesia, Malaysia, Thailand, Sri Lanka, India to Madagascar (Chairunnisa *et al.*, 2015). Betel leaf contains antimicrobial compounds including tannins, flavonoids, alkaloids, saponins, and terpenoids (Kumari and Nirmala, 2015). *Piper betle* has a characteristic that the leaves often smell aromatic or spicy taste (Munawarih and Yuzammi, 2017).

The aroma of betel leaf is due to the presence of essential oils, which consist of phenols and terpenes. In addition, it has the characteristic of containing secondary metabolite compounds that usually act as a means of self-defense so as not to be eaten by animals (pests) or as agents to compete with other plants in maintaining living space (Naidu, 2010). Classification, synonyms, and regional names as well as the morphology of green betel plants can be seen in table 1 and figure 1.

Table 1. Classification, Synonyms, and Regional Names of *Piper betle* L.

Klasifikasi	Kingdom: Plantae; Subkingdom: Tracheobionta; Super Divisi: Spermatophyta; Divisi: Magnoliophyta; Kelas: Magnoliopsida; Sub Kelas: Magnoliidae; Ordo: Piperales; Famili: Piperaceae; Genus: Piper; Spesies: <i>Piper betle</i> (Sarjani <i>et al.</i> , 2017).
Synonym	<i>Chavica auriculata</i> Miq.; <i>C. betle</i> Miq.; <i>Artanthe hixagon</i> (Dalimartha, 2006).
Region Name	Ranub (Aceh); base, sedah (Bali); sere (Madura), ganjang, gapura (Bugis); suruh (Jawa); demban (Batak); sireh, sirih, suruh (Palembang, Minangkabau); uwit (Dayak); Nahi (Bima); kuta (Sumba); mota (Flores); reman (Wendebi); manaw (Makimi) (Depkes RI, 1980).



Figure 1. Plant Morphology and Green Betel Leaves (Ekosari and Sugiarto, 2013; Sarjani et al., 2017)

Antibacterial Review

Antibacterial testing methods can be carried out by two methods, the diffusion method and the dilution or microdilution method. Diffusion methods are divided into disc diffusion, and wells while dilution methods are divided into *broth dilution* and *agar dilution*. These two methods are distinguished by their analysis and purpose of usefulness. The diffusion method is a method often used for qualitative analysis of antibacterial activity and is used to determine the diameter of the inhibitory zone of a bacterium to a particular solvent.

The results obtained are in the form of the presence or absence of clear areas formed around the paper disc which shows the inhibition zone in bacterial growth. While the microdilution method is used to determine *the Minimum Inhibitory Concentration* (MIC) / Minimum Inhibitory Concentration (KHM) or the smallest concentration of antimicrobial agents needed to inhibit microbial growth. Liquid microdilution is a suitable method for screening antimicrobial activity because it is a sensitive method with a relatively short testing time [35]

The inhibitory zones formed in the diffusion method can be categorized, including; (1) weak inhibitory zone ≤ 16 mm, (2) medium inhibitory zone 17-20 mm, (4) strong inhibitory zone ≥ 21 mm (Coyle, 2005). Meanwhile, to determine the *Minimal Inhibitory Concentration* (MIC) in the microdilution method, an antibacterial agent can be declared to have strong antibacterial activity if it has a KHM of 50-500 $\mu\text{g} / \text{mL}$, moderate if the KHM value is 600-1,500 $\mu\text{g} / \text{mL}$, and weak if the KHM value is above 1,500 $\mu\text{g} / \text{mL}$ (Aligiannis *et al.*, 2001).

In the *narrative review* in this article, we will examine the ability of green betel leaf extract to inhibit bacterial growth, both gram-positive and gram-negative bacteria using the diffusion method which will be presented in table 2 to table 7.

Table 2. Data on antibacterial activity of green betel leaf extract against *Staphylococcus epidermidis* bacteria

No.	Book	Method	Extraction Solvent	Antibacterial Activity Data
1	Kursia dkk., 2016	Difusi agar	Ethyl Acetate	Concentration 3% (D; 9.8 mm) Concentration 5% (D; 15 mm)
2	Dewi et al., 2021	Disc diffusion	Ethanol	Concentration 4.083% (D; 23.13 mm)
			Ethyl Acetate	Concentration 3.850% (D; 16.63 mm)
			N-hexan	Concentration 0.813% (D; 17.68 mm)
3	Nurdianti dkk., 2022	Difusi sumur	Ethanol	Concentration 10% (D; 11,233 mm) Concentration 15% (D; 14,533 mm)
4	Nouri and Nafchi, 2014	Difusi agar	Ethanol	Concentration 5% (D; 11.5 mm) Concentration 10% (D; 12.3 mm) Concentration 20% (D; 15.2 mm) Concentration 30% (D; 16.7 mm)

Table 3. Data on antibacterial activity of green betel leaf extract against *Staphylococcus aureus* bacteria

No.	Book	Method	Extraction Solvent	Activity Data
1	Fathoni dkk., 2019	Difusi sumur	Ethanol	Concentration 15% (D; 16.57 mm)
2	Effa and Puetri, 2015	Disc diffusion		Concentration 25% (D; 29.4 mm) Concentration 50% (D; 31.0 mm)

				Concentration 75% (D; 33.0 mm)
3	Bangash <i>et al.</i> , 2012	Difusi agar		Concentration 39.5% (D; 18.0 mm)
4	Datta <i>et al.</i> , 2011	Disc diffusion		Concentration 20% (D; 12 mm) Concentration 40% (D; 10 mm) Concentration 60% (D; 10 mm) Concentration 80% (D; 8 mm)
5	Mulang sari., 2018	Disc diffusion	Etanol daun sirih muda	Concentration 1.25% (D; 7.08 mm) Concentration 5% (D; 8.2 mm)
			Ethanol sirih tua	Concentration 1.25% (D; 7.2 mm) Concentration 5% (D; 11 mm)

Table 4. Data on antibacterial activity of green betel leaf extract against *Propionibacterium acnes bacteria*

No.	Book	Method	Extraction Solvent	Activity Data
1	Widyaningtias dkk., 2014	Disc diffusion	Ethanol	Concentration 2.5% (D; 7.01 mm) Concentration 5% (D; 8.92 mm) Concentration 10% (D; 13.28 mm) Concentration 20% (D; 21.08 mm)
2	Dewi and Wenas, 2019	Disc diffusion	Methanol	Concentration 6.25% (D; 9.05 mm) Concentration 12.5% (D; 11.50 mm) Concentration 25% (D; 12.18 mm) Concentration 50% (D; 13.53 mm)

3	Lucita and Tensika, 2021	Disc diffusion	Ethanol	Concentration 5% (D; 10.44 mm)
4	Lister, 2021	Disc diffusion	Ethanol	Concentration 1.5% (D; 9.893 mm) Concentration 3% (D; 13,103 mm) Concentration 5% (D; 16,740 mm) Concentration 7.5% (D; 16.487 mm)

Table 5. Data on antibacterial activity of green betel leaf extract against *Escherichia coli* bacteria

No.	Book	Method	Extraction Solvent	Activity Data
1	Sulastrianah dkk., 2014	Disc diffusion	Methanol	Concentration 0.5% (D; 1.6 mm) Concentration 8% (D; 10.16 mm)
2	Tirayo <i>et al.</i> , 2016	Difusi agar	Aquades	Concentration 25% (D; 23.525 mm) Concentration 50% (D; 27.05 mm)
3	Saraswati, 2011	Disc Diffusion	Aquades	Concentration 25% (D; 13.14 mm) Concentration 50% (D; 15.1 mm) Concentration 75% (D; 16.16 mm) Concentration 100% (D; 15.62 mm)
4	Pinatik dkk., 2017	Difusi sumur	Ethanol	Concentration 12% (D; 6 mm) Concentration 20% (D; 10.5 mm) Concentration 28% (D; 10.76 mm) Concentration 40% (D; 16 mm)
5	Bangash <i>et al.</i> , 2012	Difusi agar	Ethanol	Concentration 39.5% (D; 12.0 mm)

Table 6. Data on antibacterial activity of green betel leaf extract against *Bacillus subtilis* bacteria

No.	Book	Method	Extraction Solvent	Activity Data
1	Iswandana and Sihombing, 2017	Disc diffusion	Ethanol	Concentration 0.05% (D; 6 mm) Concentration 0.01% (D; 6 mm) Concentration 0.2% (D; 8 mm)
2	Nouri and Nafchi, 2014	Difusi agar	Ethanol	Concentration 5% (D; 9.6 mm) Concentration 10% (D; 10.2 mm) Concentration 20% (D; 11.3 mm) Concentration 30% (D; 15.5 mm)
3	Nguyen <i>et al.</i> , 2021	Difusi sumur	Aquades	Concentration 14.35% (D; 8 mm)
4	Lagashetty <i>et al.</i> , 2019	Difusi agar	Aquades	Concentration 10% (D; 8 mm) Concentration 20% (D; 9.5 mm) Concentration 30% (D; 11 mm) Concentration 40% (D; 13 mm) Concentration 50% (D; 14 mm)
5	Deka <i>et al.</i> , 2022	Difusi sumur	Ethanol	Concentration 20% (D; 16 mm) Concentration 40% (D; 20 mm) Concentration 60% (D; 27 mm) Concentration 100% (D; 29 mm)

Table 7. Data on antibacterial activity of green betel leaf extract against bacteria by microdilution method

No.	Extraction Solvent	Bakteri	Activity Data KHM ($\mu\text{g/mL}$)	Pustaka
1	Ethanol	<i>Escherichia coli</i>	1.250 [43], 500 [46], 650 [47], 312 [50]	(Saeloh and Visutthi., 2021) (Kulnanan <i>et al.</i> , 2021) (Valle <i>et al.</i> , 2015) (Valle <i>et al.</i> , 2016)
		<i>Staphylococcus epidermidis</i>	3.670 [44], 391 [48]	(Aminuddin <i>et al.</i> , 2018) (Sidek <i>et al.</i> , 2021)
2	Methanol	<i>Bacillus subtilis</i>	208 [45], 1.125 [49]	(Jayalakshmi <i>et al.</i> , 2015) (Rahman <i>et al.</i> , 2017)
		<i>Staphylococcus aureus</i>	312 [45], 1.125 [49]	(Jayalakshmi <i>et al.</i> , 2015) (Rahman <i>et al.</i> , 2017)
		<i>Escherichia coli</i>	312 [50]	(Valle <i>et al.</i> , 2016)

Based on the results of the study seen in Table 2, it can be seen that the research of Dewi *et al* (2021) shows the highest diameter of the inhibitory zone in antibacterial activity against *Staphylococcus epidermidis* bacteria obtained in the ethanol solvent variable, which is 23.13 mm in a concentration of 4.083% and is included in the category of strong inhibitory, when compared to other solvents, ethanol solvents have higher polarity properties than with solvents ethyl acetate and n-hexane.

The inhibitory zone formed in the antibacterial test shows that the inhibitory spectrum depends on the type and strength of the antibacterial compounds of each component extracted as well as the amount of active components extracted by the solvent used.

In the results of the study seen in Table 3, the greatest antibacterial activity against *Staphylococcus aureus* bacteria was obtained from the research of Effa and Puetri (2015) which used ethanol solvent with a concentration of 75% and had an inhibitory diameter of 33.0 mm.

The results of the above study also showed that the diameter of the inhibition zone formed was getting bigger with increasing concentration of betel leaf ethanol extract.

The higher the concentration of betel leaf ethanol extract given, the more the content of these antibacterial active compounds. The other two concentrations of betel leaf ethanol extract used in this study (50% and 25%) can also be said to have a strong inhibitory zone due to the diameter obtained ≥ 21 mm.

A number of studies in Table 4, it is said that the greatest antibacterial activity against *Propionibacterium acnes* bacteria was obtained from the research of Widyaningtias et al (2014), a concentration of 20% is an effective concentration with the largest inhibitory zone of 21.08 mm. While the results of the study seen in Table 5, the greatest antibacterial activity against *Escherichia coli* bacteria was obtained from the study of Tirayo et al (2016) which showed the average result of all treatments with a concentration of 50%, namely 27.05 mm from green betel leaf extract using aquades solvent and showed a strong inhibitory response against *Escherichia coli* bacteria.

Then, based on the results of the study seen in Table 6, the greatest antibacterial activity against *Bacillus subtilis* bacteria was obtained from the study of Deka et al (2022) which showed that green betel leaf extract using ethanol solvent at a concentration of 100% had antibacterial activity against *Bacillus subtilis* bacteria. With an inhibitory zone diameter of 29 mm it belongs to the category of strong drag.

In table 7 can be seen a number of studies on Minimum Inhibition Concentration (KHM) against several gram-positive and negative bacteria. The value of KHM which has antibacterial activity which is included in the strong category in studies using ethanol solvents against *E. coli* and *S. epidermis* bacteria respectively is 312 $\mu\text{g}/\text{mL}$ (Valle et al., 2016) and 391 $\mu\text{g}/\text{mL}$ (Sidek et al., 2021).

In addition, there are other studies with KHM values in the moderate and weak categories which can be caused by differences in the time of processing and quality of the extract. Similarly, in research using methanol solvents against 3 bacteria, namely *B. subtilis*, *S. aureus*, and *E. coli*. The value of KHM with strong antibacterial activity using methanol solvent is 208 $\mu\text{g}/\text{mL}$ (Jayalakhsmi et al., 2015), 312 $\mu\text{g}/\text{mL}$ (Jayalakhsmi et al., 2015), and 312 $\mu\text{g}/\text{mL}$ (Valle et al., 2016).

The difference in results from some of these studies is likely due to the composition of active ingredients contained in green betel leaves influenced by various conditions. The quality of active compounds contained in medicinal plants will be influenced by two factors, namely internal factors and external factors.

Internal factors include genetic quality and plant age, while external factors include growing conditions for example, land conditions, climate, altitude where it grows, pests and diseases, environmental pollution, high ultraviolet intensity, heavy metal contamination, temperature and humidity.

In some types of plants that contain essential oils, the oil content is higher with the increasing height of the growing place or the lower the ambient temperature (Katno, 2008).

Differences in antibacterial sensitivity can be caused by the selection of solution, pH, and method at the time of extract. Another thing that can affect is the storage time of green betel leaf extract on the quality of bacterial inhibition.

Betel leaf extract that is stored longer can experience a decrease in quality due to reduced antibacterial compounds. The selection of the appropriate solvent in the extraction process needs to be considered because the active compounds taken are in accordance with the solution used (Bangash et al., 2012).

The selection of pH that is less than 5.0 and more than 8.5 makes the growth of bacteria can be inhibited (Nisa et al., 2014). In addition, the method used at the time of extraction can affect the large amount of yield value which causes success in withdrawing the amount of the active compound (Vifta et al., 2017).

This decrease in antibacterial activity is thought to be due to the effect of increasing the viscosity of the extract solution due to increasing concentration, so that the extract solution has not been diffused properly in agar media. Gram positive bacteria are known to be more sensitive to antibacterial compared to Gram negative bacteria.

This is because the cell wall of Gram positive bacteria is only composed of several layers of peptidoglycan which causes the cells to be easily denatured by compounds contained in green betel leaf extract, for example bethel phenol.

While Gram negative bacteria have three wrapping polymers located outside the peptidoglycan layer such as lipoproteins, outer membranes, and lipopolysaccharides (Sadiah et al., 2022).

4. Conclusion

Based on this review, green betel leaf extract can be said to have antibacterial activity against bacteria by looking at the inhibition zone in the diffusion method and the KHM value in the dilution method. In the diffusion method, the best antibacterial activity was shown against *S. aureus* bacteria with an inhibitory zone of 33 mm using ethanol solvent, while in the dilution method, the best khm value was shown in *E. coli* and *S. aureus* bacteria of 312 µg / mL. The size of the inhibition zone and KHM values in antibacterial activity testing can be influenced by several factors that cause differences in antibacterial sensitivity. So it is expected that other researchers will pay more attention to the quality of the extract before being tested.

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