Review

**Piper betle** (L): Recent Review of Antibacterial and Antifungal Properties, Safety Profiles, and Commercial Applications

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**Abstract:** *Piper betle* (L) is a popular medicinal plant in Asia. Plant leaves have been used as a traditional medicine to treat various health conditions. It is highly abundant and inexpensive, therefore promoting further research and industrialization development, including in the food and pharmaceutical industries. Articles published from 2010 to 2020 were reviewed in detail to show recent updates on the antibacterial and antifungal properties of betel leaves. This current review showed that betel leaves extract, essential oil, preparations, and isolates could inhibit microbial growth and kill various Gram-negative and Gram-positive bacteria as well as fungal species, including those that are multidrug-resistant and cause serious infectious diseases. *P. betle* leaves displayed high efficiency on Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, Gram-positive bacteria such as *Staphylococcus aureus*, and *Candida albicans*. The ratio of MBC/MIC indicated bactericidal and bacteriostatic effects of *P. betle* leaves, while MFC/MIC values showed fungicidal and fungistatic effects. This review also provides a list of phytochemical compounds in betel leaves extracts and essential oils, safety profiles, and value-added products of betel leaves. Some studies also showed that the combination of betel leaves extract and essential oil with antibiotics (streptomycin, chloramphenicol, and gentamicin) could provide potentiating antibacterial properties. Moreover, this review delivers a scientific resume for researchers in respected areas and manufacturers who want to develop betel leaves-based products.

**Keywords:** antibacterial; antifungal; betel leaves; *Piper betle*

1. Introduction

*Piper betle* (L) commonly known as betel vine belongs to the family Piperaceae. It is a popular medicinal plant in Asia. The leaf is the most widely used and studied part of the betel vine. There are chewing habit practices of betel leaves in many countries which are believed beneficial for avoiding bad breath, strengthening the gum, preserving the teeth, and stimulating the digestive system [1,2]. In traditional medicine practices, betel leaves are used for vaginal douching in Indonesia [3], as a gargle mouthwash in India and Thailand [4], and as a treatment for dental problems, headaches, arthritis, and joint pain in Malaysia [1]. In Srilanka, the betel leaf juice is used to treat skin ailments [5]. Additionally, its boiled leaves could be used as cough medicine, tonic, or astringent [2]. Traditional applications of betel leaves are related to their antibacterial and antifungal properties.

Over the past decades, antibacterial resistance has been threatening humans and has caused a global health crisis. Some bacterial strains are resistant to antibiotics such as vancomycin intermediate *Staphylococcus aureus* (VISA), vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *S. aureus* (MRSA), and extended spectrum β-lactamase (ESβL).
enzyme producing Gram-negative bacteria, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *S. aureus*, and *Mycobacterium tuberculosis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter* spp. [6,7]. Besides bacteria, fungi can also lead to infectious diseases. Approximately 300 fungal species on Earth are known to cause illnesses such as *Candida* spp. and dermatophytes [8,9]. Moreover, in the food industry, bacteria and fungi cause problems during product processing and storage. Food spoilage due to pathogen contamination is not only harmful to consumers but also brings heavy economic losses to manufacturers [10]. Therefore, research in this area continues to develop new safe and effective antimicrobial agents that could be applied in many related fields.

In this paper, a review of the literature was conducted to display recent studies (published in 2010–2020) on the antibacterial and antifungal properties of betel leaf extract (BLE), essential oil (BLEO), preparations, and isolates. In addition, the phytochemical constituents, safety profiles, and value-added products of betel leaves are also provided. Research on antibacterial and antifungal properties of betel leaves and their safety profiles have established their application as future active and additive ingredients in the pharmaceutical and food industries. Betel leaves are highly abundant and inexpensive, thus supporting their further development in manufacturing commercial products.

2. Phytochemicals in Betel Leaves

2.1. Betel Leaves Extract (BLE)

*Piper betle* contains numerous phytochemicals depending on its botanical origin and the solvent used for extraction. A preliminary phytochemical analysis of betel leaves from Malaysia showed that alkaloids, tannins, glycosides, reducing sugars, and saponins were found in the water extract of betel leaves [11]. Moreover, a study determined the total content of phenol, flavonoid, and tannin in water, ethanol, ethyl acetate, acetone, and dichloromethane extracts of betel leaves from Mauritius [12]. The highest total phenol, flavonoid, and tannin were found in the acetone, dichloromethane, and ethanol extracts, respectively. The sample of betel leaves collected from Tamilnadu, India is known to contain steroids, tannins, proteins, amino acids, flavonoids, terpenoids, mucilage, volatile oil, saponin, carbohydrates, and fixed oil, but an absence of alkaloids [13]. Furthermore, some studies have effectively isolated bioactive compounds from BLE (Figure 1) such as phytol, acyclic diterpene alcohol, 4-chromanol, hydroxychavicol or 4-allylpyrocatechol, and allylpyrocatechols 1 [14–17].

![Figure 1. Cont.](image-url)
Figure 1. Major bioactive compounds in betel leaves extracts and essential oil. (a) phytol; (b) 4-chromanol; (c) hydroxychavicol; (d) eugenol; (e) carvacrol; (f) chavicol; (g) chavibetol; (h) allylpyrocatechols 1.

2.2. Betel Leaves Essential Oil (BLEO)

Betel leaves contain 0.15% to 0.2% essential oil which are classified as monoterpenes, sesquiterpenes, phenylpropanoids, and aldehydes (Table 1). The constituents of BLEO are strongly dependent on its botanical origin, age of the plant, and harvesting time. Various compounds of BLEO may affect its aroma, taste, and bioactivity [18]. GC-MS analysis of BLEO from different places in India showed that phenylpropanoid groups such as acetyl eugenol, eugenol, chavicol, and safrole were the major components [19]. Interestingly, Indian BLEO obtained from the Sagar Bangla cultivar contained chavicol, but not from the Magahi cultivar. The study also revealed that BLEO contained eugenol (40%) and a combination of carvacrol and chavicol (up to 40%) with chavibetol as a marker compound as depicted in Figure 1. Meanwhile, another study found additional main compounds including estragole, linalool, α-copaene, anethole, and caryophyllene α-terpinene, p-cymene, 1,8-cineole, β-caryophyllene, α-humulene, allyl pyrocatechol, allyl catechol, methyl eugenol, estragol (methyl chavicol), chavibetol, chavibetol acetate, safrol, 4-allyl-2-methoxy-phenolacetate, and 3-allyl-6-methoxyphenol [18,20,21].
Table 1. List of phytochemicals identified from betel leaf essential oil

| Classification | Compounds                  | Classification | Compounds                  |
|----------------|----------------------------|----------------|----------------------------|
| Monoterpenes   | α-Thujene                  | Sesquiterpenes | δ-Elemene                  |
|                | α-Pinene                   |                | α-Copaene                  |
|                | Camphene                   |                | β-Elemene                  |
|                | Sabinene                   |                | E-β-Caryophyllene          |
|                | Myrcene                    |                | β-Copaene                  |
|                | α-Terpine                  |                | γ-Elemene                  |
|                | β-Phellandrene             |                | Aromadendrene              |
|                | 1,8-Cineole/Eucalyptol     |                | α-Humulene                 |
|                | (E)-β-Ocimene              |                | γ-Muuroleone               |
|                | γ-Terpinene                |                | Germacrene D               |
|                | Terpinolene                |                | Germacrene B               |
|                | Linalool                   |                | β-Selinene                 |
|                | Terpinen-4-ol              |                | α-Selinene                 |
|                | α-Terpineol                |                | Bicyclogermacrene          |
|                | L-limonene                 |                | α-Muuroleone               |
|                | Linalyl acetate            |                | cis-β-Guiaiene             |
|                |                             |                | δ-Cadinene or γ-ß-<br>amorphene |
|                | Phenylpropanoids           |                | Palustrol                  |
|                | Estragole/Methyl chavicol  | Aldehydes      | Spathulenol                |
|                | Chavicol                   |                | Caryophyllene oxide        |
|                | Anethole/Isoeostragole     |                | Globulol                   |
|                | Safrole                    |                | Viridiflorol               |
|                | Chavicol acetate           |                | Cubenol                    |
|                | Eugenol                    |                | α-Cadinol                  |
|                | Methyl eugenol             |                | Ledene                     |
|                | Acetyl eugenol             |                | α-amorphene                |
|                | Phenyl acetaldehyde        |                | Cubebene                   |

3. Antibacterial Property of Betel Leaves

The extract, essential oil, preparation, and isolated compounds of betel leaves are effective against numerous Gram-negative (Table 2) and Gram-positive bacteria (Table 3). The bacteria tested included foodborne pathogens and other bacteria, including multidrug-resistant (MDR) bacteria that cause severe infectious diseases in humans. Most of the published research investigated the antibacterial activity of BLEs resulting from solvents with different polarities such as water, ethanol, ethyl acetate, acetone, and dichloromethane. Each extract contained diverse bioactive compounds which may affect their antibacterial activity [12,22]. The antibacterial tests of betel leaves were varied in methods and results, complicating the comparison between studies. Furthermore, the current review showed that the study of antibacterial activity of BLE was greater than that of BLEO.

A study showed that the ethanol extract of betel leaves was more effective than the water extract with greater inhibition zones. The ethanol extract at 50–100 µg/mL had the maximum inhibition zones (8.9–11.0 mm) on *E. coli* and moderate inhibition was observed on *P. aeruginosa* (<7.2 mm). Meanwhile, the water extract at 50 µg/mL did not actively inhibit bacterial growth [11]. Another investigation using the agar well diffusion method showed that the ethanol extract of betel leaves showed greater inhibition zones on Gram-
negative than Gram-positive bacteria [17]. A study demonstrated the antibacterial effect of five types of BLE resulting from different polarities of solvents. Among these extracts, acetone and ethyl acetate extracts demonstrated the most remarkable activity against the six bacteria tested, with S. aureus being the most susceptible one. Moreover, the antibacterial property of BLES was related to their phenol and flavonoid contents [12].

Other than the inhibition zone, the antibacterial activity was also presented as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). MIC is defined as the lowest concentration of samples that inhibits microbial growth. Meanwhile, MBC is the lowest sample concentration at which 99.9% of the bacteria are killed [23]. For easier comparison, the MIC and MBC values from published articles were recalculated from µg/mL, mg/mL, and µg/µL to percentage (w/v or v/v).

The most frequently studied Gram-negative bacteria were laboratory strains of E. coli and P. aeruginosa with MIC range from 0.03 to 0.4% and 0.05–0.4%, respectively [12,12–28]. Meanwhile, the lowest MIC (0.0156%) among Gram-negative bacteria was documented for clinical isolates of P. aeruginosa MβL(+) 3, A. baumannii MβL(+) 2, and P. aeruginosa MβL(+) 3 [29]. Additionally, S. aureus was the most commonly used Gram-negative bacteria to screen the antibacterial effect of betel leaves with MIC range from 0.00025 to 0.15% [12,24–28]. The lowest MIC among Gram-positive bacteria was recorded for an oral pathogen Streptococcus gordonii DMST 38731 (0.00005%) [30].

In this review, the MBC/MIC ratio was also measured to show the bacteriostatic and bactericidal effects of betel leaves. If the ratio is ≤2, the samples are considered to be bactericidal agents. The bacteriostatic mode of action is reflected when the ratio is ≥4 [31]. BLEO showed only a bactericidal effect and BLE was found to be bacteriostatic and bactericidal. The bactericidal action was reported against Gram-negative and Gram-positive bacteria, including those classified as MDR bacteria such as ESβL-producing Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae (CRE), Metallo-β-lactamase (MβL)-producing P. aeruginosa and A. baumannii, MRSA, and VRE. On the other hand, a bacteriostatic effect was only observed against Gram-positive bacteria Streptococcus gordonii.

A previous study proved the promising antibacterial effect of BLE against oral pathogens including Gram-positive cariogenic bacteria and Gram-negative periodontal pathogenic bacteria. The study also found that 4-chromanol was the compound responsible for the antibacterial and antibiofilm properties of BLE [17]. Another study discovered the ability of BLE to control biofilm formation of Vibrio harveyi [32]. The antibacterial effect of BLE was dose-dependent. BLE was also found to be effective in reducing biofilm formation and extracellular polymeric substance production caused by P. aeruginosa and bacterial consortium without increasing the selective pressure for the growth of microorganisms [33]. Additionally, the ethyl acetate extract of betel leaves could act as antibiofilm agents against the nosocomial pathogen Serratia marcescens through the inhibition of quorum sensing mediated virulence factors production such as protease and lipase [16].

P. betle showed an outstanding antibacterial activity compared with other plants. The previous study compared the antibacterial activity of the ethanol extract of 12 plants from the Philippines, namely Cassia alata, Centella asiatica, Curcuma longa, Psidium guajava, Piper betle, Vitex negundo, Mitrephora lanotan, Moringa oleifera, Phyllanthus niruri, Tinospora rumphii, and Zingiber officinale, against clinical isolate of MRSA, VRE, ESβL-producing Enterobacteriaceae, CRE, and MβL-producing P. aeruginosa and A. baumannii. Piper betle was the only plant that showed potent bactericidal activity against all the bacteria tested with an MBC/MIC ratio between 1 to 2 [27]. Another investigation exhibited the higher antibacterial activity of ethanol extract from betel leaves compared to other medicinal plants such as Andrographis paniculata, Momordica charantia, Phyllanthus emblica, Psidium guajava, and Sesbania grandiflora. The study also revealed that ethyl acetate fraction showed the strongest antimicrobial activity compared to hexane and ethanol fractions and crude ethanol extract. Further, the ethyl acetate fraction showed higher inhibition zones and MIC against Streptococcus gordonii than the positive control (chlorhexidine solution) [30].
Table 2. *Piper betle* against Gram-negative bacteria.

| Extract/Preparation (Unit for Activities) | Method | Bacteria Species | Activities | Recalculated (%) | MBC/MIC | Inhibition Zone (mm) | Reference |
|------------------------------------------|--------|------------------|------------|------------------|---------|----------------------|-----------|
| Ethanol                                  | Agar well diffusion | *Pseudomonas aeruginosa* | - | - | - | - | 6.7–7.2 | [11] |
| Water                                    | Agar well diffusion | *Pseudomonas aeruginosa* | - | - | - | - | 8.9–11.0 | [11] |
| Ethanol (µg/mL)                          | Disk diffusion | *Escherichia coli* | 625 | 625 | 0.0625 | 0.0625 | 1* | 16 | [27] |
| Ethanol (µg/mL)                          | Disk diffusion | *Klebsiella pneumoniae ATCC BAA-1705* | 1250 | 1250 | 0.125 | 0.125 | 1* | 17 | |
| Ethanol (µg/mL)                          | Micro dilution | *Escherichia coli ATCC 25922* | 4.00 | - | 0.4 | - | - | - | [12] |
| Ethanol (µg/mL)                          | Micro dilution | *Pseudomonas aeruginosa ATCC 27853* | 4.00 | - | 0.4 | - | - | - | [12] |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Escherichia coli ATCC 25922* | 312 | 312 | 0.0312 | 0.0312 | 1* | 20 | [29] |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Klebsiella pneumoniae ESβL(+) (CI)* | 625 | 625 | 0.0625 | 0.0625 | 1* | 20 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Klebsiella pneumoniae CRE(+) 1 (CI)* | 312 | 312 | 0.0312 | 0.0312 | 1* | 21 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Klebsiella pneumoniae CRE(+) 2 (CI)* | 312 | 312 | 0.0312 | 0.0312 | 1* | 24 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Klebsiella pneumoniae CRE(+) 3 (CI)* | 625 | 625 | 0.0625 | 0.0625 | 1* | 23 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Klebsiella pneumoniae CRE(+) 4 (CI)* | 312 | 312 | 0.0312 | 0.0312 | 1* | 23 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Serratia marcescens CRE(+) (CI)* | 312 | 312 | 0.0312 | 0.0312 | 1* | 20 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Pseudomonas aeruginosa MβL(+) 1 (CI)* | 312 | 312 | 0.0312 | 0.0312 | 1* | 17 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Pseudomonas aeruginosa MβL(+) 2 (CI)* | 312 | 312 | 0.0312 | 0.0312 | 1* | 19 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Pseudomonas aeruginosa MβL(+) 3 (CI)* | 156 | 156 | 0.0156 | 0.0156 | 1* | 28 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Acinetobacter baumannii MβL(+) 1 (CI)* | 625 | 625 | 0.0625 | 0.0625 | 2* | 23 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Acinetobacter baumannii MβL(+) 2 (CI)* | 312 | 312 | 0.0312 | 0.0312 | 1* | 24 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Acinetobacter baumannii MβL(+) 3 (CI)* | 312 | 312 | 0.0312 | 0.0312 | 1* | 24 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Acinetobacter baumannii MβL(+) 4 (CI)* | 312 | 312 | 0.0312 | 0.0312 | 1* | 23 | |
| Ethanol (µg/mL)                          | Disc dilution & Broth microdilution | *Acinetobacter baumannii MβL(+) 5 (CI)* | 625 | 625 | 0.0625 | 0.0625 | 1* | 26 | |
| Extract/Preparation (Unit for Activities) | Method | Bacteria Species | Activities | Recalculated (%) | MBC/MIC | Inhibition Zone (mm) | Reference |
|-----------------------------------------|--------|------------------|------------|-----------------|---------|---------------------|-----------|
| Methanol (µg/mL)                        | Disc dilution & Broth microdilution | *Escherichia coli ESBL(+) (CI)* | 312 | 0.0312            | 1 * 19 | [29]               |
|                                         |        | *Klebsiella pneumoniae ESβL(+) (CI)* | 625 | 0.0625            | 1 * 19 |                     |
|                                         |        | *Klebsiella pneumoniae CRE(+) 1 (CI)* | 625 | 0.0625            | 1 * 21 |                     |
|                                         |        | *Klebsiella pneumoniae CRE(+) 2 (CI)* | 312 | 0.0312            | 1 * 23 |                     |
|                                         |        | *Klebsiella pneumoniae CRE(+) 3 (CI)* | 625 | 0.0625            | 1 * 22 |                     |
|                                         |        | *Klebsiella pneumoniae CRE(+) 4 (CI)* | 312 | 0.0312            | 1 * 22 |                     |
|                                         |        | *Serratia marcescens CRE(+) (CI)* | 312 | 0.0312            | 1 * 19 |                     |
|                                         |        | *Pseudomonas aeruginosa MβL(+) 1 (CI)* | 625 | 0.0625            | 1 * 15 |                     |
|                                         |        | *Pseudomonas aeruginosa MβL(+) 2 (CI)* | 625 | 0.0625            | 1 * 18 |                     |
|                                         |        | *Pseudomonas aeruginosa MβL(+) 3 (CI)* | 156 | 0.0156            | 1 * 27 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 1 (CI)* | 625 | 0.0625            | 1 * 22 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 2 (CI)* | 625 | 0.0625            | 2 * 24 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 3 (CI)* | 625 | 0.0625            | 1 * 23 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 4 (CI)* | 312 | 0.0312            | 1 * 22 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 5 (CI)* | 625 | 0.0625            | 1 * 25 |                     |
| SC-CO₂ 15MPa (µg/mL)                    | Disc dilution & Broth microdilution | *Escherichia coli ESβL(+) (CI)* | 625 | 0.0625            | 2 * 15 | [29]               |
|                                         |        | *Klebsiella pneumoniae ESβL(+) (CI)* | 1250 | 0.125            | 1 * 15 |                     |
|                                         |        | *Klebsiella pneumoniae CRE(+) 1 (CI)* | 625 | 0.0625            | 1 * 15 |                     |
|                                         |        | *Klebsiella pneumoniae CRE(+) 2 (CI)* | 625 | 0.0625            | 2 * 20 |                     |
|                                         |        | *Klebsiella pneumoniae CRE(+) 3 (CI)* | 625 | 0.0625            | 2 * 16 |                     |
|                                         |        | *Klebsiella pneumoniae CRE(+) 4 (CI)* | 625 | 0.0625            | 1 * 16 |                     |
|                                         |        | *Serratia marcescens CRE(+) (CI)* | 312 | 0.0312            | 1 * 18 |                     |
|                                         |        | *Pseudomonas aeruginosa MβL(+) 1 (CI)* | 1250 | 0.125            | 1 * 11 |                     |
|                                         |        | *Pseudomonas aeruginosa MβL(+) 2 (CI)* | 1250 | 0.125            | 1 * 14 |                     |
|                                         |        | *Pseudomonas aeruginosa MβL(+) 3 (CI)* | 625 | 0.0625            | 1 * 12 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 1 (CI)* | 625 | 0.0625            | 1 * 20 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 2 (CI)* | 625 | 0.0625            | 2 * 20 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 3 (CI)* | 625 | 0.0625            | 1 * 19 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 4 (CI)* | 625 | 0.0625            | 1 * 18 |                     |
|                                         |        | *Acinetobacter baumannii MβL(+) 5 (CI)* | 625 | 0.0625            | 1 * 21 |                     |
| Extract/Preparation (Unit for Activities) | Method | Bacteria Species | Activities | Recalculated (%) | MBC/MIC | Inhibition Zone (mm) | Reference |
|-----------------------------------------|--------|------------------|------------|------------------|---------|---------------------|-----------|
| **SC-CO₂ 20MPa (µg/mL)**                | Disc dilution & Broth microdilution | *Escherichia coli* ESβL (+) (CI) | 625 | 0.0625 | 1 * | 16 | [29] |
| **Klebsiella pneumoniae** ESβL (+) (CI) | Disc dilution & Broth microdilution | 625 | 0.0625 | 1 * | 16 | |
| **Klebsiella pneumoniae** CRE (+) 1 (CI) | Disc dilution & Broth microdilution | 312 | 0.0312 | 1 * | 16 | |
| **Klebsiella pneumoniae** CRE (+) 2 (CI) | Disc dilution & Broth microdilution | 312 | 0.0312 | 2 * | 20 | |
| **Klebsiella pneumoniae** CRE (+) 3 (CI) | Disc dilution & Broth microdilution | 625 | 0.0625 | 2 * | 17 | |
| **Klebsiella pneumoniae** CRE (+) 4 (CI) | Disc dilution & Broth microdilution | 312 | 0.0312 | 1 * | 17 | |
| **Serratia marcescens** CRE (+) (CI) | Disc dilution & Broth microdilution | 312 | 0.0312 | 1 * | 18 | |
| **Pseudomonas aeruginosa** MβL (+) 1 (CI) | Disc dilution & Broth microdilution | 625 | 0.0625 | 1 * | 11 | |
| **Pseudomonas aeruginosa** MβL (+) 2 (CI) | Disc dilution & Broth microdilution | 625 | 0.0625 | 1 * | 15 | |
| **Pseudomonas aeruginosa** MβL (+) 3 (CI) | Disc dilution & Broth microdilution | 625 | 0.0625 | 1 * | 14 | |
| **Acinetobacter baumannii** MβL (+) 1 (CI) | Disc dilution & Broth microdilution | 625 | 0.0625 | 1 * | 22 | |
| **Acinetobacter baumannii** MβL (+) 2 (CI) | Disc dilution & Broth microdilution | 312 | 0.312 | 2 * | 22 | |
| **Acinetobacter baumannii** MβL (+) 3 (CI) | Disc dilution & Broth microdilution | 625 | 0.0625 | 1 * | 22 | |
| **Acinetobacter baumannii** MβL (+) 4 (CI) | Disc dilution & Broth microdilution | 312 | 0.312 | 1 * | 21 | |
| **Acinetobacter baumannii** MβL (+) 5 (CI) | Disc dilution & Broth microdilution | 625 | 0.0625 | 1 * | 24 | |
| **Ethanol (mg/mL)** | Agar well diffusion & Broth microdilution | Aggregatibacter actino-myctecolomamint | 1.04 | 0.104 | 2 * | ≥20 | [17] |
| **Fusobacterium nucleatum** ATCC 25586 | Microdilution plate | Vibrio harveyi | 1.30 | 0.13 | 1.6 * | ≥20 | [32] |
| **Ethyl acetate** | Broth dilution | Pseudomonas aeruginosa ATCC 27853 | 1600 | - | - | - | - | [25] |
| Extract-Ag nanoparticles | Kirby-Bauer’s Disc diffusion | | 1600 | - | - | - | 21.95 ± 0.45 | |
| **Salmonella typhi** ATCC 14028 | Agar well diffusion | | - | - | - | - | 29.55 ± 0.45 | |
| **Escherichia coli** ATCC 29922 | Agar well diffusion | | - | - | - | - | 27.12 ± 0.38 | |
| **Escherichia coli** ATCC 25922 | Microdilution plate | Pseudomonas aeruginosa ATCC 27853 | 0.5–1 | 1–1.5 | 0.05–0.1 | 1–3 * | - | [26] |
| **Klebsiella pneumoniae** MTCC 432 | Microdilution plate | *Escherichia coli* MTCC 443 | 1–1.25 | 0.1–0.125 | 0.2–0.25 | 1–2 * | - | |
| **Pseudomonas aeruginosa** MTCC 424 | Microdilution plate | | 0.5–0.75 | 1–1.5 | 0.05– | 2 * | - | |
| Extract/Preparation (Unit for Activities) | Method                        | Bacteria Species                       | Activities | Recalculated (%) | MBC/MIC | Inhibition Zone (mm) | Reference |
|-----------------------------------------|-------------------------------|----------------------------------------|------------|-----------------|---------|---------------------|-----------|
| BLEO (mg/mL)                            | Micro-dilution broth & growth inhibitory assay | *Acinetobacter baumannii (CI)*          | 8          | 8               | 0.8     | 0.8                 | 1 *       | [24] |
| BLEO + Gentamicin (mg/mL)               | Micro-dilution broth & growth inhibitory assay | *Escherichia coli ATCC 25922*            | 0.3        | 0.3             | 0.03    | 0.03                | 1 *       |         |
|                                         |                               | *Escherichia coli (CI)*                 | 2          | 2               | 0.2     | 0.2                 | 1 *       |         |
|                                         |                               | *Klebsiella pneumoniae (CI)*            | 4          | 4               | 0.4     | 0.4                 | 1 *       |         |
|                                         |                               | *Pseudomonas aeruginosa ATCC 27853*     | 0.5        | 0.5             | 0.05    | 0.05                | 1 *       |         |
|                                         |                               | *Pseudomonas aeruginosa (CI)*           | 2          | 2               | 0.2     | 0.2                 | 1 *       |         |
|                                         |                               | *Proteus vulgaris (CI)*                 | 4          | 4               | 0.4     | 0.4                 | 1 *       |         |
|                                         |                               | *Escherichia coli ATCC 25922*           | 0.5-1      | -               | 0.05–0.1| -                   | -         | [24] |

BLEO = betel leaves essential oil, ESβL = Extended spectrum β-lactamase, MRSA = Methicillin-resistant *Staphylococcus aureus*, MβL = metallo-β-lactam, - = data not available, * = bactericidal.
It is noteworthy that natural products could provide additive antimicrobial activity and modify antibiotic resistance when combining with conventional antibiotics [34]. The synergistic effect was found in a combination of ethyl acetate or acetone extract of betel leaves and streptomycin and chloramphenicol against *P. aeruginosa*, *S. aureus*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*. The highest synergy was observed when the acetone extract and chloramphenicol combination (70:30) was used against *P. aeruginosa*. However, there was no correlation between phytochemical content and the synergistic effect which indicated a different mechanism of action [12]. A study also revealed a potentiating effect of BLEO and gentamicin against *Escherichia coli* and *S. epidermidis* [24]. These results should be further confirmed to assure the effectiveness of betel leaves as an antibacterial potentiating agent.

Some research evaluated the antibacterial activity of the BLE or BLEO based preparation against different pathogens. The antibacterial activity of silver-BLE nanoparticles was found to be similar to standard drug (norfloxacin) against *S. aureus*. The nanoparticles also exhibited a bacteriostatic effect on *Salmonella typhi*, *E. coli*, and *P. aeruginosa*. Moreover, the previous study concluded that Gram-positive bacteria are more susceptible to silver-BLE nanoparticles rather than Gram-negative bacteria [25]. Another study also developed the green synthesis of CaO nanoparticles using the water extract of betel leaves. It showed maximum and minimum activity against *E. coli* and *Streptococcus mutans*, respectively [28]. Additionally, BLEO based nanoemulsion was observed to be effective against five strains of foodborne pathogens and can be used as a promising natural antibacterial agent in the food system [26].

The isolated phenolic compound of BLE, namely hydroxychavicol or allylpyrocatechols, were tested against *Streptococcus sanguinis*, a Gram-positive bacterium that contributes to caries [15]. The compound was a moderate antibacterial agent that functioned by blocking MurA that causes bacterial cell wall disruption. The result exhibited the potential of betel leaves as an alternative effective and efficient treatment for mechanical plaque removal through inhibition of bacterial growth. The isolate could also kill *Streptococcus intermedius* and *S. mutans* by a similar mechanism mentioned above. The study showed that the killing kinetic of 4-allylpyrocatechol was dose and pathogen dependent [35]. The overgrowth of these bacteria develops many serious oral infections and are the major cause of caries, gingivitis, and chronic periodontitis [36].
Table 3. *Piper betle* against Gram-positive bacteria.

| Extract/Preparation/Isolate (Unit for Activities) | Method          | BACTERIA SPECIES               | Activities | Recalculated (%) | MBC/MIC | Inhibition Zone (mm) | Reference |
|-------------------------------------------------|-----------------|--------------------------------|------------|------------------|---------|----------------------|-----------|
|                                                 |                 |                                |            | MIC              | MBC     |                     |           |
|                                                 |                 |                                |            | MBC              | MIC     |                     |           |
| Ethanol                                         | Agar well diffusion | *Bacillus subtilis*            | -          | -                | -       | 13.2–15.8            | [11]      |
|                                                 |                 | *Staphylococcus aureus*        | -          | -                | -       | 9.7–18.0             |           |
|                                                 |                 | *Micrococcus luteus*           | -          | -                | -       | 5.0–5.4              |           |
| Water                                           | Agar well diffusion | *Bacillus subtilis*            | -          | -                | -       | 4.9–6.8              | [11]      |
|                                                 |                 | *Staphylococcus aureus*        | -          | -                | -       | 5.4–12.3             |           |
|                                                 |                 | *Micrococcus luteus*           | -          | -                | -       | 3.5–4.2              |           |
| Ethanol (µg/mL)                                 | Disk diffusion  | *Staphylococcus aureus ATCC 29223* | 312        | 312              | 0.0312  | 0.0312 1*            | 30        | [27]      |
|                                                 |                 | MRSA #1 (Cl)                   | 156        | 312              | 0.0156  | 0.0312 2*            | 32        |           |
|                                                 |                 | MRSA #2 (Cl)                   | 156        | 156              | 0.0156  | 0.0156 1*            | 34        |           |
|                                                 |                 | MRSA #3 (Cl)                   | 156        | 156              | 0.0156  | 0.0156 1*            | 28        |           |
|                                                 |                 | MRSA #4 (Cl)                   | 78         | 78               | 0.0078  | 0.0078 1*            | 34        |           |
| VRE                                             |                 |                                | 19         | 19               | 0.0019  | 0.0019 1*            | 28        |           |
| Ethyl acetate (µg/µL)                           | Broth microdilution | *Staphylococcus aureus ATCC 25923* | 0.50       | -                | 0.0005  | -                    | -         | [12]      |
|                                                 |                 | *Propionibacterium acnes ATCC 6919* | 2.00       | -                | 0.002   | -                    | -         |           |
|                                                 |                 | *Staphylococcus epidermidis ATCC 12228* | 4.00       | -                | 0.004   | -                    | -         |           |
|                                                 |                 | *Streptococcus pyogenes ATCC 19615* | 4.00       | -                | 0.004   | -                    | -         |           |
| Acetone (µg/µL)                                 | Broth microdilution | *Staphylococcus aureus ATCC 25923* | 0.25       | -                | 0.00025 | -                    | -         | [12]      |
|                                                 |                 | *Propionibacterium acnes ATCC 6919* | 2.00       | -                | 0.002   | -                    | -         |           |
|                                                 |                 | *Staphylococcus epidermidis ATCC 12228* | 4.00       | -                | 0.004   | -                    | -         |           |
|                                                 |                 | *Streptococcus pyogenes ATCC 19615* | 4.00       | -                | 0.004   | -                    | -         |           |
Table 3. Cont.

| Extract/Preparation/Isolate (Unit for Activities) | Method                  | BACTERIA SPECIES                     | Activities     | Recalculated (%) | MBC/MIC | Inhibition Zone (mm) | Reference |
|-------------------------------------------------|-------------------------|--------------------------------------|----------------|------------------|---------|----------------------|-----------|
| [Dichloromethane (µg/µL)]                        | Broth microdilution     | *Staphylococcus aureus* ATCC 25923   | 1.00           | -                | 0.001   | -                    | [12]      |
|                                                 |                         | *Propionibacterium acnes* ATCC 6919  | 4.00           | -                | 0.004   | -                    |           |
|                                                 |                         | *Staphylococcus epidermidis* ATCC 12228 | 4.00          | -                | 0.004   | -                    |           |
|                                                 |                         | *Streptococcus pyogenes* ATCC 19615  | 4.00           | -                | 0.004   | -                    |           |
| Ethanol (µg/mL)                                  | Disk diffusion          | MRSA 1–7                            | 78–156         | 78–312           | 0.0078–0.0156 | 0.0078–0.0312 | 1–2 * | 28–3833          | [29]      |
|                                                 |                         | VRE 1–3                             | 19–156         | 19–156           | 0.0019–0.0156 | 0.0019–0.0156 | 1 *  | 25–3228           |           |
| Methanol (µg/mL)                                 | Disk diffusion          | MRSA 1–7                            | 78–312         | 78–312           | 0.0078–0.0312 | 0.0078–0.0312 | 1–2 * | 28–3432          | [29]      |
|                                                 |                         | VRE 1–3                             | 19–156         | 19–156           | 0.0019–0.0156 | 0.0019–0.0156 | 1 *  | 25–3226           |           |
| SC-CO₂ 15MPa (µg/mL)                             | Disk diffusion          | MRSA 1–7                            | 312–625        | 312–1250         | 0.0312–0.0625 | 0.0312–0.125 | 1 *  | 21–3025           | [29]      |
|                                                 |                         | VRE 1–3                             | 19–156         | 19–156           | 0.0019–0.0156 | 0.0019–0.0156 | 1 *  | 15–2820           |           |
| SC-CO₂ 20MPa (µg/mL)                             | Disk diffusion          | MRSA 1–7                            | 156–625        | 156–625          | 0.0156–0.0625 | 0.0156–0.0625 | 1 *  | 22–3325           | [29]      |
|                                                 |                         | VRE 1–3                             | 19–156         | 19–156           | 0.0019–0.0156 | 0.0019–0.0156 | 1 *  | 15–3124           |           |
| Ethanol (mg/mL)                                  | Agar well diffusion & Broth microdilution | *Enterobacter faecalis* ATCC 19433 | 5.21           | 8.33             | 0.521   | 0.833                | 1.6 * | 10–20             | [17]      |
|                                                 |                         | *Lactobacillus fermentum* ATCC 14931 | 4.17           | 8.33             | 0.417   | 0.833                | 2 *  | 10–20             |           |
|                                                 |                         | *Lactobacillus salivarius* ATCC 11741 | 4.17           | 8.33             | 0.417   | 0.833                | 2 *  | 10–20             |           |
|                                                 |                         | *Streptococcus sobrinus* ATCC 33478 | 1.56           | 3.17             | 0.156   | 0.317                | 2 *  | ≥20               |           |
|                                                 |                         | *Streptococcus mutans* ATCC 25175    | 1.56           | 3.17             | 0.156   | 0.317                | 2 *  | ≥20               |           |
| Hexane (µg/mL)                                   | Disk diffusion          | *Streptococcus gordonii* DMST 38731 | 1.00           | 2.00             | 0.0001  | 0.0002               | 2 *  | 8.00 ± 0.00       | [30]      |
|                                                 |                         | *Streptococcus mutans* DMST 18777    | 2.00           | 2.00             | 0.0002  | 0.0002               | 1 *  | -                 |           |
Table 3. Cont.

| Extract/Preparation/Isolate (Unit for Activities) | Method | BACTERIA SPECIES | Activities | Recalculated (%) | MBC/MIC | Inhibition Zone (mm) | Reference |
|--------------------------------------------------|--------|------------------|------------|-----------------|---------|---------------------|-----------|
| Ethyl acetate (µg/mL)                            |        |                  |            |                 |         |                     |           |
|                                                   |        | Streptococcus gordonii DMST | 0.50 | 2.00 | 0.00005 | 0.0002 | 4 ** | 12.50 ± 0.70 | [30] |
|                                                   |        | Streptococcus mutans DMST | 1.00 | 2.00 | 0.0001 | 0.0002 | 2 * | 11.00 ± 0.00 | |
| Ethanol Extract-Ag nanoparticles                  | Agar well diffusion | Staphylococcus mutans (CI) | - | - | - | - | - | 2.500–20.375 | [37] |
|                                                   | Kirby-Bauer’s Disc diffusion | Staphylococcus aureus ATCC | - | - | - | - | - | 32.78 ± 0.64 | [25] |
| Ethanol Extract-CaO nanoparticles                | Agar well diffusion | Staphylococcus aureus ATCC | - | - | - | - | - | 13 | [28] |
| BLEX-nanoemulsion (µL/mL)                       | Microdilution plate | Staphylococcus aureus MTCC 1144 | 0.5–0.75 | 1–1.5 | 0.05–0.075 | 0.1–0.15 | 2 * | - | [26] |
| BLEX (mg/mL)                                     | Micro-dilution broth & growth inhibitory assay | Propionibacterium acnes ATCC 6919 | 1 | 1 | 0.1 | 0.1 | 1 * | - | |
|                                                   |        | Staphylococcus aureus ATCC 25923 | 0.5 | 0.5 | 0.05 | 0.05 | 1 * | - | |
|                                                   |        | Staphylococcus epidermidis ATCC 12228 | 0.5 | 0.5 | 0.05 | 0.05 | 1 * | - | |
|                                                   |        | Streptococcus mutans MTCC 890 | - | - | - | - | - | 12 | |
|                                                   |        | Bacillus cereus MTCC 1272 | 0.5–0.75 | 0.75–1.5 | 0.05–0.075 | 0.1–0.15 | 2 * | - | |
|                                                   |        | Escherichia faecalis (CI) | 4 | 4 | 0.4 | 0.4 | 1 * | - | |
| BLEX+Gentamicin (mg/mL)                          | Micro-dilution broth & growth inhibitory assay | Propionibacterium acnes ATCC 6919 | 1 | 1 | 0.1 | 0.1 | 1 * | - | |
|                                                   |        | Staphylococcus aureus ATCC 25923 | 0.5 | 0.5 | 0.05 | 0.05 | 1 * | - | |
|                                                   |        | Staphylococcus epidermidis ATCC 12228 | 0.5 | 0.5 | 0.05 | 0.05 | 1 * | - | |
|                                                   |        | Streptococcus mutans MTCC 890 | - | - | - | - | - | 12 | |
|                                                   |        | Bacillus cereus MTCC 1272 | 0.5–0.75 | 0.75–1.5 | 0.05–0.075 | 0.1–0.15 | 2 * | - | |
|                                                   |        | Escherichia faecalis (CI) | 4 | 4 | 0.4 | 0.4 | 1 * | - | |
| Allylpyrocatechols I (µg/mL)                     | Kirby-Bauer disk diffusion | Streptococcus sanguinis ATCC 10566 | 39.1 | 78.1 | 0.00391 | 0.00781 | 2 * | 11.85–25.15 | [15] |
| 4-allylpyrocatechol (µg/mL)                      | Broth microdilution | Streptococcus intermedius DMST 42700 | 200 | 500 | 0.02 | 0.05 | 2.5 * | - | [35] |
|                                                   |        | Streptococcus mutans DMST 41283 | 200 | 500 | 0.02 | 0.05 | 2.5 * | - | |

BLEO = betel leaves essential oil, CI = Clinical isolate, MRSA = Methicillin-resistant *Staphylococcus aureus*, VRE = vancomycin-resistant *Enterococcus*, - = data not available, * = bactericidal, ** = bacteriostatic.
4. Antifungal Properties of Betel Leaves

Numerous methods have been applied to test the antifungal properties of betel leaves including solid dilution, broth dilution, micro-dilution, well diffusion, and solid diffusion assays, resulting in minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and inhibition zones (Table 4). Similar to antibacterial activity, recalculation of MIC and MFC, and measurement of MFC/MIC ratio to determine fungicidal and fungistatic effects, were also conducted. *Candida albicans* was the most screened fungal species with MIC ranging from 0.01% to 0.07% [2,24,30,35,38,39] The fungidal effects of BLE and BLEO against various fungal species including *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, C. albicans, Candida glabrata, Candida krusei, Candida neoformans, Candida parapsilosis, Candida tropicalis, Epidermophyton floccosum, Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis, and Microsporum gypseum* [24,30,38,40]. Meanwhile, the fungistatic effect was only recorded from hexane and ethyl acetate extract of betel leaves against *C. albicans* [30], and its isolate, hyroxychavicol, against *C. krusei* [38]. A few of these species can contaminate food and spread aflatoxin, which is harmful to humans [18,41]. Other fungal species are clinically significant human pathogens that cause dental disorders and dermatophyte infections [2,14,35,40].

Ethanol and ethyl acetate extracts of betel leaves were found to be effective against *C. albicans* isolated from oral thrush patients. The ethyl acetate extract demonstrated the highest inhibition zone compared to extracts from another plant (*Ocimum sanctum*) and a standard drug (fluconazole) [39]. Other studies have also demonstrated the greater antifungal activity of ethyl acetate extract compared to hexane and ethanol extracts of betel leaves [10,30]. The killing kinetic study revealed that the fungistatic activity of the ethyl acetate extract was concentration-dependent. Furthermore, other research showed the anticandidal action of water extract from betel leaves. This effect was possibly related to its ability to reduce the cell surface hydrophobicity of several Candida species. Adhesion of fungal species and host tissues is crucial for fungal virulence, especially for successful colonization and infection. Hydrophobic domains in fungal surface proteins which consist of non-polar amino acids are a major factor involved in fungal adhesion. Thus a deviation in hydrophobic affinity produced by *P. betle* extract may influence the adherence mechanism of the fungal cell [42].

Some research investigated the antifungal activity of BLEO. A study showed that antifungal and aflatoxin suppressor actions of BLEO are related to its main components such as eugenol [18,40]. Eugenol contains a hydroxy group that could form hydrogen bonds with the active site on fungal enzymes that are responsible for aflatoxin secretion and later causes denaturation [43]. Eugenol was also reported to induce fungal morphological abnormalities by changing or disrupting fungal cell wall structure, increasing cell membrane fluidity and permeability, and interfering with important regulator function [44]. Furthermore, docking simulation of eugenol acetate and chavicol acetate in BLEO showed strong interaction to amino acid constructing fungal protein structures, which is predicted to cause metabolic reduction and biomass breaking down, thus reducing fungal virulence [45].

The superior antifungal property of BLEO compared with essential oils from other Mauritius plants such as *Psidia argute, Psidia terebinthina*, *Pimenta dioica*, *Salvia officinalis, Cinnamomum zeylanicum*, and *Schinus terebinthifolius* has been proven. The study revealed that BLEO was the strongest fungicidal agent with the lowest MIC against all the ATCC strains and clinical isolates fungi tested [24]. A formula of BLEO based microemulsion showed tremendous fungi toxic activity against a selected mold in raw apple juice at low concentration (<0.5 µL/mL). Meanwhile, spore inactivation of *A. flavus* and *P. expansum* by BLEO was found at a greater concentration (15 µL/mL) [41].
Table 4. *Piper betle* against various fungal species.

| Extract/Preparation/Isolate (Unit for Activities) | Method | Fungal Species                | Activities | Recalculated (%) | MFC/MIC | Inhibition Zone (mm) | Reference |
|------------------------------------------------|--------|-------------------------------|------------|------------------|---------|----------------------|-----------|
| Young leaves                                   |        |                               |            |                  |         |                      |           |
| Ethanol (µg/mL)                                | Broth microdilution | *Candida albicans* (CI)      | 500        | 0.05             | -       | 8–15                 | [39]      |
| Ethyl acetate (µg/mL)                          | Broth microdilution | *Candida albicans* (CI)      | 250        | 0.025            | -       | 10–22                |           |
| Mature leaves                                  |        |                               |            |                  |         |                      |           |
| Ethanol (µg/mL)                                | Broth microdilution | *Candida albicans* (CI)      | 750        | 0.075            | -       | 5–22                 | [39]      |
| Ethyl acetate (µg/mL)                          | Broth microdilution | *Candida albicans* (CI)      | 125        | 0.0125           | -       | 17–26                |           |
| Ethyl acetate                                  | Well-diffusion   | *Aspergillus niger*          | -          | -                | -       | 28                   | [10]      |
| Hexane                                         |        |                               |            |                  |         |                      |           |
| Hexane (mg/mL)                                 | Disk diffusion | *Candida albicans* DMST 8684 | 1.00       | 0.1              | 0.2     | 2 *                  | 21.00 ± 1.40 | [30] |
|                                               |         |                               |            |                  |         |                      |           |
| Ethyl acetate (mg/mL)                          | Disk diffusion | *Candida albicans* DMST 5815 | 1.00       | 0.1              | 0.4     | 4 **                 | 20.67 ± 0.58 | [30] |
|                                               |         |                               |            |                  |         |                      |           |
| BLEO (µL/mL)                                   | Solid dilution  | *Alternaria alternate*       | 0.53       | 0.053            | -       | -                    | -         | [18] |
|                                               |         |                               |            |                  |         |                      |           |
|                                               |         | *Aspergillus candidus*        | 0.57       | 0.057            | -       | -                    | -         |       |
|                                               |         | *Aspergillus flavus*          | 0.7        | 0.07             | -       | -                    | -         |       |
|                                               |         | *Aspergillus fumigatus*       | 0.40       | 0.04             | -       | -                    | -         |       |
|                                               |         | *Aspergillus niger*           | 0.73       | 0.073            | -       | -                    | -         |       |
|                                               |         | *Aspergillus sydowi*          | 0.63       | 0.063            | -       | -                    | -         |       |
|                                               |         | *Aspergillus terreus*         | 0.60       | 0.060            | -       | -                    | -         |       |
|                                               |         | *Cladosporium cladosporoides* | 0.67       | 0.067            | -       | -                    | -         |       |
|                                               |         | *Calicularia lunata*          | 0.50       | 0.05             | -       | -                    | -         |       |
|                                               |         | *Fusarium oxysporum*          | 0.50       | 0.05             | -       | -                    | -         |       |
|                                               |         | *Mucor sp.*                   | 0.37       | 0.037            | -       | -                    | -         |       |
|                                               |         | *Mycelia sterilia*            | 0.30       | 0.03             | -       | -                    | -         |       |
|                                               |         | *Nugrospora sp.*              | 0.53       | 0.053            | -       | -                    | -         |       |
|                                               |         | *Penicillium italicum*        | 0.40       | 0.04             | -       | -                    | -         |       |
|                                               | Microdilution broth & growth inhibitory assay | *Aspergillus niger ATCC 16404* | 2         | 0.2              | 0.2     | 1 *                 | -         | [24] |
|                                               |         |                               |            |                  |         |                      |           |
|                                               |         | *Candida albicans ATCC 10231* | 1.5        | 0.15             | 0.15    | 1 *                 | -         |       |
|                                               |         | *Candida albicans (CI)*       | 2          | 0.2              | 0.2     | 1 *                 | -         |       |
|                                               |         | *Candida tropicalis ATCC 750* | 2          | 0.2              | 0.2     | 1 *                 | -         |       |
Table 4. Cont.

| Extract/Preparation/Isolate (Unit for Activities) | Method         | Fungal Species                  | Activities           | Recalculated (%) | MFC/MIC | Inhibition Zone (mm) | Reference |
|-------------------------------------------------|----------------|--------------------------------|----------------------|------------------|---------|----------------------|-----------|
| BLEO (µL/mL)                                    | Broth microdilution | *Trichophyton mentagrophytes (CI)* | 0.2–0.4 0.4 0.00002–0.00004 0.00004 | 1–2 * | -        | [40]                  |
|                                                 |                | *Trichophyton mentagrophytes*    | 0.2–0.4 0.4 0.00002–0.00004 0.00004 | 1–2 * | -        |                       |
|                                                 |                | DMST 19735                       | 0.2–0.4 0.4 0.00002–0.00004 0.00004 | 1–2 * | -        |                       |
|                                                 |                | *Microsporum canis (CI)*         | 0.2 0.4 0.00002–0.00004 0.00004 | 2 * | -        |                       |
|                                                 |                | DMST 29297                       | 0.4–0.8 0.8 0.00004–0.00008 0.00008 | 1–2 * | -        |                       |
|                                                 |                | *Microsporum gypseum (CI)*       | 0.8 0.8 0.00008 0.00008 | 1 * | -        |                       |
|                                                 |                | DMST 21146                       |                       |                  |         |                     |           |
| BLEO (%v/v)                                     | Disk diffusion | *Candida albicans ATCC 10231*    | 0.078 - 0.078 - -    | 33.83 + 0.76 | [2]     |                       |
|                                                 |                | *Candida glabrata ATCC 90030*     | 0.039 - 0.039 - -    | 33.83 + 0.76 | [2]     |                       |
|                                                 |                | *Candida krusei ATCC 6258*       | 0.078 - 0.078 - -    | 32.66 + 0.57 | [2]     |                       |
|                                                 |                | *Candida parapsilosis ATCC 22019 | 0.039 - 0.039 - -    | 33.83 + 0.76 | [2]     |                       |
|                                                 |                | *Candida pseudotropicalis (CI)*  | 0.039 - 0.039 - -    | 33.50+0.50     | [2]     |                       |
|                                                 |                | *Candida stellatoidea (CI)*      | 0.039 - 0.039 - -    | 35.50+0.86     | [2]     |                       |
|                                                 |                | *Candida tropicalis (CI)*        | 0.078 - 0.078 - -    | 30.83+0.28     | [2]     |                       |
|                                                 |                | *Aspergillus flavus*             | - 15 - 1.5 - -       | -               |         | [41]                  |
|                                                 |                | *Penicillium expansum*           | - 15 - 1.5 - -       | -               |         |                       |
| Hydroxychavicol (µg/mL)                         | Broth microdilution | *Aspergillus flavus MTCC 1973, 2799 | 250 250 0.025 0.025 | 1 * | -        | [38]                  |
|                                                 |                | *Aspergillus flavus (CI)*        | 125–500 125–500 0.0125–0.05 0.0125–0.05 | 1 * | -        |                       |
|                                                 |                | *Aspergillus fumigatus MTCC 1811 | 250 250 0.025 0.025 | 1 * | -        |                       |
|                                                 |                | *Aspergillus niger ATCC 16404*   | 125 125 0.0125 0.0125 | 1 * | -        |                       |
|                                                 |                | *Aspergillus niger (CI)*         | 125–250 125–250 0.0125–0.05 0.0125–0.05 | 1 * | -        |                       |
|                                                 |                | *Aspergillus parasiticus MTCC 2796 | 250 250 0.025 0.025 | 1 * | -        |                       |
|                                                 |                | *Candida albicans ATCC 90028, 10231 | 250 250 0.025 0.025 | 1 * | -        |                       |
|                                                 |                | *Candida albicans (CI)*          | 125–500 250–500 0.0125–0.05 0.0125–0.05 | 1–2 * | -        |                       |
|                                                 |                | *Candida glabrata ATCC 90030*    | 31.25 31.25 0.003125 0.003125 | 1 * | -        |                       |
|                                                 |                | *Candida glabrata (CI)*          | 15.62–15.62 0.001562–0.001562 | 1–2 * | -        |                       |
Table 4. Cont.

| Extract/Preparation/Isolate (Unit for Activities) | Method          | Fungal Species                  | Activities | Recalculated (%) | MFC/MIC | Inhibition Zone (mm) | Reference |
|------------------------------------------------|-----------------|--------------------------------|------------|------------------|---------|---------------------|-----------|
|                                                 |                 | **Candida krusei** ATCC 22019   | 15.62      | 62.5             | 0.001562 | 0.00625             | 4 **      | -                    |
|                                                 |                 | **Candida krusei** (CI)         | 15.62–     | 62.5             | 0.001562–| 0.001562–           | 1 *       | -                    |
|                                                 |                 | **Candida neoformans** ATCC 204092 | 31.25    | 31.25            | 0.003125 | 0.003125            | 1 *       | -                    |
|                                                 |                 | **Candida neoformans** (CI)    | 62.5       | 62.5             | 0.00625  | 0.00625             | 1 *       | -                    |
|                                                 | **Candida parapsilosis** ATCC 2019 | C. neoformans ATCC 204092     | 31.25–     | 62.5             | 0.003125–| 0.003125–           | 1 *       | -                    |
|                                                 |                 | **Candida parapsilosis** (CI)  | 15.62–     | 62.5             | 0.001562–| 0.001562–           | 1 *       | -                    |
|                                                 | **Candida tropicallis** ATCC 750 | C. tropicallis ATCC 750       | 250        | 250              | 0.025    | 0.025               | 1 *       | -                    |
|                                                 |                 | **Epidermophyton floccosum** MTCC 613 | 15.62–   | 15.62            | 0.001562–| 0.001562–           | 1 *       | -                    |
|                                                 | **Epidermophyton floccosum** (CI) | C. tropicallis ATCC 750       | 15.62–     | 31.25            | 0.003125–| 0.003125–           | 1 *       | -                    |
|                                                 | **Microsporum canis** MTCC 2820 | C. tropicallis ATCC 750       | 15.62–     | 31.25            | 0.003125–| 0.003125–           | 1 *       | -                    |
|                                                 | **Microsporum canis** (CI) | C. tropicallis ATCC 750       | 15.62–     | 31.25            | 0.003125–| 0.003125–           | 1 *       | -                    |
|                                                 | **Microsporum gypsium** MTCC 2819 | C. tropicallis ATCC 750       | 7.81–      | 15.62            | 0.000781–| 0.001562–           | 2 *       | -                    |
|                                                 | **Microsporum gypsium** (CI) | C. tropicallis ATCC 750       | 15.62      | 31.25            | 0.001562 | 0.003125            | 1 *       | -                    |
|                                                 | **Trichophyton mentagrophytes** ATCC 9333 | C. tropicallis ATCC 750       | 15.62–     | 31.25            | 0.001562–| 0.001562–           | 1–2 *     | -                    |
|                                                 | **Trichophyton mentagrophytes** (CI) | C. tropicallis ATCC 750       | 15.62–     | 62.5             | 0.00625  | 0.00625             | 1–2 *     | -                    |
|                                                 | **Trichophyton rubrum** MTCC 296 | C. tropicallis ATCC 750       | 31.25–     | 31.25            | 0.003125–| 0.003125–           | 1–2 *     | -                    |
|                                                 | **Trichophyton rubrum** (CI) | C. tropicallis ATCC 750       | 31.25      | 31.25            | 0.003125 | 0.003125            | 1 *       | -                    |
|                                                 | **Trichophyton rubrum** MTCC 613 | C. tropicallis ATCC 750       | 15.62      | 62.5             | 0.00625  | 0.00625             | 1–2 *     | -                    |
|                                                 | **Trichophyton rubrum** (CI) | C. tropicallis ATCC 750       | 15.62–     | 62.5             | 0.00625  | 0.00625             | 1–2 *     | -                    |
| **4-allylpyrocatechol** (µg/mL) | **Broth Microdilution** | **Candida albicans** DMST 8684 | 400        | 500              | 0.04     | 0.05                | 1.25 *     | -                    |

*BLEO = betel leaves essential oil, CI = clinical isolate, MIC = minimum inhibitory concentration; MFC = minimum fungicidal concentration, - = Data not available, * = fungicidal, ** = fungistatic.*
Hydroxychavicol or 4-allylpyrocatechol isolated from betel leaves was also reported to be effective against various fungi species. The compound could entirely kill *C. albicans* at a minimum concentration (400 µg/mL) [35]. The killing ability of hydroxychavicol against *C. albicans* and *C. glabrata* was dose-dependent. Hydrochavicol demonstrated fungicidal effects against other clinical isolates fungi, with the MICs ranging from 7.81 to 62.5 µg/mL for dermatophytes, 15.62 to 500 µg/mL for yeasts, and 125 to 500 µg/mL for *Aspergillus* species, while the MFCs were found to be equal or two-fold higher than the MICs [38]. Moreover, it could prevent biofilm formation and promote biofilm eradication [35,38]. The development of a biofilm, which is a network of microbial cells tightly adsorbed at the mucosal surface, is linked to a severe infection [46].

5. Safety Profiles of Betel Leaves

An acute toxicity study in both male and female ICR mice showed the safety of the methanol extract of betel leaves orally. The median lethal dose (LD50) of the extract was higher than 5000 mg/kg body weight [47]. There was also an evaluation of oral acute and sub-acute toxicity (28 days) and genotoxicity of an herbal formulation containing betel leaves alcoholic extract in rats and cellular models. This study revealed the absence of major adverse reactions [48]. Moreover, betel leaves were considered safe in terms of hematotoxicity, hepatotoxicity, genotoxicity, weights of organs, gross morphology, stress, or aversive behaviors in rats [49]. Another study discovered the nontoxicity of the ethanol extract of betel leaves on normal human dermal fibroblasts (HDFn) [29].

6. Commercial Application of Betel Leaves

There are some available commercial products containing betel leaves such as dietary supplements, mouthwash, medicinal products, and cosmetic and personal care goods including shampoo, soap, face cream, antiseptic lotions, toothpaste, and perfumes [50]. Current antimicrobial studies of betel leaves were focusing on oral pathogens, MDR Gram-negative and Gram-positive bacteria, and dermatophytes [17,29,30,38]. Thus, future development of medicinal products from betel leaves could be useful for preventing oral diseases, curing dermatophyte infections, and for the treatment and management of other infectious diseases. Additionally, a study has developed a simple, safe, cost-effective, and eco-friendly preparation of silver nanoparticles with polyaniline coating using water extract of betel leaves. The nanoparticles showed potential antibacterial properties and could be further studied in various applications such as medical devices and pharmaceutical and biomedical industries [25].

In the food industry, essential oil is a promising food additive to protect and enhance the shelf life of products during processing and storage. BLEO is an ideal food preservative agent due to its antifungal and antioxidant properties [18]. Many experiments have investigated the antimicrobial properties of BLEO against foodborne pathogens [18,26,41]. Moreover, BLEO is not only beneficial to prevent spoilage of food products but also guarantees their safety for consumer health especially due to the ability of BLEO to suppress aflatoxin production. Aflatoxin, a mycotoxin from *A. flavus*, is an example of fungal contamination in food products. The toxin is known to be hepatocarcinogenic, teratogenic, mutagenic, and immunosuppressive. An investigation revealed that BLEO in apple juice could deactivate spores or inhibit spore germination which is required to limit fungal infection and mycotoxin production [41]. Further research on the overall acceptability of sensory aspects of the essential oil-treated foodstuffs is necessary to avoid market failure of the product [51].

7. Conclusions and Outlook

The antibacterial and antifungal properties and safety profiles of betel leaves firmly support their application in the development of various products, especially in the food and pharmaceutical industries. The utilization of betel leaves in producing modern-commercial goods could increase the economy of local farmers, specifically in Asia. A good agricultural
process should be applied to the farm to yield standardized raw material and should be followed by a good manufacturing process in industries to form high-quality final products. Additionally, clinical studies should be conducted to support the use of betel leaves in medical fields. Researcher, government, and manufacturer collaboration could facilitate this necessary task.

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