Drug Design, Development and Therapy

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REVIEW

Anti-Infection of Oral Microorganisms from Herbal Medicine of *Piper crocatum* Ruiz & Pav

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Abstract: The WHO Global Status Report on Oral Health 2022 reveals that oral diseases caused by infection with oral pathogenic microorganisms affect nearly 3.5 billion people worldwide. Oral health problems are caused by the presence of *S. mutans, S. sanguinis, E. faecalis* and *C. albicans* in the oral cavity. Synthetic anti-infective drugs have been widely used to treat oral infections, but have been reported to cause side effects and resistance. Various strategies have been implemented to overcome this problem. Synthetic anti-infective drugs have been widely used to treat oral infections, but they have been reported to cause side effects and resistance. Therefore, it is important to look for safe anti-infective alternatives. Ethnobotanical and ethnopharmacological studies suggest that Red Betel leaf (*Piper crocatum* Ruiz & Pav) could be a potential source of oral anti-infectives. This review aims to discuss the pathogenesis mechanism of several microorganisms that play an important role in causing health problems, the mechanism of action of synthetic oral anti-infective drugs in inhibiting microbial growth in the oral cavity, and the potential of red betel leaf (*Piper crocatum* Ruiz & Pav) as an herbal oral anti-infective drug. This study emphasises the importance of researching natural components as an alternative treatment for oral infections that is more effective and can meet global needs.

Keywords: *Piper crocatum*, anti-infection, antibacterial, antifungal, oral pathogen

Introduction

Infections in the oral cavity can be caused by various pathogenic microorganisms such as bacteria, fungi and viruses. Oral infections are caused by pathogenic bacteria such as *S. mutans, S. sanguinis and E. faecalis* that are common in society, one of which is dental caries.[1,2] In 2017, the prevalence of dental caries in permanent teeth per 100,000 population in each country ranged from 20% to more than 50%,[3] while in 2018, the prevalence of dental caries in Indonesia reached 88.8% with root caries at 56.6%. In addition, children aged 5 to 9 years have a prevalence of up to 92.6%.[4] The infection of the oral cavity by pathogenic fungi such as *C. albicans*, which is common in the community, is called candidiasis. The prevalence of candidiasis in Indonesia is around 20–25% and can affect the hair, skin, nails, mucous membranes and other organs such as the oral cavity and oesophagus.[5,6]

Currently, many strategies to treat oral infections are carried out using a synthetic anti-infective agent. However, the use of synthetic anti-infectives has been reported to cause side effects and resistance, such as resistance to the antibiotics ampicillin, amoxicillin, penicillin, cefotaxime, cefazolin, methicillin, erythromycin, lincomycin, clindamycin and vancomycin against *S. mutans*, with the greatest resistance in 87 adults being to amoxicillin (14.8%) and lincomycin (28.7%), and the greatest resistance in 87 children being to penicillin (27.6%) and vancomycin (42.5%).[7–9] With regard to the resistance of several antifungal agents to *C. albicans*, it has been reported that *C. albicans* has a relatively high level of resistance to several antifungal agents, such as nystatin, fluconazole, flucytosine and echinocandin.[10–13]

The ongoing development of anti-infective drugs underscores the paramount importance of efficacy and safety in identifying compounds with no adverse effects. Indonesia's diverse plant life, meantime, affords ample potential as a source of active oral anti-infective compounds.[14] Betel leaf has shown several activities such as antibacterial, antifungal

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and antiviral.[15–17] Ethnobotanical and ethnopharmacological studies have shown that *P. crocatum* Ruiz & Pav has the potential to be exploited as a raw material for oral anti-infective purposes.[18–20]

Anti-Infections

Anti-infective is a broad term that refers to any type of drug that can inhibit or kill the spread of infectious microorganisms. Viruses, bacteria, parasites, and fungi are the four organisms that cause infection. Each organism can cause different health problems. To treat infections, the use of anti-infectives must be adjusted to the organisms that cause infections in certain parts of the body. Anti-infective agents that target the microorganism that causes the infection, such as including antibacterial, antifungal and antiviral.

Antibacterial

Antibacterials are substances that can both inhibit and kill pathogenic bacteria.[21] Antibacterials are classified into two types: those that restrict bacterial growth (bacteriostatic) and those that kill bacteria (bactericidal).[18] Bacterial inhibition take place via a variety of synthetic pathways, including the DNA replication pathway, the transcription, the protein biosynthesis pathway, and the bacterial cell wall biogenesis pathway.[22] Bacterial cell death is caused by the destruction of the peptidoglycan layer. Antibiotics that target the peptidoglycan synthesis pathway are β-lactams and glycopeptides.[23] In addition, the cytoplasmic membrane can also be a viable target for inhibiting bacterial activity. Damaged membranes are usually very difficult to repair. Cationic polymers inhibit many bacteria through electrostatic interactions to the cell membrane followed by hydrophobic bonds to the lipid tails which cause membrane lysis. DNA synthesis is the basis for bacterial replication, DNA damage will have a negative impact on DNA synthesis and replication. Furthermore, protein synthesis occurs in bacterial ribosomes, making ribosomes become one of the targets for inhibitory compound inhibition.[24]

But today some bacteria have developed various strategies to damage or tolerate attacks from an antibiotic, where bacteria work directly to damage or modify the structure of antibiotics so that it can avoid inhibition of growth and bacteria can carry out their lives. One of them is through degradation and enzymatic modification, this enzymatic degradation and modification becomes an effective means of antibiotic resistance and has caused resistance to several main classes of antibiotics that exist today, including β-Lactam and Aminoglycoside antibiotics. The hydrolysis process, carried out by a variety of hydraulics, is known to be able to deactivate some antibiotics. Co-evolution of β-Lactamase and β-Lactam antibiotics is an example of the portrayal of competition between antibiotics and antibiotic resistance. In the process β-Lactamase acts as a weapon to degrade β-Lactam type antibiotics, for example such as penicillin, carbapenem, and cephalosporin. β-Lactamase works by breaking the β-Lactam core ring, both through serine nucleophilic attacks or through metal-based activation from water molecules.[25]

Anti-Fungal

Fungal infections can occur in people who have been exposed to any circumstances in their lives. Predisposing factors for this infection can occur for no apparent reason. However, people are often exposed because of their environment, behaviour, or a weakened immune system. Clinically, fungal infections can be classified according to the site of infection,[26] that is:

Systemic mycoses (systemic fungal infections) include deep mycoses (eg aspergillosis, blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, mucormycosis, paracoccidioidomycosis and candidiasis) and subcutaneous mycoses (eg chromomycosis, mycetoma and sporotrichosis).[27–29]

Dermatophytes, fungal infections of the skin, hair and nails, usually caused by Epidermophyton and Microsporum.[30–33]

Mucocutaneous mycoses, which are fungal infections of the mucous membranes and moist skin folds, usually caused by Candida.[34–36]

Antifungals, also known as antimycotics, are commonly used to kill/deactivate fungi and treat fungal infections. According to clinical indications, antifungal drugs are classified into two groups, the first group is antifungals for systemic infections, including: amphotericin B (**1**), flucytosine (**2**), ketoconazole (**3**), fluconazole (**4**), miconazole (**5**), and

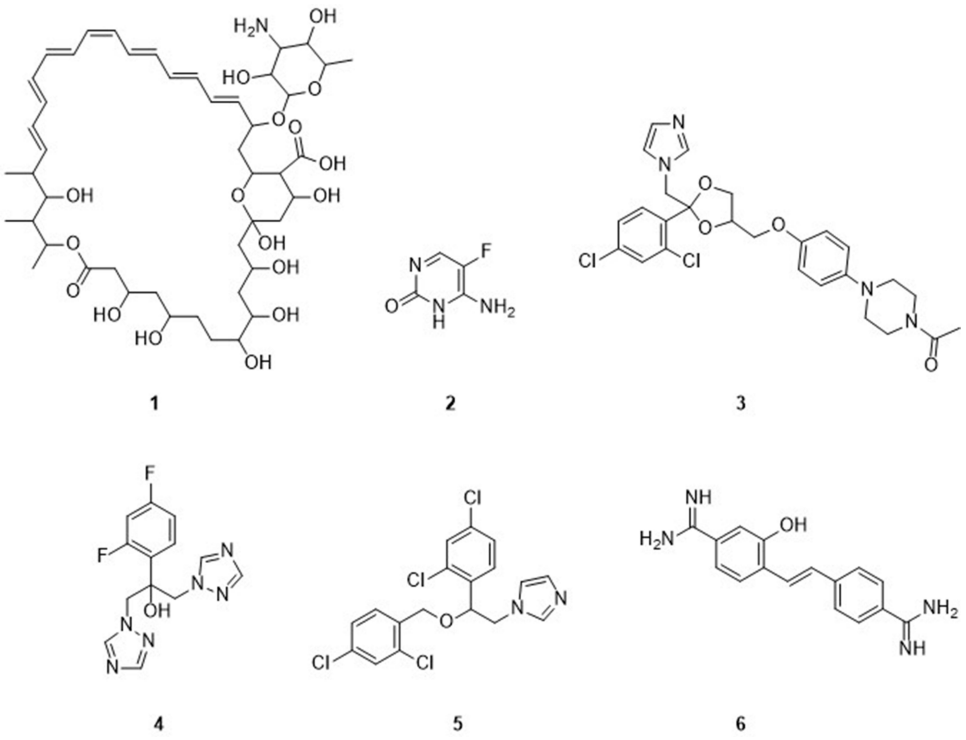

Figure 1 Structure of an antifungal compound with a mechanism of systemic infection Amphotericin B (**1**), Flucytosine (**2**), Ketoconazole (**3**), Fluconazole (**4**), Miconazole (**5**), and Hydroxystilbamidine (**6**).[27]

hydroxystilbamidine (**6**),[27] the structure of the compounds are as shown in Figure 1. The second group of antifungals for dermatophyte and mucocutaneous infections includes griseofulvin (**7**), clotrimazole (**8**), econazole (**9**), isoconazole (**10**), tioconazole (**11**), bifonazole (**12**), nystatin (**13**), tolnaftate (**14**) and other topical antifungals candicidin (**15**), undecylenic acid (**16**) and natamycin (**17**),[37] the structure of the compounds is shown in Figures 2–3.

The azole group can be separated into two categories depending on the number of nitrogens in the azole ring. The imidazole group, comprising ketoconazole, miconazole, and clotrimazole, has two nitrogens, while the triazole group, including itraconazole, fluconazole, voriconazole, and posaconazole, has three nitrogens.[38,39] Both groups share the same range and mode of action. Triazoles are metabolised at a slower rate and result in fewer side effects compared to imidazoles. Due to this advantage, researchers are attempting to develop a new class of triazoles instead of imidazole.[40–42] In general, the azole group serves to inhibit the biosynthesis of ergosterol, which is the primary sterol responsible for maintaining the integrity of the fungal cell membrane. Azole group antibiotics function by inhibiting the cytochrome P 450 enzyme, C-14-α-demethylase. This particular enzyme is responsible for converting lanosterol into ergosterol, as shown in Figure 4. This makes the fungal cell wall permeable, leading to fungal damage.[43]

Polyene molecules contain numerous conjugated double bonds. Macrocyclic polyenes with hydroxylated ring components in a conjugated system are known as polyene antifungals.[45–47] These antifungals bind to sterols in the fungal cell membrane, specifically ergosterol. This results in a decreased fluidity of the inner membrane and a more crystalline state due to an alteration in the transition temperature of the fungal cell membrane. Conversely, in its typical state, the sterol membrane enhances the toughness of the phospholipid bilayer, making the plasma membrane denser. Nevertheless, the sterol-polyene group antifungal bond induces a reduction in the stickiness of the phospholipid bilayer.[40–42,48] Thus, the fungal cell contains monovalent ions, such as K^+, Na^+, H^+, and Cl^-. Organic molecules exit the cell due to membrane permeability, resulting in cell death.[45,49] It is important to note that this process occurs due to a leaky membrane and is not a voluntary act of the cell.

A new class of synthetic antimycotics referred to as allylamines are identified by their capacity to function as squalene epoxidase inhibitors.[51–53] Naftifine serves as an exemplar of an allylamine antifungal and was the first

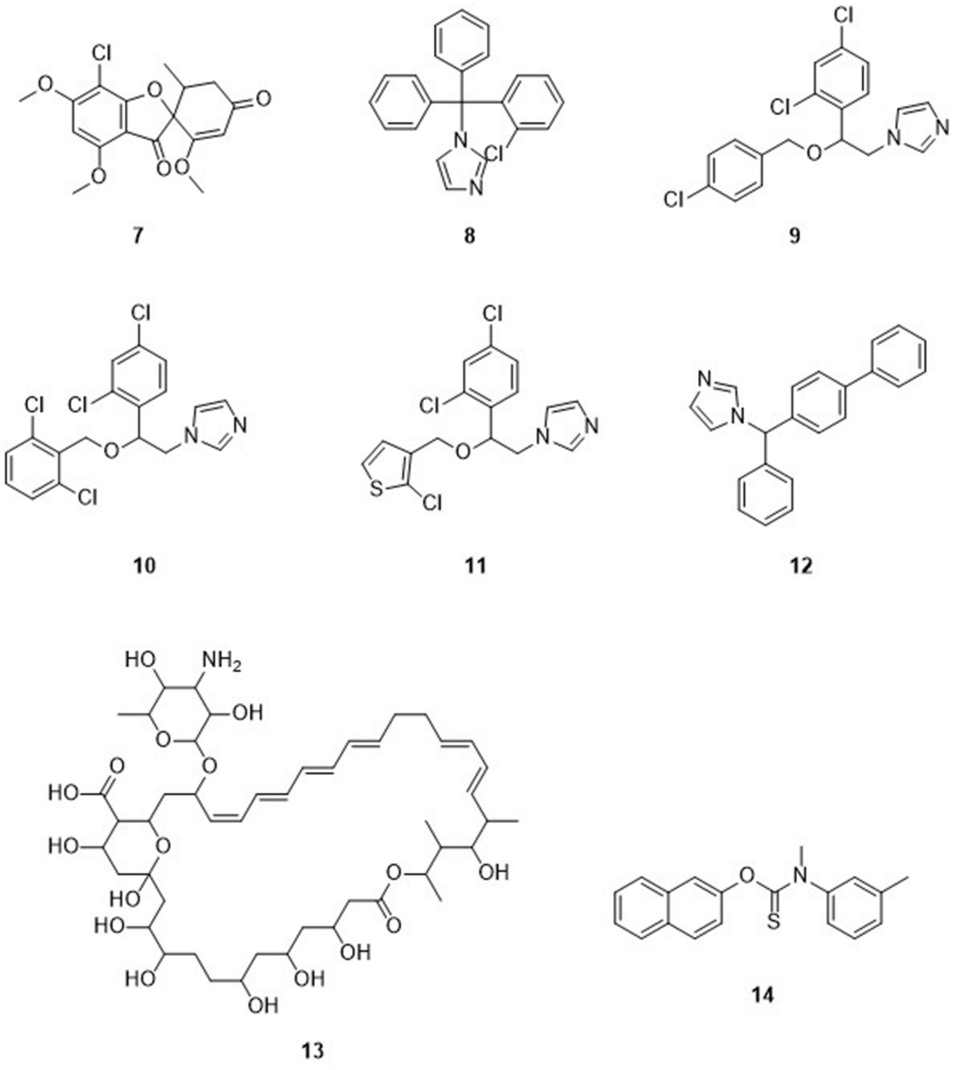

Figure 2 Structure of an antifungal compound with a mechanism of systemic infection: Griseofulvin (**7**), Clotrimazole (**8**), Econazole (**9**), Isoconazole (**10**), Tioconazole (**11**), Bifonazole (**12**), Nystatin (**13**), and Tolnaftate (**14**).[37]

substance discovered in 1974, during routine research, to possess antifungal properties. Naftifine's potent antifungal activity both in vitro and in vivo led to its clinical development as a topical antimycotic.[54] Naftifine is the basis of a significant project which aims to enhance the effectiveness of antifungals, especially for oral administration. Allylamine antifungals work akin to azoles, but they act earlier on the ergosterol production pathway.[53,55,56] They impede squalene epoxidase, an enzyme responsible for converting squalene to ergosterol, therefore disrupting the development of the fungal cell wall.[57]

Semisynthetic cyclic lipopeptides known as echinocandins prevent cell wall formation by non-competitive inhibition of the (1,3)-β-D-glucan synthase complex, as shown in Figure 5.[58–60] The target inhibition of 1,3-β-D-glucan synthase is specific for fungi because the enzyme 1.3-β-D-glucan synthase is absent in humans, this demonstrates the good tolerability and safety of echinocandins compared to other antifungal classes.[61] Most Candida species are killed by echinocandins both in vitro and in vivo, whereas Aspergillus species are only inhibited. Globally, the MIC necessary to prevent the growth of 90% of bacteria (MIC90) is 2 g/mL. Additionally, the MIC required to inhibit the development of 50% of microorganisms, including echinocandins to the Candida group of fungi, is 0.5 g/mL. It should be noted that the MICs for *C. lusitaniae* and *C. parapsilosis* were higher than those for *C. albicans*.[23,26,62]

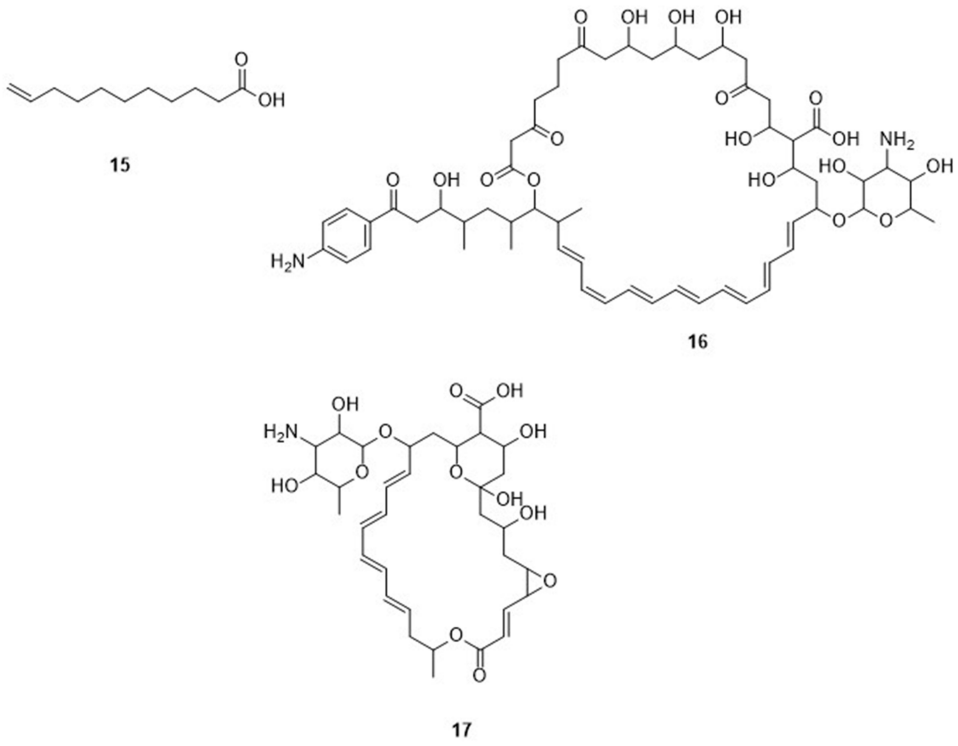

Figure 3 Structure of an antifungal compound with a mechanism of dermatophyte and mucocutaneous infections: Candicidin (**15**), Undecylenic acid (**16**), and Natamycin (**17**).[37]

Piper crocatum Ruiz & Pav as Anti-Infection Herbal

Piper crocatum Ruiz & Pav

Numerous research studies have reported the effectiveness of Red Betel leaf in both ethnobotanical and ethnopharmacological contexts. Red Betel has been applied in numerous traditional treatments, including but not limited to treating toothaches, vaginal discharge caused by fungi, ulcers, diabetes, sore eyes, and shortness of breath, as well as other traditional remedies.[64,65] Ethnopharmacological studies have shown that Red Betel exhibits a range of activities, including antifungal, antibacterial, antioxidant, antihyperglycemic, antiinflammatory, and others.[66–69] These findings led to the reclassification of Red Betel from an ornamental plant to a medicinal plant.[70] The phytochemical analysis revealed that *P. crocatum* Ruiz and Pav contain secondary metabolites, such as flavonoids, essential oils, alkaloids, saponins, tannins, terpenoids, and phenolic compounds, which substantiated the various bioactivities previously reported. The shape and characteristics of the Red Betel leaf are shown in Figure 6.

Several experiments isolated the components of bioactive compounds from Red Betel were carried out by Emrizal et al, (2014), Arbain et al, (2018), Li et al, (2019), and Chai et al, (2021), where each of these researchers has succeeded and reported the results of their isolation, namely in the study of Emrizal et al, 2014 two compounds were obtained from the Red Betel plant which were later identified and known as β-sitosterol (**18**) and compound 2 -(5'),6'- dimethoxy-3', 4'-methylenedioxyphenyl)-6-(3',4',5 trimethoxyphenyl)-dioxabiclo [3,3,0] octane (**19**). In the n-hexane, ethyl acetate, butanol, and methanol fractions, the IC_{50} values of both of these compounds were reported to be 2.04; 1.34; 2.08; and 27.40 g/mL, respectively.[71] According to a study done by Arbain et al, (2018) isolated and reported the biclo [3.2.1] neolignan octanoid compounds of the guianine type, (1'*R*, 2'*R*, 3'*S*, 7*S*, 8*R*)-Δ5',8'-2'-acetoxy-3,4,5,3', 5'-pentamethoxy -4'-oxo-8'.1,7.3-neolignan (**20**) and (1'*R*, 2'*R*, 3'*S*, 7*S*, 8*R*)-Δ5', 8'-2'-hydroxy-3, 4, 5, 3', 5'-pentamethoxy-4'-oxo-8.1', 7.3'-neolignan (**21**).[72] Whereas in the study of Li et al, (2019) it was reported that seven compounds had been isolated consisting of four phenolic compounds (**22, 23, 24, 25**), one mono-terpene compound (**26**), one sesquiterpene compound (**27**), one flavonoid compound C-glycosides (**28**) of the species Red Betel.[73] In addition, in a study by Chai et al, (2021)

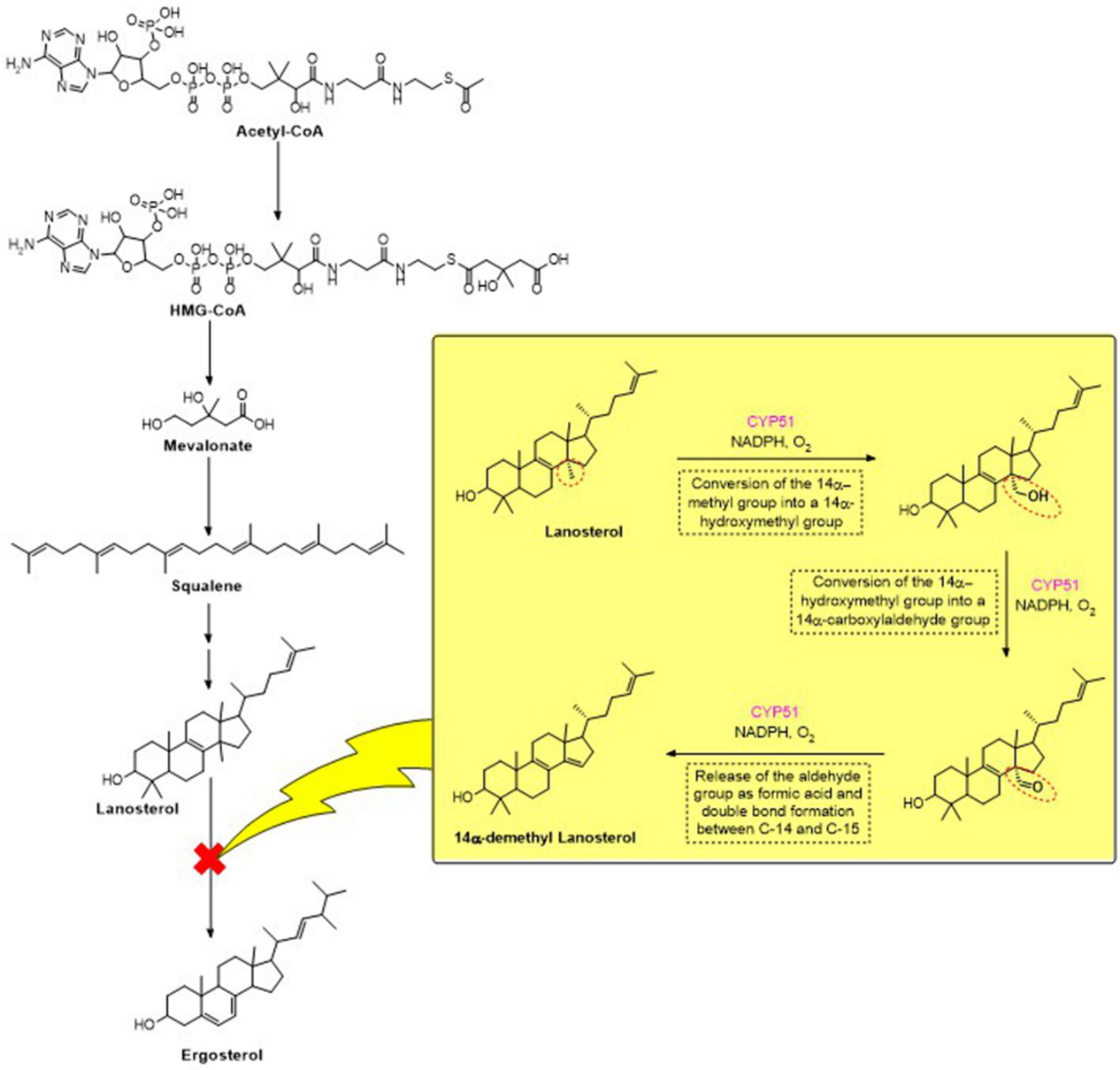

Figure 4 Summarized synthesis of ergosterol, the fungal sterol, and detailed steps of CYP51 conversion of lanosterol to 14α-demethyl lanosterol.[44]

reporting the results of isolating MeOH extract from *P. crocatum* Ruiz & Pav, a macrophylline-type neolignan compound was found, namely pipcroside A (**29**), pipcroside B (**30**), pipcroside C (**31**), and crocatin B (**32**).[74] The structures of the isolated compounds in previous studies are illustrated in Figures 7 and 8 respectively.

Anti-Infection Activity of Red Betel Extract

Infections can be caused by various microorganisms, including bacteria, fungi, and viruses. Such infections can cause long-lasting health problems due to pathogenic microorganisms that are never fully resolved. The variety of infectious agents highlights the seriousness of this problem. Furthermore, the lack of strategic planning in treatment can lead to resistance and side effects, emphasizing the need to find new, effective, and efficient oral anti-infective agents. Red betel leaves have been used to treat various infections caused by bacteria, fungi, and viruses. Several research reports have identified the potential of red betel leaves as antifungal, antibacterial, and antiviral, as discussed in more detail in their respective sections.

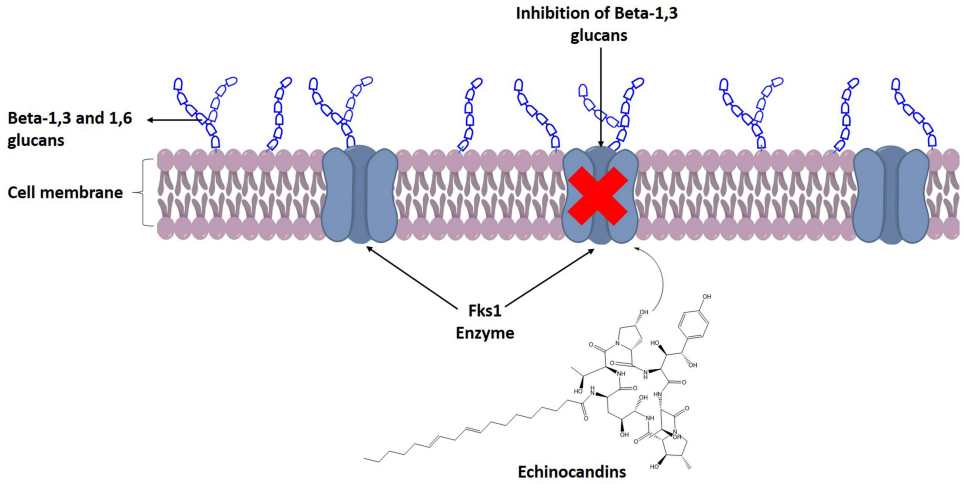

Figure 5 Mechanism of inhibition by antifungal echinocandins by inhibiting the synthesis of beta-1,3 glucan synthase.
Notes: Data from De Cândido et al.[63]

Figure 6 Red Betel leaves.

Anti-Fungal Activity of Red Betel Extract

Several bioactivities of Red Betel leaf extract have been reported, including the inhibition of several species of pathogenic fungi. This demonstrates the potential for Red Betel leaf as an anti-fungal agent. The anti-fungal activity of *P. crocatum* Ruiz & Pav is derived from the secondary metabolite compounds contained therein,[75] as reported by multiple previous studies listed in Table 1.

The anti-fungal activity of secondary metabolites in Red Betel leaves has also been demonstrated in the study conducted by Golam et al (2022). This study tested several secondary metabolites from Red Betel leaves, listed in Table 2, via molecular docking at the Sap 5 (Secreted Aspartyl Proteinase) receptor, which is one of the virulence factors of the fungus *C. albicans*.

The researchers used pepstatin ligand (CID_5478883) as a standard inhibitor, which has a binding energy of 9.484 kcal/mol. Molecular docking results indicate that two test ligands, CHEMBL216163 (CID_44418672) and MLS000557666 (CID_1077234), have binding energies above pepstatin. The binding energies of the two test ligands are −9.644 kcal/mol (CID_44418672) and −9.525 kcal/mol (CID_1077234). The CHEMBL216163 ligand

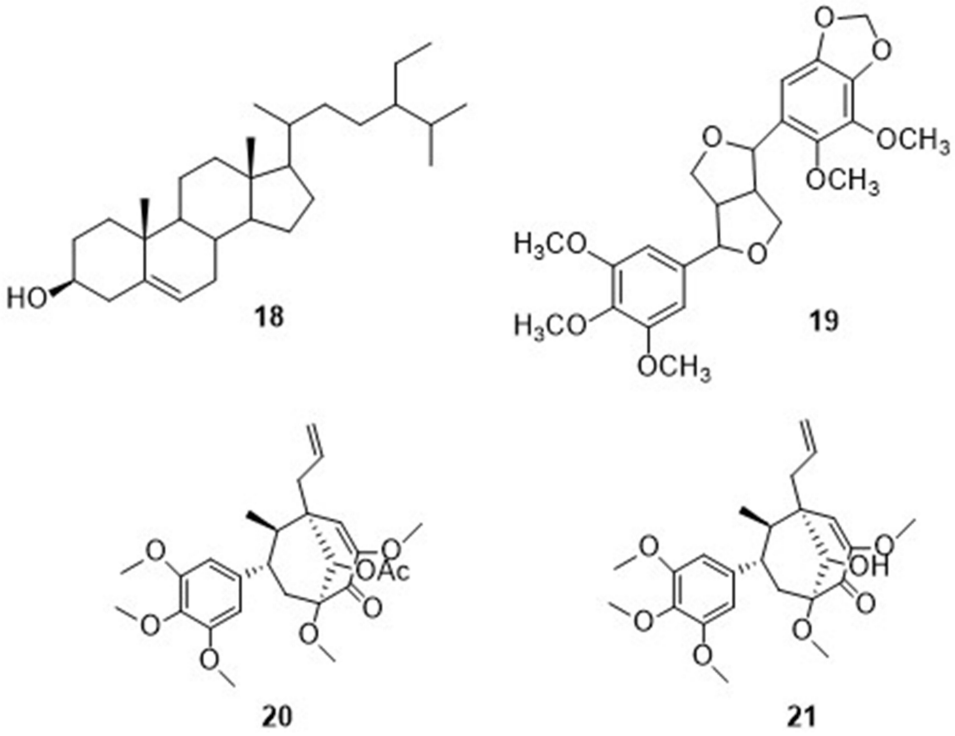

Figure 7 Structure of the compound β-Sitosterol (**18**), 2 -(5'),6'-dimethoxy-3', 4'-methylenedioxyphenyl)-6-(3',4',5 trimethoxyphenyl)-dioxabiclo [3,3,0] octane (**19**).[71] Structure of (1'*R*, 2'*R*, 3'*S*, 7*S*, 8*R*)-Δ5',8'-2'-acetoxy-3,4,5,3', 5'-pentamethoxy-4'-oxo-8'.1,7.3-neolignan (**20**) and (1'*R*, 2'*R*, 3'*S*, 7*S*, 8*R*)-Δ5', 8'-2'-hydroxy-3, 4, 5, 3', 5'-pentamethoxy-4'-oxo-8.1', 7.3'-neolignan (**21**).[72]

(CID_44418672) interacts with the Sap 5 receptor through hydrogen bonds, electrostatic bond type salt bridges, and attractive charges on the amino acid residue Asp303. Electrostatic bonds form at amino acid residues Asp86, Asp218, and Tyr225. Hydrogen bonds also form at amino acid residues Gly85, Asp86, Tyr225, and Gly34, with bond distances of 2.31, 2.96, 2.42, and 2.28 Å, respectively. Additionally, ligand MLS000557666 (CID_1077234) binds to the Sap 5 receptor, forming a hydrogen bond with the Gly3 amino acid residue (2.86 Å). Apart from hydrogen bonds, two electrostatic bonds are also formed on the amino acid residue Asp86, with distances of 3.53 and 3.86 Å. Hydrophobic interactions occur at amino acid residues Tyr84, Ile305, Lys193, and Tyr225.

Additionally, a small Kd value indicates a stronger bond between the ligand and the receptor.[80] The ligand efficiency of CHEMBL216163 and MLS000557666 is 0.2922 and 0.3528 respectively, while that of pepstatin is 0.1976 (crystallographic ligand). The ligand efficiency range for pepstatin (based on binding energy) is 0.2506 to 0.7214. The research results indicate that Red Betel leaf extract contains several components, including CHEMBL216163 and MLS000557666, which have potential as Sap 5 inhibitors, thereby reducing the virulence of *C. albicans*.[81] The presence of Sap 5 plays a crucial role in supporting the dimorphic nature of *C. albicans*. Sap 5 is involved in the adhesion mechanism.

The results of the isolation of antifungal compounds from *P. crocatum* leaves will be reported by Tessa et al, 2023. The isolated compounds include two new active compounds, **33** and **34**, as well as the previously known compound 35. The structures of these compounds were identified using spectroscopic methods, and their bioactivity was studied in vitro against the fungus *C. albicans* ATCC 10231 and in silico against the ergosterol enzyme, which is an important component of fungal cell membranes. In vitro antifungal studies were conducted against *C. albicans* ATCC 10231 using ketoconazole as a positive control, while methanol and sterile water were used as negative controls. The inhibition zone test results for compounds 33, 34, 35 and ketoconazole at a concentration of 2.5% (w/v) were 8.9, 9.4, 9.7 and 30.0 mm, respectively. At a concentration of 5% (w/v), the inhibition zones of compounds 33, 34, 35 and ketoconazole were 10.0, 12.4, 12.8 and 31.3 mm, respectively. Inhibition zones of 11.9, 13.0, 14.5 and 32.2 mm were produced by

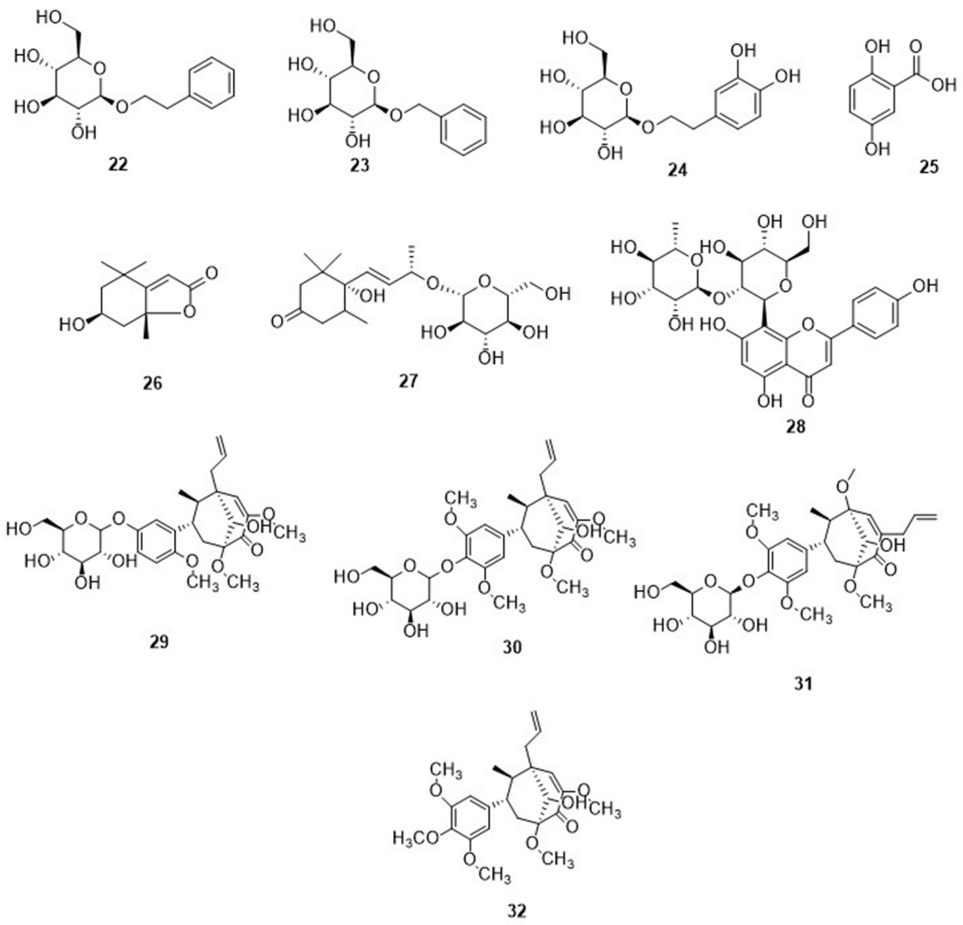

Figure 8 Structure of the compound β-phenylethyl β-D-glucoside (**22**), Benzyl-β-D-glucoside (**23**), hydroxytyrosol-1glucopyranoside (**24**), Gentisic acid (**25**), Loliolide (**26**), (6S,9S)-roseoside (**27**), Vitexin 2″-*O*-rhamnoside (**28**),[73] and structure of Pipcroside A (**29**), Pipcroside B (**30**), Pipcroside C (**31**), Crocatin B (**32**).[74]

concentrations of 10% (w/v) compounds **33, 34, 35** and ketoconazole. Compound 35 exhibited the best potential in inhibiting *C. albicans* compared to compounds **33** and **34**, with a strong inhibitory category at a concentration of 10%, as seen from the results of the inhibition zone test.

The MIC/MFC testing of each compound resulted in 0.46/1.8, 0.62/2.5, 0.31/1.2 and 0.00005/0.0001% b/v for compounds **33, 34, 35** and ketoconazole, respectively. The results of the inhibition zone tests indicate that antifungal compound **35** is the most active compared to **33** and **34**. In addition, in silico research showed that compound **35** had a higher ΔG than the positive control, with compounds **33** and **34** having values of −11.14, −12.78, −12.00 and −6,89 Kcal/mol for ERG1, ERG2, ERG11 and ERG24, respectively. Compound **35** also has the best Ki values of $6.8x10^{-3}$, $4x10^{-4}$, 1.6×10^{-3} and 8.88 μM. This occurs because ligand 35 interacts with the receptor, specifically on thirteen residues with the same amino acids as ketoconazole leucine B: 376 on π-alkyl. Van der Waals bonds bind phenylalanine B:233, B:380, and B:228, proline B:230, serine B:378 and B:507, histidine B:310, threonine B:311, leucine B:121, glycine B:65, tyrosine B:505, and serine B:506.

Furthermore, the research conducted by Tessa et al, 2023 is supplemented by ADMET analysis of compounds **33**–**35**, which are predicted to be safe as a potential new drug candidate and meet the five Ro5 parameters. Based on the reported data, *P. crocatum* shows promising potential as an antifungal agent. It can be considered as a new treatment option for *C. albicans* infections, with a mechanism of action similar to that of azole antibiotics, by inhibiting fungal ergosterol.[82] The three components' structure is as illustrated in Figure 9.

Table 1 Anti-Fungal Activity of Several Groups of Secondary Metabolites in Red Betel Leaves

No.	Secondary Metabolite	Types of Fungi	Result	References
1	Alkaloids Flavonoids Polyphenols Quinones Saponins	*C. albicans*	MIC and MBC values ranged as follows *C. albicans* (1.25–2.5% w/v).	Kusuma et al, 2017[76]
2.	Alkaloids Tannins Saponins Flavonoids	*C. albicans*	*P. crocatum* Ruiz & Pav leaf extract was able to inhibit of *C. albicans* with MIC at a concentration of 25% and MBC at a concentration of 100% of *P. crocatum* Ruiz & Pav extract.	Rezeki et al, 2017[77]
3.	Essential Oil Flavonoids Tannins Saponins	*C. albicans*	At 50, 60, and 70% concentrations, a methanol extract of Red Betel leaves was able to inhibit with inhibition diameters of 12.17, 13.17, and 21.17 mm, respectively. At 30, 40, 50, 60, and 70% concentrations, the inhibition zone diameters were 7.83, 8.40, 9.00, 9.87, and 7.87 mm, respectively.	Rachmatiah et al, 2018[78]
4.	Flavonol Chalcone Anthocyanin	*C. albicans* ATCC 10231	The ethanol extract of red betel leaves at a concentration of 40% v/v had the most effective inhibition against the growth of the fungus *C. albicans* ATCC 1023, with a maximum diameter of the inhibition zone of 13.3 mm.	Suri et al, 2021[79]

Table 2 The Test Ligand Used Was Compounds of Red Betel (*Piper crocatum* Ruiz & Pav)

Compounds (Ligand Test)	PubChem ID
Glabrescione	44,257,338
Catechin	73,160
Caryophyllene	5,281,515
Germacrene	5,317,570
Elemicin	10,248
Propionic acid	1032
Neophytadiene	10,446
Butyl ethanoate	31,272
Alfa pinene	82,227
Limonene	22,311
Cineole-1,8	2758
Terpinene-4-ol	11,230
6XO32ZSP1D	75019
Ethyl L-serinate hydrochloride (1:1)	2,729,185
Schisandrin B	108130
Columbin	188,289

(*Continued*)

Table 2 (Continued).

Compounds (Ligand Test)	PubChem ID
ZINC8756459	6,070,252
MLS000557666	1,077,234
Oprea1_462146	2,865,476
CHEMBL216163	44,418,672
1,1'-(1,4-Butanediyl) bis (2,6- dimethyl-4-[(3-methyl-1,3- benzothiazole-2(3H)- ylidene)methyl]pyridinium)	3,414,657
Methyl eugenol	7127
4-methoxyindole	138,363
Leucylleucinamide hydrochloride (1:1)	16,219,591
5-isopropyl-3- pyrazolidinecarbohydrazide hydrochloride (1:1)	61,440,504
1H-pyrazole-1- carboximidamidmidhydrochloride	2,734,672
Protocatechuic acid	72
N1-(5-methylisoxazole-3- yl)ethanediamide	2,805,645
CHEMBL3217136	90,665,169
2-(4-morpholinylmethyl)aniline sulfate hydrate	45,595,316
SCHEMBL569003	14,839,452
L-Arginine hydrochloride	66,250
1-(1,4-Dithian-2-ylmethyl)-3- (3-methoxypropyl)thiourea	116,510,220
ALBB-026042	1,511,955

Antibacterial Activity of Red Betel Extract

Many studies have highlighted the ethanol and methanol extracts of Red Betel leaves as a potent antibacterial source against several gram-positive and gram-negative bacteria. Some reports on the antibacterial potential of red betel leaf extracts are summarised in Table 3 below.

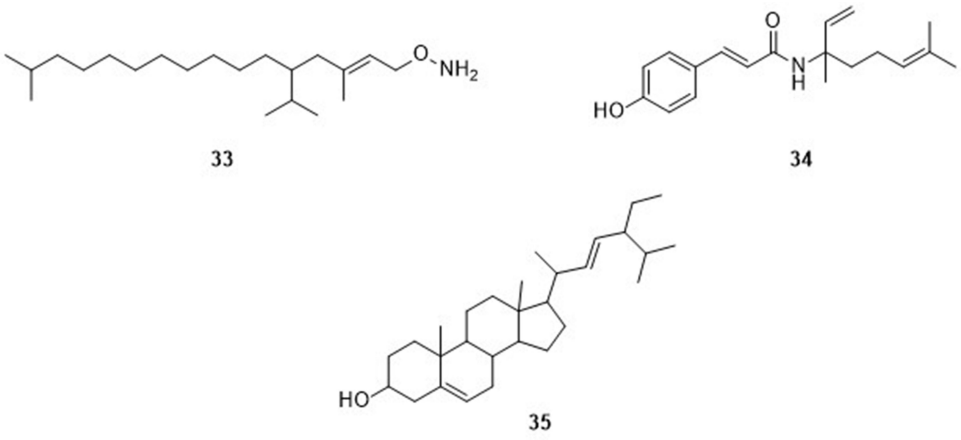

Figure 9 Structure of antifungal constituents of *P. crocatum*, Piperyamide A (**33**), Piperyamine A (**34**), and Stigmasterol (**35**).[82]

Table 3 Antibacterial Activity of Several Groups of Secondary Metabolites in Red Betel Leaves

No.	Extract	Secondary Metabolite	Bacteria	Result	References
1	Red Betel ethanol extract	Flavonoids Tannins Alkaloids Essential oils	*Staphylococcus epidermidis*	Red Betel leaf extract (*P. crocatum* Ruiz & Pav) at concentrations of 20%, 40% and 80% were able to inhibit the growth of *Staphylococcus epidermidis* which causes urinary tract infections.	Sawitri et al, 2022[83]
2.	*n*-hexane fraction	Tannins	*Escherichia coli* pBR322	The best antibacterial activity was produced by the *n*-hexane fraction at a concentration of 1000 ppm with an inhibition zone of 2.40 mm ± 0.14. The best minimum inhibitory concentration was produced by the *n*-hexane fraction at a concentration of 100 ppm with an inhibition zone of 0.60 mm ± 0.56	Chairunisa et al,2022[84]
3	Red Betel ethanol extract	Tannins Flavonoids Saponins Triterpenoids	*Fusobacterium nucleatum* ATCC 25586	Red Betel leaf ethanol extract appeared to have weak inhibition at 10 and 15% concentrations and strong inhibition at 20, 25, and 30% concentrations (11.4, 15.6 and 19.3 mm) against *F. nucleatum* ATCC 25586.	Ramadhan et al, 2022[85]
4	Red Betel ethanol extract	Alkaloids Steroid Tannins	*B. Subtilis* *P. aeruginosa*	The best zone of inhibition at a concentration of 100 mg/mL is 1.12–1.32 mm (*B. subtilis*) and 1.03 mm (*P. aeruginosa*).	Puspita et ai., 2019[86]
5	Red Betel ethanol extract	Alkaloids Tannins PolyphenolEssential Oils	*Staphylococcus aureus*	Red Betel leaf extract concentration of 12.5% improved histopathology infected with *S. aureus*.	Wurlina et al, 2019[87]
6	Red Betel methanol extract	Tannins	*Staphylococcus aureus*	Methanol extract at 100% concentration inhibited 12.3 mm	Soleha, 2018[88]
7	Red Betel ethanol extract	Alkaloids Flavonoids Polyphenols Quinones Saponins	*E. coli* *P. aeruginosa* *S. aureus*	Concentrations of 60 and 80% inhibited *E. coli* 12.33 and 13.17 mm, *P. aeruginosa* 15.33 and 17.23 mm and *S. aureus* 14.73 and 17.33 mm, respectively.	Kusuma et al, 2017[76]
8	Red Betel methanol extract	Alkaloids Tannins Essential Oils Flavonoids	*S. viridans* and *Porphyromonas gingivalis*	Red betel leaf extract was able to inhibit the growth and infection of *S. viridans* and *P. gingivalis* at concentrations of 8.42 and 10.34 mm respectively	Pujiastuti et al, 2015[89]

Ramadani's research (2018) states that red betel leaves have total tannins containing active compounds, namely methyl eugenol, *L*-(+)-arginine hydrochloride and protocatechuic acid. Eugenol can function as an antimicrobial, antiseptic, and other medicinal raw materials.[84] Arginine is one of the amino acids that has a great influence on peptide activity for antibacterial.[90] Tannin is a type of polyphenol compound that is soluble in water and organic solvents and is widely found in plants. Tannins act as antibacterial agents by inhibiting the enzymes reverse transcriptase and DNA topoisomerase so that bacterial cells cannot form. Tannins also target cell wall polypeptides so that the cell wall becomes less perfect.[91,92] In addition, tannin compounds can damage bacterial cell membranes by disrupting extracellular proteins and forming complex compounds. Microorganisms growing under anaerobic conditions require iron for various functions, including the reduction of DNA ribonucleotide precursors. The strong iron binding capacity of tannins may also interfere with the iron binding process required by bacteria.[93]

The mechanism of antibacterial action of the flavonoid compounds found in red betel leaf works by inhibiting the synthesis of nucleic acids so that bacterial cells cannot form, as well as inhibiting the function of the cytoplasmic membrane and the energy metabolism of bacteria.[94] In addition, flavonoids that are lipophilic can also act as antibacterials by forming complexes with extracellular proteins and bacterial cell walls that interfere with the permeability of bacterial cell walls.[95]

Red betel leaf extract contains steroidal compounds. Steroid compounds inhibit bacterial growth through their role in binding to lipid membranes, disrupting membrane sensitivity and causing leakage in bacterial liposomes.[96] Alkaloid compounds are also found in red betel leaf extract. Alkaloids are compounds containing one or more nitrogen atoms that are formed and usually exist in combined form as part of a cyclic system. Alkaloid compounds can inhibit the growth of gram-positive and gram-negative bacteria by inducing cell lysis and changes in bacterial morphology.[97]

The mechanism of saponin as an antibacterial is that it can associate with lipopolysaccharide in the bacterial cell wall, thereby increasing the permeability of the cell wall and reducing the surface tension of the cell wall, causing the wall to lyse and the antibacterial substance to easily enter the cell, resulting in the death of the bacteria.[98] The mechanism of action of triterpenoid compounds as antibacterial agents is to react with porins, which are transmembrane proteins in the outer membrane of the bacterial cell wall, forming strong polymer bonds and damaging the porins. Damage to the porins, which are the entry and exit points for the compounds, reduces the permeability of the bacterial cell walls and causes the cells to lack nutrients, so that bacterial growth is inhibited or killed.[99]

Furthermore, this study specifically tries to summarise the antibacterial potential of *P. crocatum* Ruiz & Pav, focusing on the impact of Red Betel on *S. mutans, S. sanguinis*, and *E. faecalis*, bacteria that cause oral infections. Rizkita et al (2017) distilled green and red betel leaf oil, identified its components, and tested its activity against *S. mutans* bacteria. The gas chromatography analysis of green betel oil revealed 16 compound peaks, including camphena (**36**), Sabinene (**37**), cariophyllen (**38**), α-Humulen (**39**). The gas chromatography analysis of red betel oil revealed the presence of 35 compound peaks. Two main peaks were identified as belonging to the terpenoid group, namely Sabinene (**37**) and Mirsen (**40**).

Antibacterial activity test was conducted on Red and Green Betel Oil against *Streptococcus mutans* using varying concentrations of 100, 75, 50, and 25%. Propylene glycol solvent was used as a negative control, and amoxicillin was used as a positive control. Research has demonstrated that red betel leaf oil can effectively inhibit the growth of *S. mutans* bacteria. The diameter of the inhibition zone decreases as the concentration of the oil decreases, with values of 7.1, 6.2, 4.3, and 3.6 mm for the highest to the lowest concentrations, respectively. The active compound's structure is illustrated in Figure 10.

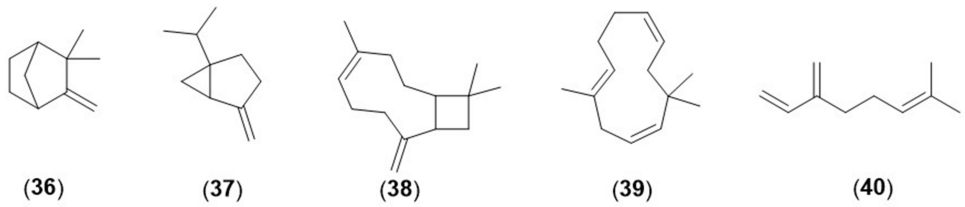

Figure 10 Structure of the compound camphene (**36**), sabinene (**37**), caryophyllene (**38**), α-Humulen (**39**), Mirsen (**40**).[100]

The inhibitory effect of green betel leaf oil was observed, with inhibition zones ranging from 7.1 mm to 10.5 mm for the smallest to largest concentrations, respectively. *Piper betle* and *Piper crocatum* have similar content, but the yield difference between green betel oil (*Piper betle* L.) and red betel oil (*Piper crocatum*) is 0.8 and 0.3%, respectively. It is suggested that Piper betle has a higher concentration of active substances than *Piper crocatum*, which may contribute to its superior effectiveness against *Streptococcus mutans*.

The compounds identified in the GC-MS analysis, namely Sabinene, Kamfen, β-Kariophyllen, β-Salinen, α-Salinen and Mirsen, belong to the monoterpene and sesquiterpene groups and possess antimicrobial properties. Sesquiterpene compounds are hydrophobic and can disrupt bacterial cell integrity by reducing intracellular ATP reserves and lowering cell pH. These compounds are absorbed and penetrate into bacterial cells, causing bacterial cell death. The deposition and denaturation of proteins cause lysis of bacterial cell membranes. Compounds found in red and green betel leaf oil are believed to inhibit bacterial growth by destroying and inhibiting bacterial cell walls. Gram-positive bacteria are more susceptible to penetration by antibacterial agents as they do not have an outer membrane in their cell walls. Bacteria, including *S. mutans*, which is a Gram-positive bacterium, have simple cell walls consisting of 60–100% peptidoglycan, which is made up of *N*-acetyl glucosamine and *N*-acetyl muramic acid. The report suggests that red betel leaves, particularly red betel leaf oil, have potential as an antibacterial agent with moderate inhibition criteria against *S. mutans*. It is important to note that this evaluation is based on objective criteria and not subjective opinions.

Antiviral Activity of Red Betel Extract

Red betel plants (*Piper crocatum* Ruiz & Pav) have been proven to inhibit the growth of pathogenic bacteria and fungi. According to Akbar et al (2022), *in silico* testing of Red Betel leaf components against the SARS CoV-2 virus showed anti-viral activity. This research used four receptors from SARS CoV-2, including 5R7Y, 7JKV, 7TLL, and 7VH8. The 5R7Y receptor has 12 test compound ligands with lower docking scores than natural ligands, including Proanthocyanidin, Catechin, Asiaticoside, Myricetin, Quercetin, Fisetin, Rhamnazin, Isorhamnetin, Pachypodo, Kaempferol, Linalool, and Aurone. This shows that the test compound's ligand for the 5R7Y receptor has high potential to be a candidate for anti-SARS CoV-2.

None of the tested ligands for the 7JKV receptor had lower docking values than the natural ligands. When compared to the docking scores of comparator drugs, the scores are still far behind, especially for remdesivir, nirmatrelvir, and molnupiravir. However, the docking scores for favipiravir were much better. Because there is no ligand of the test compound that has a lower docking score than the natural ligand of the 7JKV receptor, it is unlikely that the ligand of the test compound will be a candidate for anti-SARS CoV-2 at the 7JKV receptor. On the other hand, only one ligand of the test compound, Asiaticoside, had a lower docking score than the natural ligand at the 7TLL receptor. The results suggest that the test compound ligand is unlikely to be a strong candidate for anti-SARS CoV-2 treatment at the 7TLL receptor. Additionally, only one ligand in the test compound, Asiaticoside, had a lower docking score than the natural ligand at the 7VH8 receptor. The ligand docking score of the test compound on the 7VH8 receptor was smaller than that of the natural ligand, indicating that the ligand is unlikely to be a viable anti-SARS CoV-2 candidate. Research reports show that compounds contained in the red betel plant (*Piper crocatum* Ruiz & Pav) show antiviral activity, especially at the 5R7Y receptor.[101]

Diniatik et al (2011) reported that the ethanol extract of Red Betel leaves inhibited infections caused by the Newcastle Disease virus at a concentration of 10 µg/mL. The mechanism of action of the virus suggests that the ethanol extract of red betel leaves interferes with viral mRNA, inhibiting the formation of viral capsids. This is because the Newcastle Disease virus is an RNA virus. Flavonoids, saponins, and tannins are compounds that function as antivirals. Flavonoids are a group of natural phenolic compounds that have antiviral activity, specifically as reverse transcriptase. It is important to note that this text already meets the desired characteristics and is free from errors. Flavonoids cause protein denaturation at low levels and protein coagulation at high levels, leading to cell death. Flavonoids are believed to act as antivirals by inhibiting the viral reverse transcriptase enzyme, preventing the synthesis of viral RNA into cDNA and the replication of the virus. This, in turn, prevents the production of necessary viral proteins and enzymes, ultimately leading to the death of the virus.[102] Similarly, saponin compounds exhibit antiviral activity by inhibiting the formation of capsids in viruses and increasing the resistance of host cells. Meanwhile, tannin compounds in plants can inhibit the

interaction between host cell surface proteins and viral proteins, thereby preventing virus attachment and penetration into the plasma membrane. In other words, tannins bind to both viral and host cell proteins to form a complex, which prevents the virus adsorption process. This extract has potential antiviral properties.[103]

Although some compounds in betel leaf have been shown to have antiviral activity against certain viruses, there are no reports on the activity of betel leaf constituents against viruses that cause oral health problems.

Pathogenic Bacteria That Cause Oral Infections

Streptococcus mutans

Streptococcus mutans is a bacterium belonging to the *Streptococcaceae* family and the *Streptococcus* genus. Its name is derived from the change in coccal morphology to cocco-bacilli. *S. mutans* is a gram-positive bacterium with facultative anaerobic properties. During growth, this spherical bacterium typically forms pairs or chains with a cell diameter of 0.5–0.7 µm. Additionally, *S. mutans* possesses a thick cell wall structure (15–80 nm) and a single coat. This bacterium is capable of surviving in low pH environments and thrives at temperatures ranging from 18 to 40°C. The primary habitat of *S. mutans* bacteria is the tooth surface, particularly near the gums and in dental carious lesions. A conducive environment for *S. mutans* can facilitate population growth and pathogenicity.[104]

One of the health issues caused by *S. mutans* is dental caries, which takes 6–24 months to develop. In the oral cavity, *S. mutans* bacteria can organize themselves in the bacterial community through cell-cell contacts and connections with other medium components, including polysaccharides, proteins, and DNA, leading to the formation of biofilms in the mouth. Technical terms such as biofilms will be explained when first used to ensure comprehensibility. The objective language used herein aims to avoid biased expressions. The cariogenicity of *S mutans* can be impacted by various factors such as diet, sucrose, antibiotics use, mouthwashes with antiseptics, and overall oral hygiene conditions or oral cavity area.[105,106]

During dental caries, *S. mutans* bacteria produce enzymes that actively ferment carbohydrates, including glucosyltransferase, dextranase, and fructosyltransferase.[107] These molecules break down sucrose, converting it into glucan, dextran, and fructan. Sucrose is a disaccharide linked by β-2,1 consisting of glucose and fructose. Research has revealed it to be the most cariogenic carbohydrate.[108] Virulence significantly relies on glucan production because it fosters biofilm formation and generates a polysaccharide matrix. Fructans constitute a type of extracellular carbohydrate, which is metabolized through the action of FruA fructanase enzyme that produces fructose as a source of energy.[109,110]

ATP-binding cassette (ABC) transporters, like the Msm and MalXFGK transport systems, have the primary responsibility of transporting oligosaccharides into cells. On the other hand, phosphoenolpyruvate and phosphotransferase (PTS) sugars are responsible for transporting monosaccharides and disaccharides. Several PTS can transport the same carbohydrate in *S. mutans*, with at least three PTS involved in fructose transport and numerous PTS and permeases involved in glucose transport.[111] Carbohydrates are phosphorylated and converted into fructose-6-phosphate (Fru-6-P) within cells, where they are fermented by glycolysis to produce organic acids, particularly lactic acid.[112] Moreover, Fru-6-P is transformed into glucosamine-6-phosphate (GlcN-6-P), which acts as the primary precursor for the synthesis of cell walls. When additional carbohydrates are stored as intracellular granules and utilised as a reserve energy source during times of hunger, cells have the ability to generate intracellular polysaccharides (IPS), which are polymers of the glycogen-amylopectin kind.

Streptococcus sanguinis

Streptococcus sanguinis is a Gram-positive bacterium that is a facultative anaerobe and lacks spores. It is part of the pathogenic bacteria that can cause infections in the oral cavity, with the most frequent being the creation of biofilms that could eventually result in dental health issues such as dental caries. *S. sanguinis* undergoes cell division along a single axis, leading to the production of chains or pairs of cocci. The circular DNA molecule of *S. sanguinis* SK36 has 2274 protein codes and 2,388,435 base pairs, obtained from dental plaque in humans.[113,114] tRNA has 61 genes, expected to

generate 50 carbohydrate transporters and 20 amino acids, including the phosphotransferase enzyme. This enzyme is capable of transporting fructose, glucose, mannose, lactose, cellobiose, glucosides, maltose and trehalose. *S. sanguinis* can grow using different carbohydrate sources.

The initial stage in producing an oral biofilm involves the attachment of *S. sanguinis* and other primary colonies to the large molecular complex created on the tooth surface coated with saliva.[115,116] Apart from *S. mutans, S. sanguinis* is also a significant contributor to biofilm development and serves as a fundamental species in the ecology of oral biofilms.[117–119] *S. sanguinis* bacteria, in contrast, may have advantageous effects by generating H_2O_2 as a mechanism for producing extra O_2 and performing as a broad-spectrum antibacterial agent to hinder the expansion of *S. mutans* and other anaerobic bacteria that contribute to periodontal disease.[120]

Bacterial adhesion to the tooth surface is crucial to the formation of the Acquired Enamel Pellicle (AEP). This process is aided by negatively charged residues and electrostatic interactions with hydrophilic areas of salivary proteins.[121] While *S. sanguinis* can adhere to salivary-free hydroxyapatite, the initial attachment is probably due to surface interactions between streptococci and salivary components.[122] The mechanism for salivary protein binding is mediated through interactions between compounds of protein-carbohydrate or protein-protein and receptors present on the bacteria's surface. The dental plaque and AEP show the detection of amylase, which is the most common salivary protein, to which *S. sanguinis* attaches via long filamentous pili in a specific manner.[123,124]

Enterococcus faecalis

Enterococcus faecalis is a gram-positive, non-motile bacterium with a spherical shape. These bacteria are facultative anaerobes with a fermentative and non-sporadic metabolism.[125] The ovoid cells of *E. faecalis* exhibit characteristics of single, paired, or short chain formations and typically elongate in the direction of the chain. The bacteria have a diameter of 0.5–1μm[45] and are commonly found in the root canal region of teeth. *E. faecalis* bacteria demonstrate the capacity to exist in highly extreme surroundings, such as those that possess very alkaline pH and elevated salt concentrations.[126,127] Additionally, the resistance of *E. faecalis* to several antimicrobial agents poses a serious concern, as it enables survival within root canals following treatment procedures.[50]

The pathology of *E. faecalis* bacteria commences when these bacteria invade the dental pulp tissue either by direct invasion (caries), crown or root fractures, attrition, abrasion, erosion and cracks in the crown, invasion of blood vessels (open lymphatics linked with periodontal disease) or through infectious disease (transient bacteremia).[128,129] The said bacteria then infiltrate the root canal and produce metabolic products that may incite reactions in the periapical tissues. There are numerous virulence factors responsible for the survival of *E. faecalis* in the dental canal, including the Aggregation substance factor, which binds leukocytes and the extracellular matrix, conferring immunity protection.[130]

Adhesive surface factors, including attachment to dentine collagen or body tissue and biofilm formation, have been found to have an impact.[130] Lipoteichoic acid factor, with its attachment to body tissues, is found to stimulate cytokine production from monocytes, leading to inflammation and resistance to root canal medication. Additionally, extracellular factors have contributed to the production of superoxide, which has had a detrimental effect on cells and tissues during the inflammatory process.[131] Gelatinase factor is an extracellular zinc metalloprotease capable of hydrolysing collagen and hyaluronidase lysis enzymes present in damaged dentine and periapical tissue. Finally, the ability to produce toxins and suppress the growth of other bacteria is exhibited by Cytolysin, AS-48, and bacteriocins.[131–133]

Pathogenic Fungi That Cause Oral Infections

Candida albicans

The fungus *C. albicans* naturally resides in both the digestive tract and vagina. It coexists in harmony with the flora that also dwells in the intestines under usual circumstances. As long as the body remains healthy, the fungus does not cause any issues since it is counterbalanced by the probiotic bacteria that also inhabit these regions. The *Candida albicans* microorganism can become pathogenic, causing disease under certain circumstances, and is therefore considered an opportunistic pathogen, as seen in cases of candidiasis.[134] It is typically found in the oral cavity, on the skin surface, within the genitourinary tract, and also in the gastrointestinal tract. Candidiasis is a mucocutaneous infection that triggers

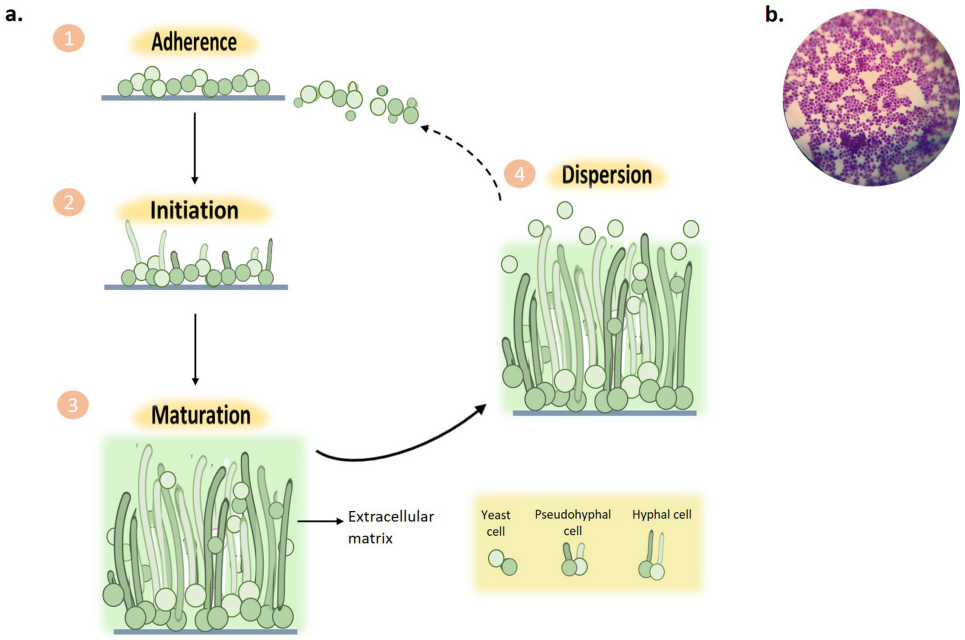

Figure 11 Biofilm formation by *Candida albicans*.
Notes: *C. albicans* biofilm formation is divided into four main stages: the first begins with the attachment of the round yeast-shaped cells to the surface; thesecond stage of initiation of biofilm formation; the third stage of maturation of complex structured biofilms; and the fourth stage dispersion of the yeast-forming cells from the biofilm to the new seed bed (**a**). *Candida albicans* stained with Gram stain (**b**). Data from Lohse et al.[136]

physiological alterations and tissue harm that can be similar to thrush, but it is characterised by symptoms such as irritation, itching and discharge.[135] Formation of *C. albicans* in oral biofilms illustrated in Figure 11.

The morphology of *C. albicans* comprises shapes ranging from oval, round, to elliptical, with sizes that vary from 2–5μ x 3–6μ to 2–5μ x 5–28.5μ.[137] Clamydospora is an infrequently found species that distinguishes *C. albicans* from other *Candida* species. Clamydospores, which are spores formed by hyphae, exist on the lateral or terminal part of the hyphae, and have enlarged, rounded, and thick walls.[137,138] *C. albicans* has a cell wall structure that is complex. The cell wall of *C. albicans* has a thickness ranging from 100 to 400 nm. Its essential functions include shaping and safeguarding the cell from the surrounding environment, comprising glucan, mannan and chitin as the primary components.[139] The multiplication of *C. albicans* occurs via blastospores that emerge from shoots. Blastospores are generally round or oval in shape and located around the septum. They exist in small numbers and continue elongating, subsequently developing pseudo-hyphae or chlamydospores with thick walls that measure approximately 8–12μ in diameter. Technical abbreviations such as "μ" will be explained upon first use.[140]

Candida albicans is capable of growth over a broad pH range, however, it exhibits optimal growth between pH 4.5 and 6.5. The yeast can also flourish in temperatures between 28–37°C. Organic compounds are an essential carbon and energy source for *C. albicans* metabolic processes and growth.[141–143] As a facultative anaerobic organism, it can perform cell metabolism during both anaerobic and aerobic conditions. The virulence factor in *C. albicans* commences with the attachment process, hyphae formation, thigmotropism, protease secretion, and phenotypic changes. Its ability to produce and secrete the enzyme, aspartyl protease, to activate the virulence factor is one of the crucial factors contributing to *C. albicans* virulence.[144–147]

Candida albicans is capable of infecting through interactions with multiple microorganisms present in the oral cavity, resulting in the development of several oral health issues over several years. These health problems include oral candidiasis, endodontic disease, dental caries, periodontitis, and biofilm-associated oral diseases.[148] The cross-kingdom interactions between these microorganisms play a crucial role in the development of such diseases. Technical term abbreviations will be explained upon first use. Physical attachment to the fungal cell wall (eg surface proteins and EPS), cross-feeding of metabolites, extracellular signalling, and changes in the environment enable *C. albicans* to interact with oral bacteria. Figure 12 provides an illustration of the interaction of *C. albicans* with various inhabitants of the oral cavity.

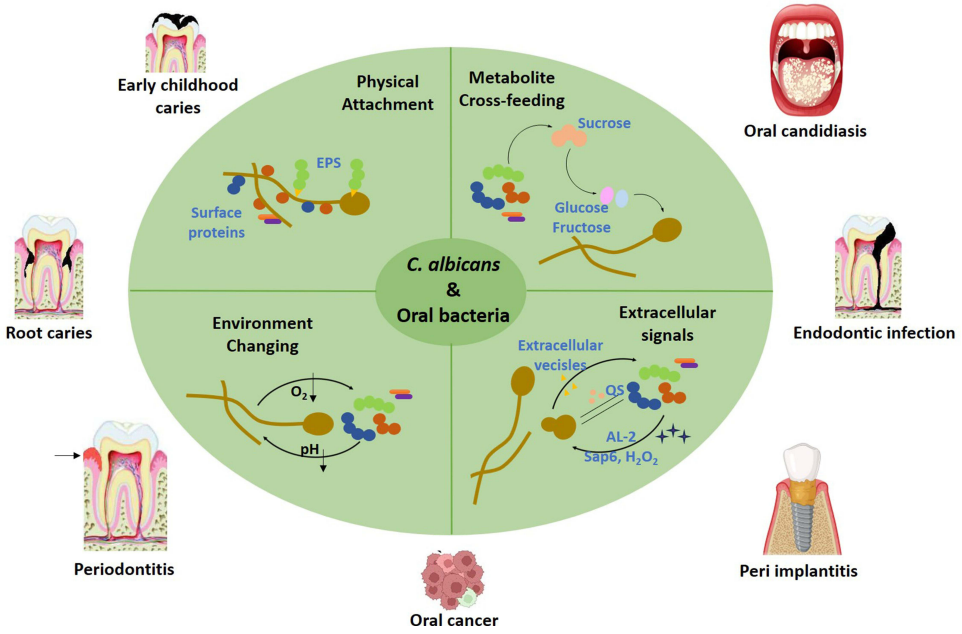

Figure 12 Pathogenic mechanism of cross-kingdom interaction between *C. albicans* and oral bacteria.
Note: Adapted with permission from Du, Q., Ren, B., Zhou, X., Zhang, L., & Xu, X. (2022) Cross-kingdom interaction between Candida albicans and oral bacteria. Frontiers in Microbiology. 13(November), 1–14.[149]

Conclusion

The conclusion that can be drawn from several literatures is that the Red Betel plant has significant potential for use in the development and application of medicine. It can be used as a candidate for herbal medicine or a raw material for medicinal mixtures to treat infections caused by bacteria, fungi or viruses. The anti-infective potential of Red Betel leaves is mainly determined by the quantity and composition of its secondary metabolites. This has been proven through in vitro and in silico testing. These factors also collectively contribute to the bioactivity of Red Betel, resulting in anti-infective effects. This review highlights the inhibition of pathogenic oral bacteria and fungi responsible for oral infections. The components in Red Betel have been proven to be able to inhibit the growth of *S. mutans* bacteria, which are pioneer bacteria in oral health problems. In addition, the active compounds in Red Betel leaves can inhibit *C. albicans*, the dominant fungus in the oral cavity, as shown by in vitro, in silico, and ADMET evidence. This scientific article presents valuable data that can contribute to the advancement of drug research and development, as well as the exploration and identification of potential new anti-infective agents.

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Author Contributions

All authors have made a substantial contribution to the work reported, be it in the conception, design, conduct, acquisition, analysis, and interpretation of data, or in all these areas; all authors have also been involved in the preparation, revision, or critical review of the article reported; have given final approval to the version to be published; have approved the journal to which the article has been submitted; and agree to take responsibility for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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