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Review Article

Phytochemistry and Antibacterial Efficacy of *Kelakai* (*Stenochlaena palustris* (Burm.f.) Bedd.) Extract: A Review

Muhammad R.F. Muslim[1], Lutfi Chabib[1]*, Hasyrul Hamzah[2]

[1]*Master Program of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia, Yogyakarta, Indonesia*
[2]*Department of Pharmacy, Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda, Indonesia*

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ABSTRACT

In many countries, the prevalence of infectious diseases has posed a significant challenge to public health. A conventional approach to disease treatment involves antibiotic administration. However, protracted and uninterrupted medication use can lead to the development of antibiotic resistance. *Kelakai (Stenohclaena palustris* (Burm.f.) Bedd) is a species of fern commonly found in the regions of Kalimantan and Sumatra. This review aims to critically examine the antibacterial properties of *Kelakai* and elucidate the specific components in *Kelakai* that exhibit antibacterial activity. The research method involved conducting a comprehensive literature review of articles from prominent academic databases, including Google Scholar, PubMed, and ScienceDirect. The search process resulted in 8 articles that were deemed eligible for inclusion based on the predetermined criteria. These articles demonstrate the efficacy of *Kelakai* extract in inhibiting the growth of gram-positive bacteria in comparison to gram-negative bacteria. The isolates derived from *Kelakai* extract, especially stenopalustrosides A-D, show notable efficacy in suppressing the proliferation of gram-positive bacteria.

Keywords: Stenochlaena palustris (Burm.f) Bedd, Antibacterial activity, *Kelakai*, Literature review

Introduction

Infection is a major health concern in developing countries, including in Indonesia, and antibiotics are commonly employed to treat infection. However, extended and repeated use of antibiotics may lead to harmful consequences and adverse effects on individuals, including the development of antibiotic resistance. In 2018, approximately 500,000 people were believed to have contracted bacterial infections and encountered resistance to antibiotics.[1] Utilizing and evaluating medicinal plants with potential antibacterial characteristics present an alternative approach to combat antibiotic resistance.

The *Kelakai* plant, scientifically known as *Stenohclaena palustris* (Burm.f.) Bedd., is a medicinal plant widely spreading across the regions of Kalimantan and Sumatra. *Kelakai*, a member of the *Blechnaceae* family, typically grows horizontally on the ground or vertically on trees. *Kelakai* is an indigenous species found in tropical countries, such as India, Malaysia, Indonesia, and Australia.[2] Young *Kelakai* leaves, which have a brownish red color, are consumed as a vegetable in Malaysia, Thailand, the Philippines, and Indonesia.[3] In western India, *Kelakai* leaves, with their antibacterial properties, are commonly used as a medicinal remedy for such ailments as fever, gastric acidity, sore throat, and various skin conditions. In addition, the rhizome has therapeutic properties that can be utilized for the treatment of burns.[4]

*Corresponding author. E mail: lutfi.chabib@uii.ac.id
Tel: +6281575074356

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The pharmacological and phytochemical properties of *Kelakai* plants have been examined. A study demonstrated that *Kelakai* water extract exhibits significant antioxidant activity and is rich in polyphenols and flavonoids.[5] The study also showed that *Kelakai* water extract is a promising α-glucosidase inhibitor compared to quercetin as the reference. However, there is limited knowledge of the phytochemicals of *Kelakai*. Research indicates that *Kelakai* leaves in isolation contain stenopaluside, cerebroside, lutein, and *β*-sitosterol. Further investigation revealed that stenopalustrosides A-E and kaempferol glucoside have notable antibacterial properties against gram-positive bacteria.[6] Therefore, a comprehensive examination of the phytochemical composition, antibacterial attributes, and mechanism of action of *Kelakai* against bacteria necessitates a literature review.

Research Methods

The research approach entailed an observational strategy with a comprehensive study of the existing literature and a thorough analysis of the putative antibacterial properties of *Kelakai* extract and its constituent components with their antibacterial effects. The researchers conducted a literature search on the Google Scholar, PubMed, and ScienceDirect databases. The search terms included in the PubMed and ScienceDirect database queries were "*Stenochlaena palustris*" OR "*Stenochlaena palustris* extract" AND "Antibacterial" OR "Antimicrobial". Meanwhile, the search terms employed in Google Scholar were "*Stenochlaena palustris*" ATAU "Kelakai extract" DAN "Antibacterial" ATAU "Antimicrobial". From the search, a total of nineteen journals were obtained. After the inclusion criteria were applied, only eight journals were deemed suitable. The eligibility criteria for this study included complete articles in either national or international publications, articles written in Indonesian or English published from 2018 to 2023, and those focusing on experimental research. Additionally, the exclusion criteria were review articles, articles published before 2018, articles without a full-text format, and those not containing the specific keyword "Kelakai Extract".

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Results and Discussion

The journal search identified eight publications that satisfied the specified inclusion criteria. They were both national and international in scope, contained full-text articles, were published between 2018 and 2023, and focused on experimental research. Phytochemical screening of *Kelakai* leaves was found in all the journals. Six of these journals specifically examined the antibacterial activity of *Kelakai* while two journals explored the toxicity, antioxidants, and antibacterial activity of *Kelakai* plant as presented in Table 1.

Of the eight articles, three performed and analyzed phytochemical screenings for *Kelakai* leaves. The phytochemical composition of *Kelakai* leaf extract was analyzed using the maceration technique with 96% ethanol. The findings indicated the presence of alkaloid, flavonoid, tannin, steroid, and triterpenoid components, whereas no saponin compounds were detected.[7] A separate investigation demonstrated that the ethanol extract derived from *Kelakai* leaves exhibited the presence of alkaloids, flavonoids, saponins, tannins, phenolics, steroids, and triterpenoids in favorable amounts.[8] The water infusion of *Kelakai* leaves yielded a positive result for various metabolite components, including alkaloid, flavonoid, saponin, phenol, steroid, triterpenoid, and tannin contents.[9] Variations in secondary metabolite molecules in plants are determined by two elements: internal and external variables. External variables can be categorized into abiotic and biotic factors. Abiotic factors, such as salt, water availability (drought or excess), extreme temperatures, and solar radiation, contribute to environmental stress.[10] Microorganisms and herbivores exert an influence on biotic variables. Meanwhile, internal variables encompass the genetic elements, the many stages of plant growth and development, and the specific plant components utilized. The presence of secondary metabolite chemicals is also affected by several other factors, including variations in plant collecting locations, extraction methodologies, and solvent selection.[11]

Kelakai leaves contain secondary metabolite chemicals that employ various mechanisms to inhibit bacterial proliferation. They are distinct due to the synergistic impact of secondary metabolite chemicals, which depends on the specific types and morphology of bacteria.[8] Flavonoids inhibit bacterial growth by disrupting cell walls, deactivating enzymes, binding to cell adhesions, and damaging cell membranes.[12] Impairment of bacterial cell wall and cytoplasm stability leads to compromised selective permeability, active transport function, and control over the bacterial cell protein structure. Due to this disturbance, macromolecules and ions are released from the cell, leading to the loss of shape and an eventual lysis of bacterial cells. Meanwhile, phenolic chemicals inhibit bacterial growth by obstructing protein enzymes in cell membranes. Phenolic chemicals that bind to proteins through hydrogen bonds cause protein structure destruction. Since the cell wall structure and bacterial cytoplasm mainly consist of protein and fat, this damage significantly affects them.[13] In addition, tannins specifically interact with cell wall polypeptides, resulting in cell wall degradation due to the phenolic nature of tannins. Tannin compounds can also act as an iron chelating agent in a medium, thus preventing bacteria from acquiring iron.[8]

Meanwhile, the antibacterial activity of terpenoid compounds is achieved by destructing bacterial cell membranes. Subsequently, terpenoids interact with the active region of the membranes, leading to the dissolution of their lipid components and an increase in permeability.[12] The compound make-up of terpenoids, particularly the hydroxyl groups of terpenoid phenolics and the quantity of individual components, plays a crucial role in determining their antibacterial activity.[8] Furthermore, saponin compounds, which are intricate glycoside chemicals, exhibit antibacterial properties by destabilizing the integrity of bacterial cell membranes, leading to the breakdown of bacterial cells.[14]

In a recent study, the chemical composition of the ethanol extract of *Kelakai* leaves was analyzed using the liquid chromatography-mass spectrophotometry (LC-MS).[8] The analysis revealed the presence of 81 peaks, corresponding to 66 different chemicals, with retention durations ranging from 0.873 to 27.498 minutes. However, only 53 peaks of 44 compounds, or approximately 29 compounds, had a quality level of more than 85%. Approximately 29 of these chemicals possessed the ability to serve as antibacterial agents. The analysis revealed that the ethanol extract derived from *Kelakai* leaves contained the following compounds: alkaloids (2.19%), alcohol (23.56%), amine (3.50%), amine alcohol (1.32%), amino acids (34.25%), fatty acids (10.88%), flavonoids (0.74%), glycosyl glucose (1.89%), lipid derivatives (2.94%), monocarboxylic acids (0.40%), saponins (0.13%), steroids (0.58%), and terpenoids (1.44%). The ethanol extract of *Kelakai* leaves exhibited significant potential as a reservoir of antioxidants and antibacterial agents. These findings suggest that the secondary metabolites can work effectively with other chemicals or function as an antibacterial agent on their own.[8]

The researchers in the eight articles reviewed conducted antibacterial tests on *Kelakai* leaf extract against different species of bacteria with varied gram characteristics. There were six types of gram-positive bacteria used, including *Staphylococcus aureus*, *Bacillus cereus*, *L. monocytogenes*, *Streptococcus mutans*, *Streptococcus sobrinus*, and *Enterococcus faecalis.*[7,9,15–17] The study identified six gram-negative bacteria involved consisting of *Salmonella typhi*, *Porphyromonas gingivalis*, *Parahaemolyticus*, *Escherichia coli*, *Ralstonia solanacearum*, and *Aeromonas hydrophila*.[7–9,16,18]

The findings showed that the extract of *Kelakai* leaves had superior bactericidal activity against gram-positive bacteria rather than gram-negative bacteria.[16] The difference in the cell structure of gram-negative and gram-positive bacteria accounts for this phenomenon. Gram-negative bacteria exhibit greater resistance to antibacterials due to the impermeable barrier created by their outer membrane. Therefore, antibacterial chemicals can be hindered from accessing targeted bacterial cells.[19] Meanwhile, the cell walls of gram-positive bacteria are typically less complex and composed of a thin layer of lipids (1 to 4%). The peptidoglycan has a thickness range of 20 to 80 nanometers, thus facilitating the infiltration of bioactive substances into bacterial cells.[20] This is further corroborated that the substances in *Kelakai* leaves, specifically stenopalustrosides A-D, have a noteworthy capacity to hinder the growth of gram-positive bacteria.[2]

Table 1: Summary of the Articles in Review

Parts Used	Bacteria Used	Country	Methods	Results	Reference
Leaves	*Salmonella typhi* and *Staphylococcus aureus*	Indonesia	An antibacterial activity test using the disc diffusion method to evaluate the effectiveness against *S. aureus* and *S. typhi*	The *Kelakai* leaf ethanol extract exhibited a Minimum Inhibitory Concentration (MIC) of 10.6% (w/v) against the growth of *S. aureus* and 9% (w/v) against *S. typhi*. The Minimum Kill Concentration (MKC) of *Kelakai* leaf ethanol extract against the growth of *S. aureus* was 11%	7

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				(w/v) and 10.8% (w/v) against *S. typhi*.	
Leaves	*Porphyromonas gingivalis*	Indonesia	A bacterial growth inhibition test using the diffusion method	The *Kelakai* leaf extract exhibited the greatest inhibitory effect at a dose of 100 mg/ml, resulting in an inhibition zone of 14.61 mm.	[18]
Fronds	*Bacillus cereus, Staphylococcus aureus, L. monocytogenes, Salmonella typhi, Parahaemolyticus*	Indonesia	An antibacterial activity test by employing the well diffusion method	The IC_{50} of the ethyl acetate extract of *Kelakai* was 50.63 ± 0.46 µg/mL in the DPPH method and 60.03 ± 0.65 µg/mL in the Nitric oxide method. The outcome of the antiplasmodial test was 11.06 ± 0.45 µg/mL. The toxicity test yielded an LC_{50} value greater than 1000 µg/mL. The ethyl acetate extract of *Kelakai* exhibited antibacterial activity at a concentration of 500 µg/disc against *B. cereus*, *parahaemolyticus*, *L. monocytogenes*, and *S. typhi*.	[16]
Leaves	*Streptococcus mutans*	Indonesia	An antibacterial efficacy test using the liquid dilution and solid dilution methods	The Minimum Inhibitory Concentration (MIC) was 12.5% while the Minimum Kill Concentration (MKC) was 50%.	[17]
Old and Young Leaves	*Staphylococcus aureus, Escherichia coli* and *Candida albicans*	Indonesia	An antimicrobial activity test using the diffusion method	The *Kelakai* leaves and *Katuk* leaves exhibited antimicrobial properties. The *Kelakai* leaves exhibited superior antibacterial activity to the *Katuk* leaves. The antibacterial activity of mature *Kelakai* leaves surpassed that of young *Kelakai* leaves. The antibacterial activity of young *Katuk* leaves was superior to that of mature *Katuk* leaves.	[9]
Leaves	*Ralstonia solanacearum* and *Streptococcus sobrinus*	Indonesia	An antimicrobial activity test using the agar well diffusion method	The inhibitory diameter results indicated that the ethanol extract of *S. palustris* leaves and *P. caudatum* at concentrations of 0.5%, 1%, and 2%, had no inhibitory effects on the growth of *R. solanacearum*. However, it successfully inhibited *S. sobrinus*, with the largest diameter of 13.7 mm seen at a concentration of 2%.	[21]
Leaves	*Enterococcus faecalis*	Indonesia	An antibacterial activity test using the diffusion method	The findings demonstrated notable differences between the cohorts administered with *Kelakai* leaf extract in comparison to those treated with a 2.5% sodium hypochlorite solution. The average inhibition values at the concentrations of *Kelakai*	[15]

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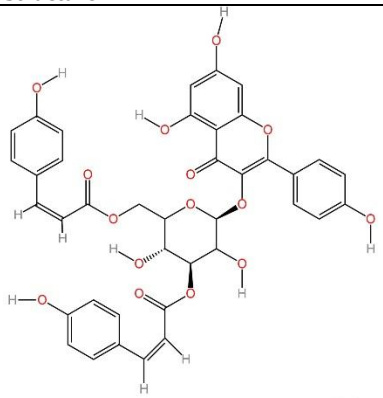
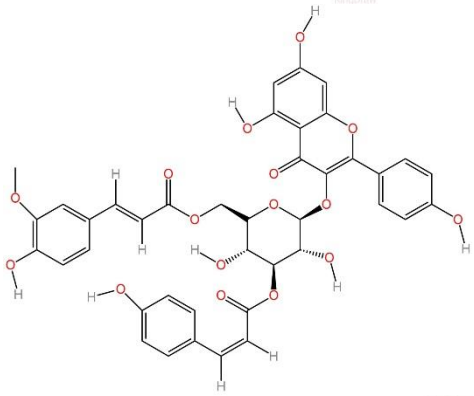
				leaf extract of 25%, 50%, 75%, and 100% and the 2.5% sodium hypochlorite solution were 9.47 mm, 14.64 mm, 17.91 mm, 21.24 mm, and 23.27 mm, respectively.	
Leaves	*Aeromonas hydrophila*	Indonesia	An antibacterial activity test using the broth dilution method	The findings demonstrated that the extract of *Kelakai* leaves effectively suppressed the growth of *A. hydrophila*. The *Kelakai* leaf extract exhibited a highly potent IC_{50} antioxidant activity of 42.47 ± 0.98 µg/mL, along with a total phenol content of 193.97 ± 0.11 mg GAE/g, total flavonoids of 23.45 ± 0.14 mg QE/g, and total alkaloids of 11.74 ± 0.10 CE/g.	[8]

Table 2: Chemical Constituents Isolated from *Kelakai*

No.	Structure	Name
1.		Stenopalustroside A[2]
2.		Stenopalustroside B[2]

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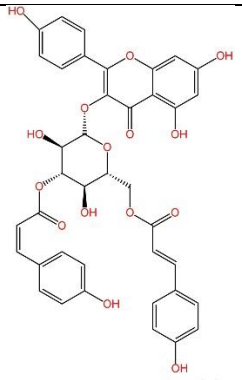
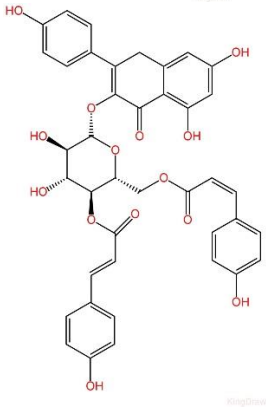
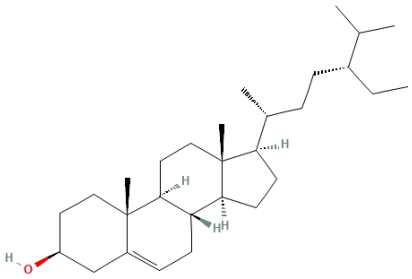
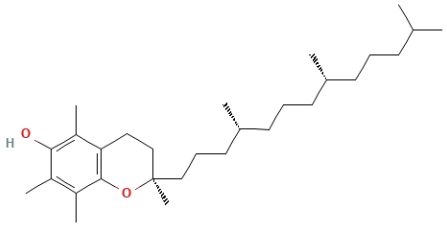
3.  Stenopalustroside C[2]

4.  Stenopalustroside D[2]

5.  *β*-sitosterol[22]

6.  *α*-tocopherol[22]

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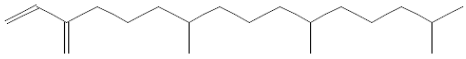
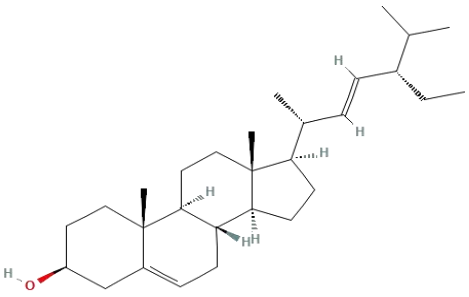
7.  Neophytadiene[22]

8.  Stigmasterol[22]

Conclusion

In addition to its antibacterial characteristics, the *Kelakai* plant possesses secondary metabolites such as alkaloids and flavonoids. The stenopalustrosides A-D found in *Kelakai* leaves act as an antibacterial agent. Therefore, the *Kelakai* plant has the potential to be a prospective option for future antibacterial and antioxidant therapy.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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